Determination of the physiological function of a *Zea mays* pentatricopeptide repeat protein through cultural studies

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DECLARATION

I, Mmamorena Dorothy Mojanaga solemnly declare that this work entitled ‘**’Determination of the physiological function of a *Zea mays* pentatricopeptide repeat protein through cultural studies**” is my work and has not been submitted to any institution of higher learning other than at North-West University for examination or other purposes.

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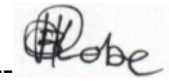
STUDENT



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Dr BT Dikobe ..... **DATE**
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DEDICATION

This work is dedicated to my mother Mrs Mananki Mojanaga, my father Mr Oreeditse Mojanaga, my daughter Omaatla Mojanaga and my siblings Malerato, Masego, Tefo, Mathapelo and Othusitse, thank you all for the support and love that you offered throughout my studies.

TO GOD BE THE GLORY

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To Mr Ezekiel Mosiane thank you a lot uncle for paving a way for me to enrol as a first year student (undergraduate) now I am this far because of you. I would also like to thank the Plant Biotechnology group for their support on my laboratory work and keeping a smile on my face during the most difficult time's thank you all for sharing this academic journey with me. To my friends all would have not been easy without you and lastly to my parents and siblings, I am truly blessed to have a family like you, indeed a family that prays together stays together. I love you all.

Most importantly I thank you Lord for the love and knowledge that you bestowed upon me. I thank you for surrounding me with all the positive people in my life. You have never failed nor forsaken me. It has not been an easy journey you gave me hope, strength and courage to persevere and to make my daughter proud, indeed your love endures forever I will honour and uphold your name forever.

DEFINITIONS OF TERMS

Abiotic stress: The negative effects of non-living factors on living organisms in a specific environment.

Adenosine triphosphate: A molecule that carries energy within cells an end product of many processes such as photophosphorylation, cellular respiration, and fermentation.

Adenylate cyclases: Enzymes that are capable of converting adenine 5′-triphosphate (ATP) to cyclic 3′,5′-adenosine monophosphate (cAMP).

Annotation: A determination of the locations of genes, their coding regions in a genome and the functions of such genes.

Climate change: The long-term shift in weather patterns in a specific region or globally.

Cultivar: A type of plant that is bred for desired traits which are reproduced in each new generation by method.

Culturing: The propagation of microorganisms in a growth medium.

Cyclic adenosine 3′,5′-monophosphate (cAMP): A second messenger cyclic nucleotide formed from adenosine triphosphate by the action of the enzyme adenylate cyclase that participates in signal transduction.

Maize: A tall growing cereal grain that is staple food crop.

Mutation: A change that occurs in a DNA sequence to alter the genetic message carried by the gene.

Plant morphology: The study of the physical form and external structure of plants.

Plant physiology: The study of functions and behaviour of plants.

Salt stress: The accumulation of excessive salt contents in the soil which eventually results in the inhibition of crop growth.

Second messenger: A biological molecule capable of transmitting external cellular signals within the cell for the development of appropriate cellular responses through regulated gene expressional and metabolic events.

Signal transduction: The pathway of molecular signals from a cell's exterior to its interior.

LIST OF ABBREVIATIONS

AC	**:**	Adenylate cyclase
ANOVA	**:**	A one-way analysis of variance
ATP	**:**	Adenosine 5′-triphosphate
cAMP	**:**	Cyclic 3′,5′-adenosine monophosphate
cm	**:**	Centimeter
CO_2	**:**	Carbon dioxide
FOV	**:**	Field of view
IRGA	**:**	Infra-red gas analyzer
mM	**:**	Millimolar
mt	**:**	Mutant-type
MVD	**:**	Mutant variety database
NaCl	**:**	Sodium chloride
NADPH	**:**	Nicotine adenine dinucleotide phosphate
PPR	**:**	Pentatricopeptide repeat protein
ROS	**:**	Reactive oxygen species
SEM	**:**	Standard errors of the means
SNK	**:**	Student Newman Kuehls
SOS	**:**	Salt overly sensitive
T-DNA	**:**	Transfer DNA
TPR	**:**	Tetratricopeptide repeat
wt	**:**	Wild-type
ZmPPR	**:**	*Zea mays* Pentatricopeptide repeat protein

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ABSTRACT

Farming of crop plants for food and feed purposes makes agriculture a vital system that sustains both human beings and animals in general. Plants are often affected by various biotic and abiotic stress factors, leading to reduced growth and production. Salinity is one of the major abiotic stresses that severely affects the morphological, biochemical, and physiological processes of plants. Generally, plants naturally display complex interactive and adaptive cellular responses when exposed to salinity or any other stress factor. Therefore, in order to gain a better understanding of the response mechanisms through which plants utilize and survive when exposed to salinity, we targeted the pentatricopeptide repeat (PPR) protein that was previously confirmed as an adenylate cyclase (AC) and known to be involved in various key cell signalling and plant developmental processes under varying environmental stresses. In order to carry this out, wild type (wt) and mutant (mt) maize plant lines for this PPR protein were developed and grown under salt stress, followed by assessment and evaluation of their associated morphological and physiological responses under such conditions, for 16 days. Results obtained indicated that salt stress had severe negative effects on various morphological parameters such as plant height, shoot and root lengths, leaf width, shoot and root weights in mt plant lines than in wt plants. In contrast, increased leaf area in mt plants was observed. In addition, salt stress also affected the physiological parameters such as stomatal density and stomatal count, photosynthesis, respiration, and transpiration in mt plant lines than in wt plants. The study therefore, has successfully managed to determine the physiological and morphological functions of the PPR protein in plants when challenged with salt stress. Thus the protein could be potentially used in breeding and cultivar development programs aimed at developing varieties that are resistant and/or tolerant to salinity.

Keywords: Salt stress, pentatricopeptide repeat protein, mutant-type, wild-type, physiology, morphology, cultural studies, *Zea mays*.

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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Agriculture is the world's leading source of food and has long been the backbone of human survival both directly and indirectly, however due to the environmental changes and population growth, food security has become one of the hot topics worldwide. Therefore, the agricultural sector is under massive strain to strike a balance between food production and consumption rates, hence advances in plant biotechnology have been adapted to maintain and ensure stable food production.

Since agriculture and biodiversity are strongly interrelated, plants as multicellular life forms, utilize the process of photosynthesis to manufacture their own food that benefit humans and other organisms. They serve as a significant source of proteins that are fundamental for human consumption and also provide various raw materials that can be used worldwide. Furthermore, since plants are sessile, this makes them incapable to keep away from ominous conditions that influence their development and result in diminished efficiencies and harvest misfortunes worldwide (Bray, 2002).

Food security has become one of the most pressing issues in the world in relation to environmental changes that bring about new extraordinary plant diseases. It has been anticipated that crop production will decrease with population pressure, if abiotic and biotic stress keep on increasing due to cataclysmic events (Abah *et al*., 2010). Anticipated expansion in the total population to more than 9 billion by the year 2050, will bring about additional development in food interest. Currently, food creation embraces the utilization of 279 kcal consumed daily by every individual, henceforth the degree of food demand will heavily affect the economy. In addition, it has been predicted that the food costs would increase by 70-90% in 2030 worldwide, despite the effect of environmental change, which could make costs two-fold and affect food security (Kwasek, 2012).

Abiotic stress is the negative effect of the non-living life forms on living organisms in a particular climate and this include factors such as thermal heating under outrageous cold environments, extreme high temperatures, dry spells, floods, mineral and soil nutrient constituent (Murphy, 2011). In addition to the other abiotic stresses, drought and salinity

influence over 10% of the arable land, which brings a decrease in the normal yield of significant harvests worldwide (Bray, 2002). The tremendous effects of abiotic stresses on plants and crop productivity have gained popularity these days since most researchers need to understand the plant's response and adaption mechanisms. To further investigate their mechanisms, the use of model plant systems such as *Zea mays* has been adapted. *Zea mays* L. is used to investigate various developmental, morphological, physiological, biochemical and molecular pathways.

Maize is one of the most important grain crops in South Africa produced throughout the country under diverse environments. About 8.0 million tons of maize grain is produced in South Africa annually on approximately 3.1 million ha of land (Du Plessis, 2003). According to Iqbal *et al*. (2020), maize is a cross-pollinated, polymorphic plant in nature. It is a tall, monoecious annual plant and/or grass that produces large narrow, opposite leaves along its stem. Maize or corn belongs to the family of grasses (*Poaceae*), while its genus *Zea* consists of four species of which *Zea mays* L. is of economic importance (Kumar *et al*., 2011).

The supply and demand of maize production continues to increase globally due to its economic importance, a greater shift was predicted to it for the year 2020 that it will surpass the demand for wheat and rice (Pingali, 2001). The processing and consumption of maize varies from country to country, whereby in most countries, the extraction rate varies from 60% to 100%, depending on the product. Maize production continues to show a slightly steady increase throughout the years, which leads to more readily available food, since it is expected that world population will continue to grow and exceed nine billion by 2050 (Kendal and Pimentel, 1994; Ranum *et al*., 2014).

Maize is a versatile crop having a wider adaptability (Kumar *et al*., 2011), which has been reported to be moderately sensitive to salt stress (Farooq *et al*., 2015). Stress in plants, as defined by Litchtenthaler (1998) is any unfavourable condition or set of circumstances that affects or blocks a plant's metabolism, growth or development. Salinity stress is one of the major abiotic stress factors that affect the physiological characteristics and plant growth of maize (Iqbal *et al*., 2020). Soil salinity is a serious threat to maize production in arid and semi-arid regions. Soil salinization is referred to as the accumulation of excess soluble salts in the soil (Bockheim and Gennadiyev, 2000), which results in osmotic stress, reduction in plant growth and crop production (Cicek and Cakirlar, 2002). The use of poor irrigation practices and soil salinization are continuously affecting approximately 20% of the cultivated land worldwide (about 45 hectares) (Gupta and Huang, 2014). Similarly, several physiological

processes such as photosynthesis, respiration, starch metabolism and nitrogen fixation are affected (Davenport *et al*., 2005).

Previous studies have been performed through advanced methods such as cultural procedure, which is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition (Mathe, 2012). Thus cultural studies are important as they can be used to conceptualize the physiology and advancement of higher plants.

This study therefore, mainly focused on the determination of growth/ developmental roles and (cellular level) physiological function of maize in response to salinity stress through cultural studies in particular maize pentatricopeptide repeat (PPR) protein which is a target candidate molecule for biotechnology. This candidate molecule has been previously annotated as an adenylate cyclase which is referred to as an enzyme capable of catalyzing the conversion of adenosine 5'-triphosphate (ATP) into a signalling molecule cyclic 3', 5 adenosine monophosphate (cAMP), which in turn acts as a second messenger in various cellular and metabolic pathways (Gehring, 2010). Their functional roles in higher plants have remained to be a matter of serious debate.

Pentatricopeptide encodes a functional adenylate cyclase (Ruzvidzo *et al*., 2013), however the physiological roles of this specific gene remains largely unknown in cell signal transduction and particularly plant responses. In this regard since PPR is an AC, this possibly indicates its ability to be involved in plant stress responses such as salinity and cell signalling. Thus in our study, it was significant to investigate its growth, development and physiological functions in response to induced salt stress. Under normal circumstances as the levels of cAMP are affected, plants will exhibit numerous responses to stress and result in constraints on the growth, development and productivity of plants (Blanco *et al*., 2020).

Despite previous studies on other plant species that have been reported on mutational analysis of the pentatricopeptide repeat protein (Bentolila *et al*., 2002; Desloire *et al*., 2003), no study so far has demonstrated its physiological functions relating to plant development and abiotic stress response, in particular salt stress through cultural approach on maize.

1.2 Literature Review

1.2.1 Maize Production, Utilization and Consumption

Maize, being the world's most important grain crop and for its successful production, depends on the correct application of production methods such as soil tillage, adapted cultivar, disease control, plant population and fertilization that will sustain the environment as well as agricultural production (Du Plessis, 2003). Due to its importance, it has various uses including human food, animal feed and the manufacturing of pharmaceutical products. The consumption of maize varies greatly from country to country and this can be estimated by the extraction rate of maize, which is the proportion of the flour or meal produced from the whole-grain cereal. The rate differs from 60% to 100% in most countries, depending on the product targeted and intended by the consumer (Ranum *et al*., 2014).

1.2.2 Overview of Maize Growth Stages and Development

Plant growth is referred to as an increase of plant volume and/or mass with or without formation of new structures such as organs, tissues, cells or cell organelles. It is mostly associated with development (cell and tissue specialization) and reproduction (production of new individuals) (Brukhin and Morozova, 2010).

There are various criteria that are utilized for the determination of plant growth rates for example plant height/width, mass, cell number whilst cell differentiation plays a fundamental role in development and morphogenesis as it results in formation of new cells, tissues and organs (Brukhin and Morozova, 2010). Generally maize, requires a warm sunny weather, soil with adequate depth and drainage, high water holding capacity, adequate organic matter and humus content (Du Plessis, 2003). There are two key growth/developmental stages of maize, which are the vegetative and reproductive stages for the successful growth of a mature maize (Figure 1.1). The vegetative growth stage starts from seedling emergence, where a plant develops leaves until flowering initiation of male flowers. The reproductive stage then follows, where there is silk appearance, involving the formation of female flowers, through which soft-dough stage commences and grains start forming until hard-dough stage that shows the dried out leaves and silks of the plant (Tripathi *et al*., 2011).

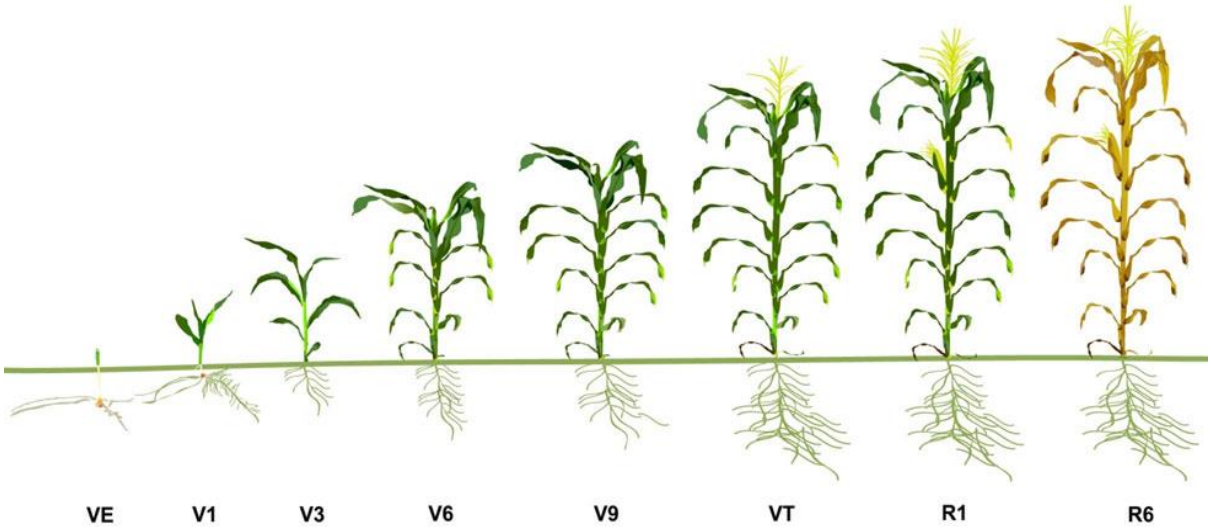

Figure 1.1: Vegetative and reproductive stages of maize from seed emergence to a mature maize plant. VE represents the emergence of seedling, followed by **V1-VT** where a plant develops leaves and visibility of tassel (male flower). The reproductive stages include **R1-R6** where a plant develops silk, followed by the formation of female flowers and dough stage which illustrates a mature plant with dried leaves and silk (https://www.pioneer.com/us/agronomy/staging_corn).

1.2.3 The Physiological Responses of Plants under Stress

It is well-known that plant photosynthesis and respiration are the two fundamental physiological processes that occur in plants. Plant growth and development relies on water for gaseous exchange to take place, however growth is continuously affected by diverse environmental and genetic factors (Kafi, 2009).

Photosynthesis is the fore-most vital process by which green plants convert solar energy into the form of organic compounds synthesized by fixation of climatic carbon dioxide, to generate glucose and oxygen, whilst respiration occurs when glucose and oxygen combine together to generate energy, carbon dioxide and water. Photosynthesis occurs in chloroplasts and utilizes low energy, unreactive carbon dioxide, whilst respiration occurs in mitochondria and cytoplasm and use high energy from adenosine triphosphate (ATP), nicotine adenine dinucleotide phosphate (NADPH) and reactive carbohydrate (Holding and Streich, 2013).

According to Omoto *et al*. (2012), carbon fixation in maize is very sensitive to salt stress. There are possible contributing factors that lead to a decline in photosynthesis. The mechanisms of photosynthesis involve various components such as photosynthetic pigments and

photosystems, the electron transport system and carbon dioxide reduction pathways (Ashraf and Harris, 2013).

Salinity disturbs the balance between production of reactive oxygen species (ROS) and antioxidant defense, resulting in accumulation of reactive oxygen species, which induce oxidative stress. Sodium increase in plant tissues causes ion toxicity, which leads to a decrease in leaf growth and ultimately leaf abscission, which reduces carboxylation. Limited carbon dioxide uptake in a plant results in reduction of photosynthesis rates (Gong *et al*., 2011).

Decrease in stomatal conductance and photosynthetic pigments are due to salt stress, which causes a decline in photosynthesis (Farooq *et al*., 2015). Photosynthesis decreases due to inhibited leaf development and expansion as well as early leaf abscission (Iqbal *et al*., 2020). Figure 1.2 below represents a schematic diagram of the possible mechanisms that leads to salt stress effect on photosynthesis.

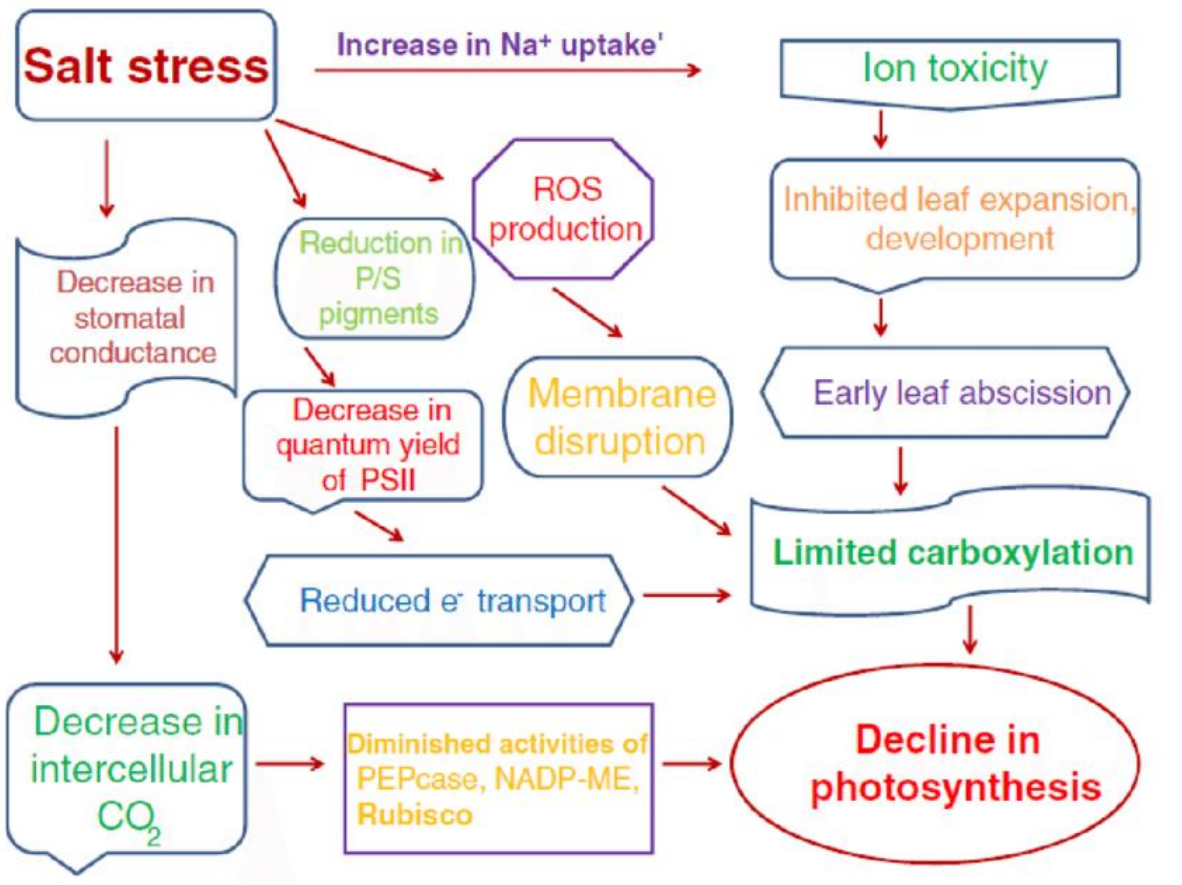

Figure 1.2: Possible effects of salt stress that leads to a decline in photosynthesis. Salinity disturbs the balance between production of reactive oxygen species (ROS), which induce oxidative stress. An increase in sodium ion uptake in plant tissues results in ion toxicity, which decreases leaf growth and leads to early abscission, then ultimately, a decrease in photosynthesis rate (Farooq *et al.*, 2015).

The effect of salt stress on photosynthesis and respiration rates may differ in plants, this is mainly controlled by whether a plant is salt-tolerant or salt-sensitive. Physiological processes, such as photosynthesis and respiration are affected at vegetative and growth stages of the plant and this has been confirmed through gas exchange analysis, which indicated that reductions in net photosynthetic rates are associated with the limited availability of intercellular carbon dioxide due to reduced rates of transpiration and stomatal conductance. This phenomenon was also observed in salt-treated maize plants, where the rates of respiration were reduced (Iqbal *et al*., 2020).

1.2.4 Morphological and Biochemical Responses of Crop Plants to Salt Stress

The impacts of plant response depend on the concentration and duration of the stress imposed upon them (Gupta and Huang, 2014). Salinity stress affects plant growth, development and productivity by posing threats, which results in osmotic stress, oxidative stress, ion toxicity and nutrient deficiency (Ngara *et al*., 20120. It causes changes in gene expression, which ultimately affects the expression of gene products and proteins. Furthermore, different physiological and metabolic processes of plants are affected (Chen *et al*., 2018). Various morphological responses of plants against salinity are demonstrated by different symptoms such as decrease in leaf area, increase in leaf thickness and abscission of leaves (Gucci and Tattini, 1997).

The response of plants varies with the degree of stress and crop growth stage. Short-term exposure of maize plants to salinity influences plant growth, resulting with osmotic stress in the first phase of salt stress without reaching toxic sodium concentrations. Germination and seedling development are more sensitive to salt stress exposure compared to later developmental stages. Osmotic stress is linked with ion accumulation in the soil solution, whereas nutritional imbalance and sodium/chloride ion builds up and lead to toxic levels that prevent water uptake. This accumulation interferes with the availability of elements in the soil such as calcium and potassium needed for plant growth (El-Bassiouny and Bekheta, 2004; Munns *et al*., 2006; Hussain *et al*., 2013).

High osmotic stress level and toxic effects inhibit successful germination (Farooq *et al*., 2015). Toxic levels of sodium in plant organs damage biological membranes and subcellular organelles, which leads to the reduction of growth and causing abnormal development before plant mortality (Davenport *et al*., 2005; Quintero *et al*., 2007). Salinity stress may also displace calcium from plasma membrane binding sites thus resulting in membrane leakiness.

An increase in salinity stress brings stomatal modulation disturbances, which affect potassium translocation from root to shoot tissues in maize. During salinity stress, mineral uptake inhibited in a plant due to sodium/chloride ions build up in the soil. Stomatal disturbances affect gas exchange in maize, which results in reduced stomatal conductance and photosynthetic pigments that limits carbon fixation capacity. The decreased plant growth, plant height and leaf area is due to the accumulation of toxic ions and less availability of water as was observed in five *Coleus* species subjected to salinity stress (Kotagiri and Kolluru, 2017).

1.2.5 Salt Tolerance Mechanisms in Maize

Plants require various mechanisms in order to tolerate or adapt to diverse environmental changes. Maize undergo various adaptations at subcellular, cellular and organ levels for successful growth under salinity. Those adaptations include osmotic adjustment, activation of antioxidant defence system, toxic ion exclusion, hormonal regulations, maintenance of water tissue contents and also stomatal regulation (Iqbal *et al*., 2020).

These mechanisms are observed at various levels in maize plants. Osmotic adjustment or osmoregulation is the key adaptation of plants at cellular level to minimize the effects of salinity in the first phase, whereby the accumulation of organic osmolytes such as proline, glycine betaine, sugar alcohols, polyamines and proteins lowers water potential of plants (Turkan and Demiral, 2009). Osmotic tolerance involves the plant's ability to tolerate salinity induced stress and to maintain leaf expansion and stomatal conductance (Nandal and Hooda, 2013). The exclusion of excessive salt is also an adaptive trait of plants to acquire salt resistance or its compartmentation into vacuoles. Furthermore, it assists the plant to avoid toxic effects, lowers potential osmotic stress hence contributing to osmoregulation.

Salinity alters the general metabolic processes and enzymatic activities leading to over generation of reactive oxygen species. These results in oxidative stress, increased enzymatic activities and non-enzymatic antioxidants in maize which helps to maintain growth by removing the oxidative damages. Hormone regulation also plays a role in maintaining growth and development under salt stress. For instance, modifications to synthesis of growth substances such as higher abscisic acid levels in salt stressed maize, helps to lower water loss and also lowers sensitivity of leaf growth (Farooq *et al*., 2015).

Plants exposed to salt stress develops various adaptive mechanisms at molecular level to mitigate the effects of salinity stress, whereby accumulation or inhibition of various proteins and the up-regulation /down-regulation of gene transcripts are vital, in this regard up-regulation of antioxidant defense genes and expansion of proteins are vital in maize salt resistance (Zorb et al., 2004). Figure 1.3 distinguishes and summarises the various types of plant tolerance mechanisms to salinity stress as it results in osmotic and ionic stress.

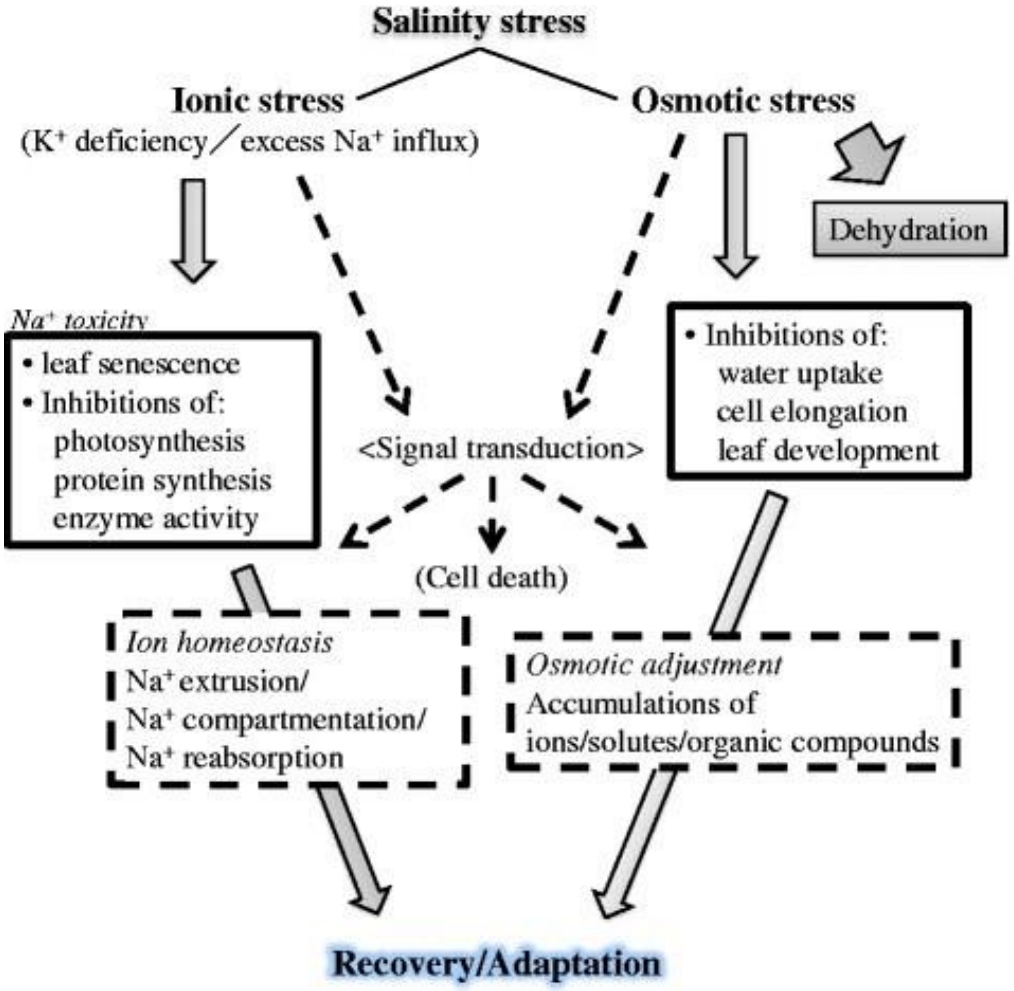

Figure 1.3: A schematic representation of the stresses that plants suffer from under high salinity growth condition as well as the corresponding responses that plants utilise in order to survive these harmful effects (Horie *et al*., 2012).

1.2.6 Significance of Mutational Studies in Plants

Mutation in plants occurs when a DNA gene is damaged or changed to alter the genetic message carried for desirable traits therefore, creating new variants of genes and can occur potentially anywhere (Kharkwal, 2012). Classification of mutations are based on the extent of the DNA sequence affected by the mutational event pertaining to either small-scale or large scale mutations. This DNA changes may also be grouped further into diverse occurrence for instance small-scale can include point mutations, deletions and insertions, whilst large-scale may include inversions and gene duplication (Lee *et al*., 2012).

Mutations can be used as a tool to determine gene functional studies and to create genetic variability, these changes in species are important for adaptation to the natural environment and also for crop improvement to increase food production. Furthermore, this assist by overcoming the challenges of food security and ensuring the provision of sustainable nutrition, through developing new cultivars hence this has a positive socio-economic impact worldwide (Goyal *et al*., 2009; Wani *et al*., 2011; Oladosu *et al*., 2016).

In addition, mutational studies have developed and improved different traits such as resistance to biotic and abiotic stress, increased yield components, quality, nutritional and agronomical traits in diverse plants (Sarsu, 2020).

1.2.7 Pentatricopeptide Repeat (PPR) Protein

The pentatricopeptide repeat (PPR) protein is one of the largest protein families in higher land plants of the modular RNA-binding protein consisting of about 466 genes (Aubourg *et al*., 2000). It has been identified and analysed in multiple organisms, with more than 400 members in land plants. This protein constitutes of tandemly repeated sequenced motif which are found in all eukaryotic lineages. It appeared to have undergone expansion in terrestrial plants, although reports have shown that a small number of PPR encoding genes are also present in prokaryotes (Manna, 2015).

It has further been established that the PPR family is divided into two sub-families: the P and PLS sub-families, with members of the P sub-family abundantly distributed in eukaryotes, while the PLS sub-family are strictly restricted to plants (Lurin *et al*., 2004). The PPR motif consists of three closely related motifs: the canonical PPR motif (P motif) that is common to all eukaryotes, and two variants specific to plants, the PPR-like S motif (for short) and the PPR-

like L motif (for long). These motifs appear to be related to tetratricopeptide (TPR) motifs known to mediate protein interactions (Small and Peeters, 2000).

The PPR and TPR motifs can be easily distinguished since the PPR family are mostly abundant in eukaryotes specifically in the flowering plants such as *A. thaliana* with about 441 genes and in rice with more than 655 genes (Lurin *et al*., 2004). As compared to the PPR motifs, TPR are generally found in both prokaryotes and other eukaryotes such as yeast (*Saccharomyces cerevisiae*) and Drosophila (*Drosophila melanogaster*) (Desloire *et al*., 2003).

Systematic bioinformatic analysis for maize PPR gene family has been performed and a total of about 521 members have been identified in the maize genome. This clearly indicates that this protein plays broad and vital roles in maize organelle gene expression and stress response.

1.2.8 The Role of PPR Protein in Plant Growth and Development

Plants rely on gene expression regulation to achieve growth and development, under the control of both intrinsic and extrinsic factors. Previous studies have shown that PPR proteins are localised in the organelle, with 80% target in the chloroplast or mitochondrion (Lurin *et al*., 2004). PPR domain-containing proteins have shown to play a role in several developmental processes in plants for instance in chloroplast RNA processing, mitochondrial RNA processing, RNA translation and RNA editing these processes ensures the regulation of successful growth and development in plants (Saha *et al*., 2007).

In Arabidopsis for instance, EMB175 is targeted to the plastid and essential for plant embryogenesis and mutation in the EMB175 caused defects in the rate of cell division (Xing *et al*., 2018).

According to Manna, (2015), proteins containing the PPR motifs are known to have roles in transcription, RNA processing, splicing, stability, editing and translation of gene expression. As a result, they are important for the expression of organelle genome and organelle biogenesis. A maize protein CRP1 has been shown to have 13 PPR motifs which localise in the chloroplast stroma and facilitates the processing and translation of *pet*D and *pet*B mRNAs (Fisk *et al*., 1999). In addition, a protein from Arabidopsis and radish: a P67 with two PPR motifs have shown to be involved in RNA processing and translation (Lahmy *et al*., 2000).

Furthermore, a rice protein *OsPPR1* targeting the chloroplast has shown to play a role in early biogenesis of plastids (Gothandam *et al*., 2005). An Arabidopsis CRR4 protein localized in

the chloroplast has been found to be involved in RNA editing of the *ndhD* gene. The PPR motifs can also act as non-catalytic adaptors by mediating interaction between cognate transcripts and their effectors.

On that note, this protein was previously annotated to be an AC by Gehring, (2010), followed by an experimental confirmation three years later as a bonafide adenylate cyclase (Ruzvidzo *et al*., 2013). This AC molecule is important for the synthesis of second messenger in response to a variety of extracellular signals which converts adenosine 5′-triphosphate (ATP) to cyclic adenosine 3′, 5′-monophosphate (cAMP) (Gehring and Turek, 2017). Different hormones and neurotransmitters are able to balance or regulate the cAMP binding to members of the transmembrane family. The levels of the intracellular cAMP are also affected by various mechanisms that utilizes other signalling pathways to modulate the cyclase activity; calcium and protein kinase which function in a discrete second messenger pathways that can independently regulate the activity of cyclase (Levin and Reed, 1995).

Several studies have reported the importance and involvement of cAMP in essential plant cellular processes such as growth, development and response to stress (Choi and Xu, 2010; Thomas *et al*., 2013; Ito *et al*., 2014; Swiezawska *et al*., 2014). Since PPR protein has been found to be involved in important cellular processes as an AC, it was crucial in our study to investigate its growth, development and stress response in an economically important grain crop maize to understand its role and response in relation to the current unstable global climate changes and food shortage.

1.2.9 Mutational Approach and *Zea mays* PPR Mutant Lines

Mutants refers to individuals with a phenotype that varies from the normal population, formed due to a mutational event of a gene or a chromosome. The FAO/IAEA Mutant Variety Database (MVD) collects information on plant mutant varieties (cultivars) has reported on the developed and officially released mutants of about a total of 3,332 accessions from 228 crop species (Sarsu, 2020).

Gene knockout generated by insertional mutagenesis for gene silencing is increasing in maize species, insertion of a transposon or T-DNA into a structural gene (into an exon or an intron) will usually interrupt gene expression completely and give a null mutation, in this manner a particular enzyme is totally absent meaning that the target gene transcript is missing (Thorneycroft *et al*., 2001).

There are various maize PPR mutants that have been reported, for instance the identification of a small kernel 1 (smk1) mutant encoding PPR protein that is targeted to mitochondria, loss of SMK1 function removes the C→U editing at the nad7-836 site leading to retention of a proline codon in the wild type and in *Oryza sativa* (rice) the smk1 mutant sustain the function of C→U editing of the mitochondrial nad7-836, the knock-out mutants showed an abnormal embryo and endosperm development (Li *et al*., 2014). In the study of *Dek41* (defective kernel) gene encoding a P-type protein revealed that *dek41* mutations cause reduced splicing efficiency of mitochondrial nad4 intron 3, displaying defective seed development (Zhu *et al*., 2019).

In general, gene modification through deletion or silencing involves the removal of specific unwanted genes in order to obtain germlines with desirable traits and these are mostly applied in the agricultural sector to improve the adaptability/resistance of different crop species to survive unfavourable conditions. Thus in our study, maize mutant type lacking PPR protein synthesized through gene knockout process was used to compare the growth, development and physiological roles against the wild type (having the PPR protein) under environmental stress condition particularly salinity. This study was therefore, conducted to determine the physiological function of a maize pentatricopeptide repeat protein through cultural studies as there is limited information.

1.3 Problem Statement

Farmers grow maize as an important food grain in all climates ranging from tropical to warm temperate areas. In addition, it is the third most important cereal crop after rice and wheat used for human nutrition and animal feed, which is known to be moderately sensitive to salinity (Iqbal *et al*., 2020). However, with the escalating population growth its production demand is increasing rapidly. Soil salinity is among the major environmental stresses that affects plant growth and development, thus result in a decline in crop productivity (Cicek and Cakirlar, 2002).

Despite the fact that some previous studies on mutational analysis approaches have demonstrated a sole involvement of the PPR protein in important plant developmental processes such as chloroplast RNA processing, mitochondrial RNA processing, RNA translation, RNA editing and stress response. All of these processes ensure the regulation of successful growth and development in plants and are strictly dependent on the enzymatic activity of adenylate cyclases (Bentolila *et al*., 2002; Desloire *et al*., 2003; Saha *et al*., 2007).

However, no study so far has demonstrated the maize PPR proteins' physiological function relating to plant development and abiotic stress response. In this regard, this study sought to determine the physiological function of PPR protein in relation to plant development and response to abiotic stress particularly salinity through cultural approach. Hence through cultural studies, this study can relate the annotated PPR functions to the physiological and growth/developmental features. The information obtained from this study will help to understand the physiological function and growth responses of maize PPR protein in mitigating abiotic stresses and to develop salinity stress tolerant varieties through genetic engineering of the PPR protein and provide management strategies of maize and related crops worldwide.

1.4 Research Aim

The aim of this study was to determine the physiological function of a maize pentatricopeptide repeat protein in maize plants under stress through cultural approach.

1.5 Objectives

1. To cultivate two lines of the maize plants i.e. wild type (wt) and mutant (mt) plants.
2. To expose the cultivated maize plant lines (wild type and mutant) to salt stress.
3. To determine the effect of this salt stress on the two plant lines.
4. To assess the physiological role of the PPR protein in mitigating the salt stress effect.

1.6 Significance of the Study

1. This study will contribute to plant development and physiological function knowledge on PPR protein from maize.
2. Additionally this study will improve the coping strategies of salt stress in maize globally.
3. This will also help to improve the genetic potential of maize cultivars to obtain improved yield and crop production.

CHAPTER TWO

RESEARCH METHODOLOGIES

2.1 Generation and Maintenance of Maize Plants

2.1.1 *Zea mays* Seed Sterilization

The *Zea mays* seeds (wild-type (wt) and mutant-type (mt)) used in this study were commercially acquired from Nottingham University (Nottingham, UK), whereby the mutant variety was generated from the wt variety through the knock-out of a gene responsible for the expression of a pentatricopeptide (ZmPPR) protein. A total of 18 (wt) and 18 (mt) seeds were collected and transferred into separate 50 ml sterile falcon tubes and washed with 2 ml of 70% (v/v) ethanol through vortexing for 1 minute, followed by subsequent removal of ethanol. The seeds were further washed with 2 ml of 1.25% sodium hypochlorite solution (bleach) for 10 minutes. The surface sterilized seeds were further washed with 3 ml of sterile distilled water. After washing, the seeds were transferred into falcon tubes with sterile distilled water at room temperature for 20 minutes, to absorb water and promote quick germination.

2.1.2 Germination of the Seeds

Six seeds were sown in each of the 6 plastic plant pots (16 cm diameter), filled with a 3:2 (v/v) mixture of sterilized organic soil (Culterra potting mix, Builders, South Africa) and vermiculite. The pots were divided into equal numbers, i.e., 3 for the wt variety and 3 for the mt variety and placed in a sterile growth chamber at 23°C. The sown seeds were watered with 200 ml of sterile tap water every 2 days until emergence on day 7.

2.1.3 Maintenance and Treatment of the Seedlings

After germination, the maize seedlings were maintained under greenhouse conditions for periods of 8/16 hours night/day at 10 000 light lux and 23°C for a total of 28 days. During this period, the seedlings were watered with a 200 mM NaCl solution after every 2 days. Immediately after the treatment period, plants were harvested to measure and record the morphological and physiological parameters.

2.2 Determination of the Morphological Parameters

In order to evaluate the morphological responses of the seedlings to salt stress parameters such as plant height, leaf size (area and width), leaf number per plant pot, shoot (weight and length) and root (weight and length) were measured. The projected leaf size (area and width) was calculated using the formula: Leaf area = leaf length x leaf width x K, whereby K = 0.75 as coefficient constant (Musa and Usman, 2016), followed by counting and recording the total leaf number per plant pot. Plant height, leaf length and width, shoot length and root length were all measured in centimetres using a ruler. The shoot and root fresh weights were measured in grams by weighing the respective tissues on an electrical weighing balance (Radwag, model no: PS 750/C/2, Radom, Poland). All of the evaluated morphological parameters were measured in nine biological replicates, whereby three plants from each of the three wt or mt plant lines were analyzed.

2.3 Determination of the Physiological Parameters

2.3.1 Measurement of the Stomatal Number and Density

To determine the leaf stomatal number and density a method described by Volenikova and Ticha (2001) was followed, whereby fully developed healthy maize leaves were randomly selected and carefully detached from the plants. Smooth leaves with few trichomes were selected and the upper and lower surfaces of the leaf were identified. A thin layer of clear nail polish was spread on each surface, and allowed to dry. Then a strip of a clear sticky tape was placed over the nail polish. The tape was pressed over both leaf surfaces to make a good leaf impression. The sticky tape was then peeled off from the leaf surfaces and placed on a microscope slide to be viewed at x40 magnification using a Primo Star light microscope (Carl Zeiss Microscopy, Germany). Images were taken with a digital camera on the microscope (Axiocam 208 color, Zeiss, Germany) and this microscopic analysis then determined the sought stomatal number. On another note, the diameter of the field of view for the used microscope was 0.05 mm and therefore, its area was calculated using the formula: $A = \pi r^2$ where π =3.14 and r = radius of the field of view. Then to determine the stomatal density, the number of stomata noted in the entire field of view (FOV) was divided by the area of FOV.

2.3.2 Measurement of the Photosynthetic, Transpiratory and Respiratory Rates

The plants were evaluated for the photosynthetic, transpiratory and respiratory rates, using the LCpro-SD infra-red gas analyzer (IRGA) (ADC BioScientific, Hertfordshire, UK). From each plant, three leaves were selected and a midrib section of each leaf enclosed in the leaf chamber of the portable LCpro-SD system, which was set to keep all other environmental factors at ambient state (PAR =1600 $\mu molm^{-2}s^{-1}$, CO_2 = 300 ppm and temperature = 23°C). The readings were displayed on the device screen of the machine and recorded during the day at 10-second intervals for 3 minutes (180 seconds).

2.4 Statistical Analysis

Analysis of the morphological and physiological data were obtained from 3 plants of each of the 3 pots for either the wt or mt plant lines. The data were then subjected to analysis of variance (ANOVA) (Super-Anova, Statsgraphics Version 7, 1993, Statsgraphics Corporation, USA). In addition, a post hoc Student Newman Kuehls (SNK), multiple range test ($p \leq 0.05$) was performed to separate the significant differences between treatments (n = 9).

CHAPTER THREE

RESULTS

3.1 Effects of Salt Stress on the Morphological Traits of Maize

The effects of salt stress on growth parameters of maize wild-type (wt) and mutant-type (mt) plant lines for the PPR protein were assessed and ascertained. Various morphological parameters including plant height, shoot length, root length and leaf width (Table 3.1) were measured. Plant height and shoot length of the wt plant lines showed a significant increase compared to the mt plant lines (Figure 3.1A - Figure 3.1B). Furthermore, an increase in root length of the wt lines was observed compared to the mt plants (Figure 3.1C). A moderate increase was also evident on leaf width for the wt compared to mt plants respectively (Figure 3.1D).

Table 3.1: Morphological parameters of wt and mt maize plant lines for the PPR protein subjected to 200 mM NaCl for 16 days.

Treatment concentration	Plant height (cm)	Shoot length (cm)	Root length (cm)	Leaf width (cm)
Wild type 1 (200 mM)	48	11.2	15.6	2
Wild type 2 (200 mM)	47	14.5	19.5	4.4
Wild type 3 (200 mM)	38	13.6	18	1.2
Mutant type 1 (200 mM)	38	9.4	6.7	1.2
Mutant type 2 (200 mM)	48	7.5	8.9	2.2
Mutant type 3 (200 mM)	43	10.4	4.5	1.1

The mean averages of these growth parameters were used to construct bar graphs.

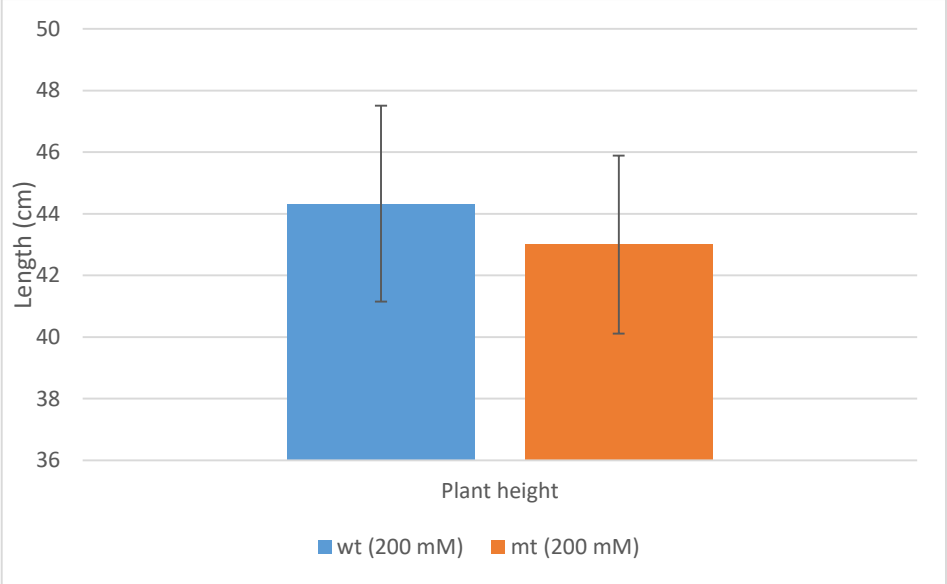

Figure 3.1A: Effects of Salt Stress on Plant Height. Maize plant height in response to 200 mM salt treatment for wt and mt plant lines for the PPR protein was measured after 16 days of stress exposure and ascertained. Error bars indicate the standard errors of the means (SEM) of nine independent seedling treatments.

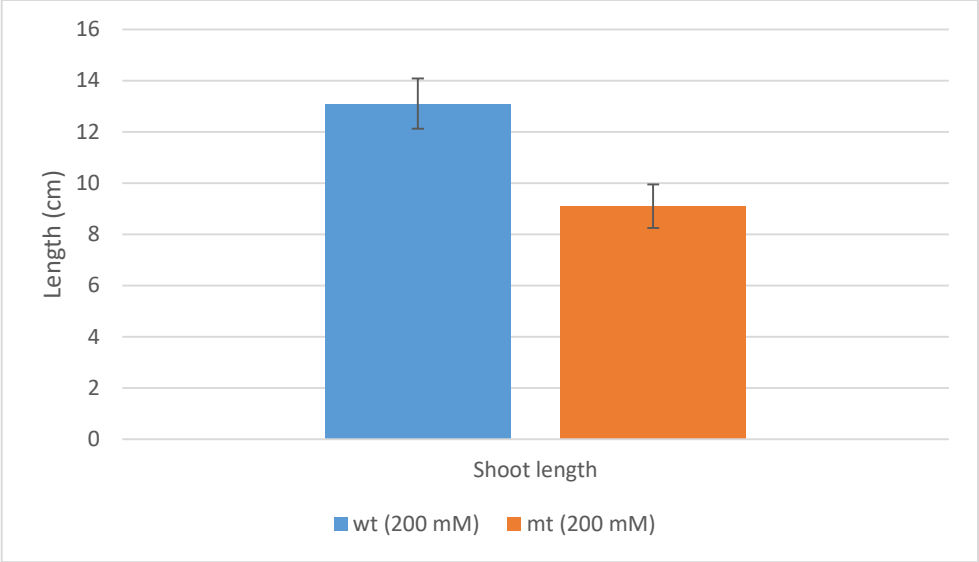

Figure 3.1B: Effects of Salt Stress on Shoot Length. Maize shoot length in response to 200 mM salt treatment for wt and mt plant lines for the PPR protein was measured after 16 days of stress exposure and ascertained. Error bars represent the standard errors of the means (SEM) of nine independent seedling treatments.

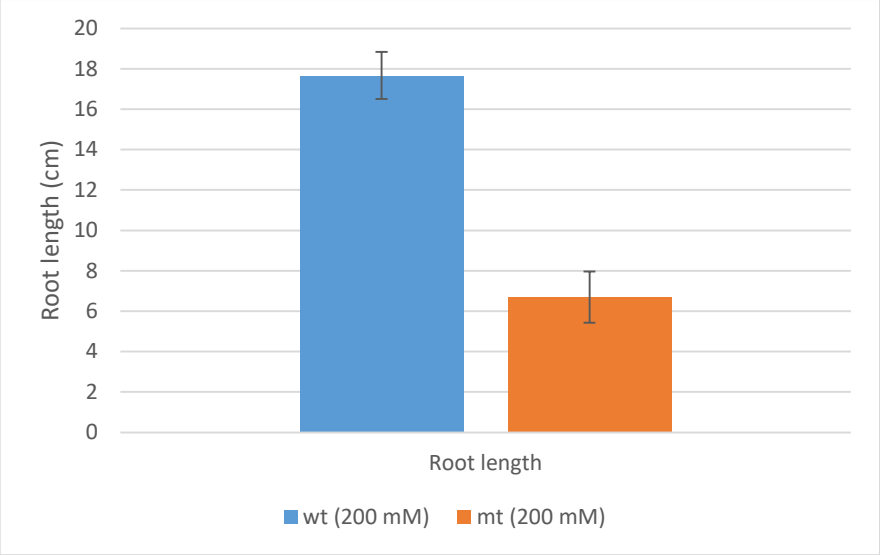

Figure 3.1C: Effects of Salt Stress on Root Length. Maize root length in response to 200 mM salt treatment for wt and mt plant lines for the PPR protein was measured after 16 days of stress exposure and ascertained. Error bars represent the standard errors of the means (SEM) of nine independent seedling treatments.

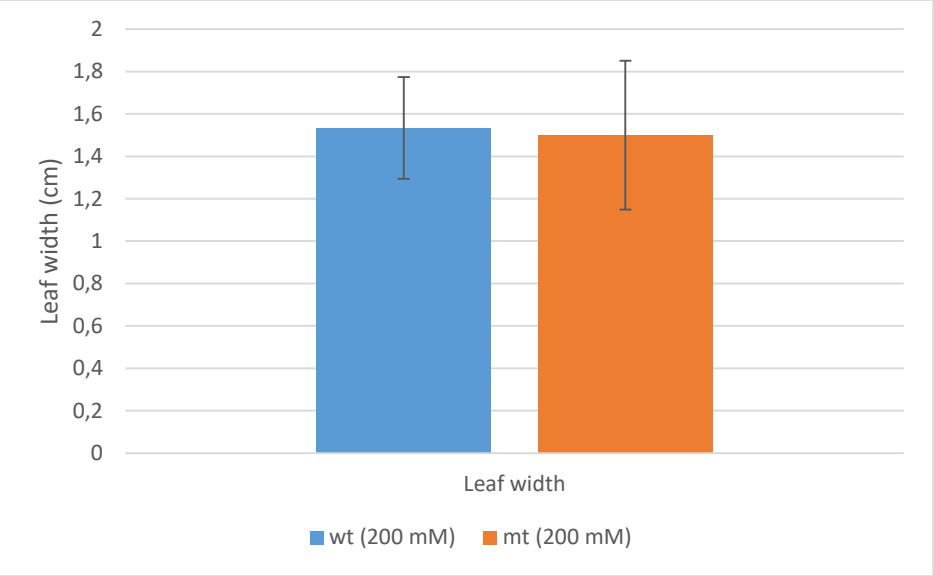

Figure 3.1D: Effects of Salt Stress on Leaf Width. Maize leaf width in response to 200 mM salt treatment for wt and mt plant lines for the PPR protein was measured after 16 days of stress exposure and ascertained. Error bars represent the standard errors of the means (SEM) of nine independent seedling treatments.

3.2 Effects of Salt Stress on Leaf Area

The effects of salt stress on wt and mt maize plant lines for the PPR protein were assessed and ascertained (Table 3.2). A relative increase in leaf area was observed in the mt plant lines, while the wt showed a decrease (Figure 3.2).

Table 3.2: Leaf area response of wt and mt maize plant lines for the PPR protein subjected to 200 mM NaCl for 16 days.

Treatment concentration	Leaf area (cm^2)
Wild type 1 (200 mM)	45
Wild type 2 (200 mM)	33.6
Wild type 3 (200 mM)	26.1
Mutant type 1 (200 mM)	26.55
Mutant type 2 (200 mM)	69.2
Mutant type 3 (200 mM)	24.75

The leaf area mean averages was used to construct a bar graph.

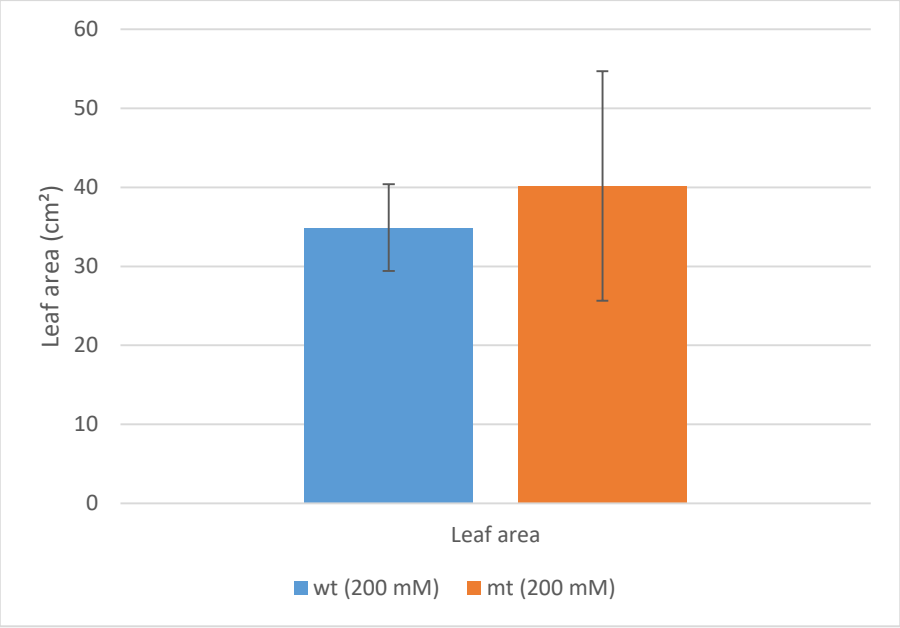

Figure 3.2: Effects of Salt Stress on Leaf Area. *Zea mays* leaf area in response to 200 mM salt treatment was measured in wt and mt plant lines for the PPR protein after 16 days of stress exposure and ascertained. Error bars on the graph represent the standard errors of the means (SEM) of nine independent seedling treatments.

3.3 Effects of Shoot and Root Weights Response to Salt Stress

The effects of salt stress on shoot and root biomass of wt and mt maize plant lines for the PPR protein were assessed and ascertained (Table 3.3). Both the shoot and root weights showed a significant increase in wt plant lines compared to the mt plants (Figure 3.3 – Figure 3.4).

Table 3.3: Root and shoot weight responses of wt and mt maize plant lines for the PPR protein subjected to 200 mM NaCl for 16 days.

Treatment concentration	Shoot weight (g)	Root weight (g)
Wild type 1 (200 mM)	1.64	0.56
Wild type 2 (200 mM)	1.74	0.71
Wild type 3 (200 mM)	1.27	0.59
Mutant type 1 (200 mM)	0.74	0.29
Mutant type 2 (200 mM)	0.47	0.37
Mutant type 3 (200 mM)	0.88	0.18

Mean averages were used to construct bar graphs.

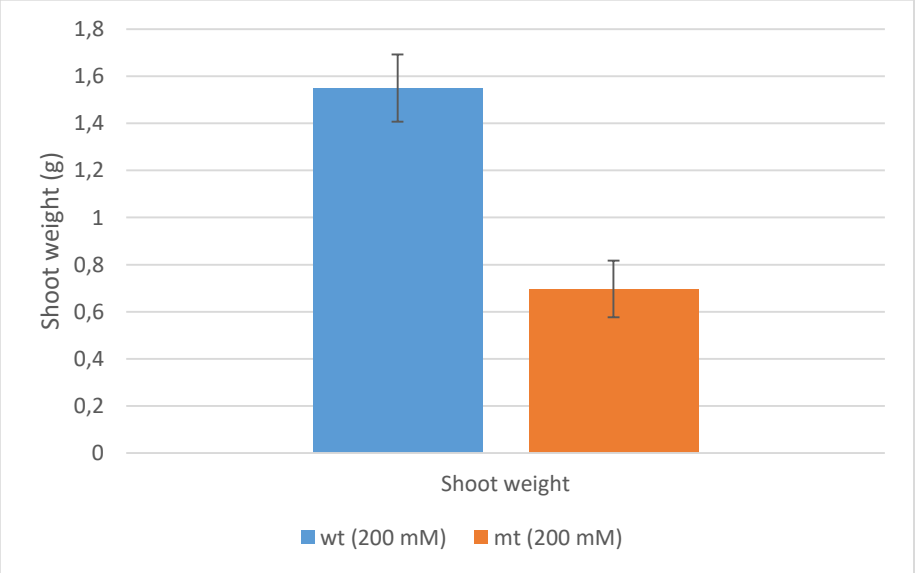

Figure 3.3: Effects of Salt Stress on Shoot Weight. After 16 days of salt stress exposure, shoot weights of wt and mt maize plant lines for the PPR protein were measured and ascertained. Error bars indicate the standard errors of the means (SEM) of nine independent treatments.

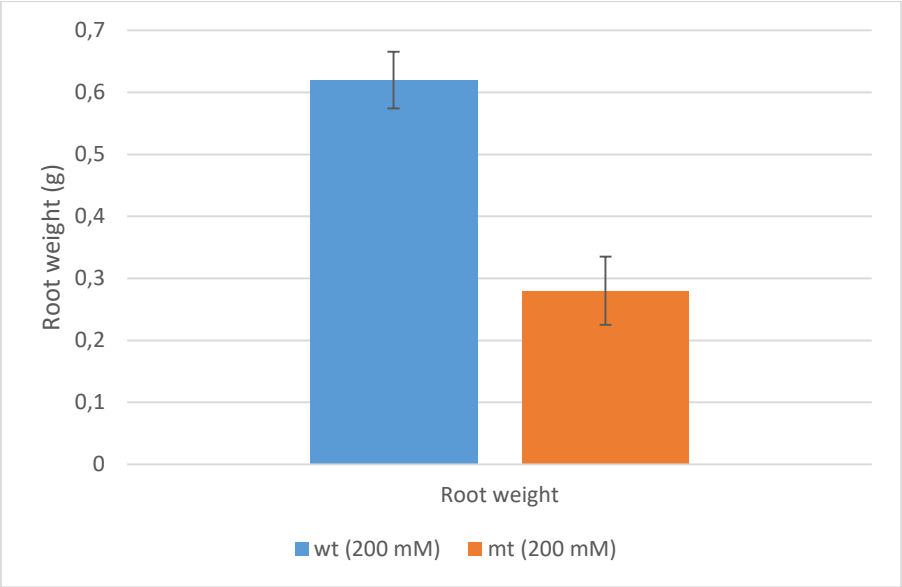

Figure 3.4: Effects of Salt Stress on Root Weight. After 16 days of salt stress exposure, root weights for wt and mt maize plant lines for the PPR protein were measured and ascertained. Error bars indicate the standard errors of the means (SEM) of nine independent treatments.

3.4 Effects of Salt Stress on the Physiological Parameters of *Zea mays*

3.4.1 Evaluation of the Stomatal Number and Stomatal Density

The effects of salt stress on various physiological parameters, including stomatal number, stomatal density, photosynthesis, respiration and transpiration of the maize wt and mt plant lines for the PPR protein were assessed and ascertained. The recorded readings for the stomatal number and density from both leaf surfaces (abaxial and adaxial) of both plant lines are represented in Table 3.4. The stomatal number for the wt upper surfaces showed a significant increase compared to the mt upper surfaces, while the stomatal number for the wt lower surfaces evidently showed a moderate increase compared to the stomatal number of the mt lower surfaces (Figure 3.5 & Figure 3.6). A significant increase on the stomatal density was also observed on the wt upper surfaces compared to the mt upper surfaces, whereas there was a relative decrease on the mt lower surfaces compared to the wt lower surfaces (Figure 3.5 & Figure 3.6).

Table 3.4: The effects of salt stress on maize stomatal number and stomatal density of wt and mt maize plant lines for the PPR protein.

Sample number and NaCl concentration	Magnification (ocular x objective)	Leaf surface (upper/lower)	FOV#	Stomatal number in the entire FOV (mm^2)	Stomatal Density (mm^2)
wt leaf 1 (200 mM)	100x	Upper	1	5	2632
		Lower	1	7	3684
wt leaf 2 (200 mM)	100x	Upper	1	5	2632
		Lower	1	4	2105
wt leaf 3 (200 mM)	100x	Upper	1	5	2632
		Lower	1	6	3158
mt leaf 1 (200 mM)	100x	Upper	1	4	2105
		Lower	1	3	1578
mt leaf 2 (200 mM)	100x	Upper	1	4	2105
		Lower	1	6	3158
mt leaf 3 (200 mM)	100x	Upper	1	5	2632
		Lower	1	7	3684

Mean averages were used to construct bar graphs.

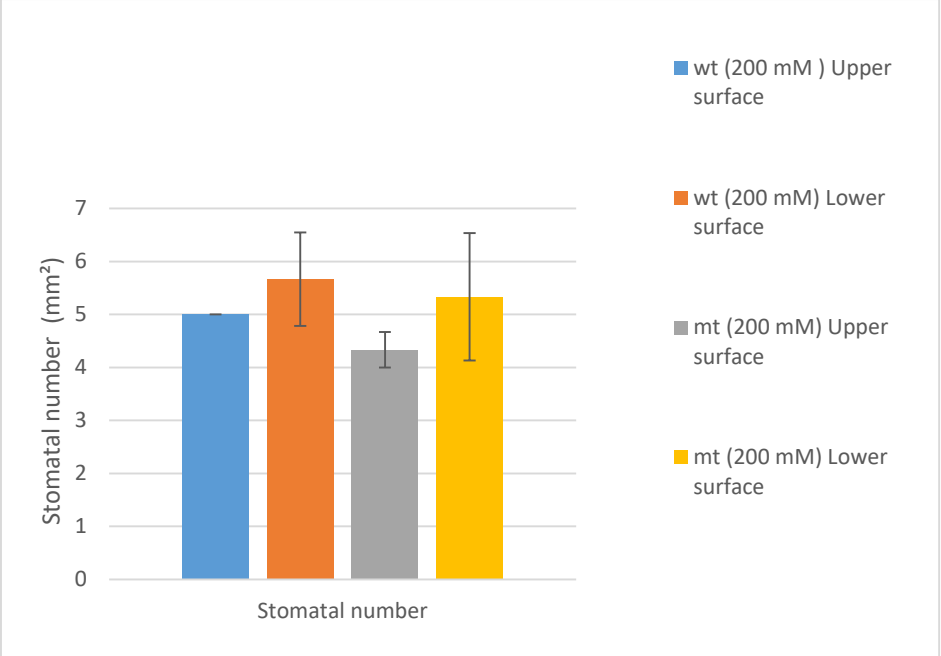

Figure 3.5: Effects of Salt Stress on Stomatal Number of the Upper and Lower Surfaces of wt and mt Maize Plant Lines for the PPR Protein. After 16 days of salt treatment, stomatal numbers were counted and determined on both surfaces of the selected leaves of the two plant lines (wt and mt). Error bars indicate the standard errors of the means (SEM) of nine independent treatments.

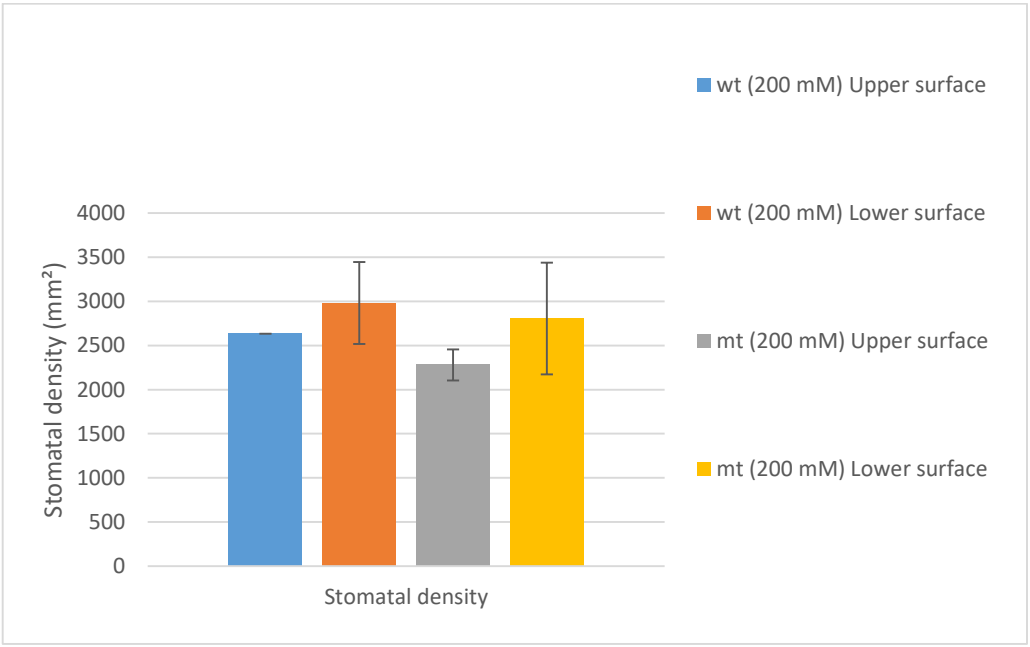

Figure 3.6: Effects of Salt Stress on Stomatal Density of the Upper and Lower Surfaces of the wt and mt Maize Plant Lines for the PPR Protein. After 16 days of salt treatment, stomatal densities were calculated and determined on both surfaces of the selected leaves of the wt type and mt type plant lines. Error bars indicate the standard errors of the means (SEM) of nine independent treatments.

3.4.2 Effects of Salt Stress on the Photosynthesis Rate

The effects of salt stress on the rate of photosynthesis in wt and mt maize plant lines for the PPR protein were assessed and recorded as is shown in Appendix A (Table 3.5). After 16 days of salt treatment, photosynthesis rates were measured; both the wt and mt plant lines showed low but stable rates from 0 to 60 seconds. For the next 120 seconds, the wt lines showed a fluctuating increase in their rates while the mt lines continuously showed decreased constant rates from 110 to 180 seconds. Additionally, notable decreased rates in the wt plants were observed from the 130th to 180th seconds. Overall, notable increased photosynthetic rates were observed for the wt compared to the mt plant lines (Figure 3.7).

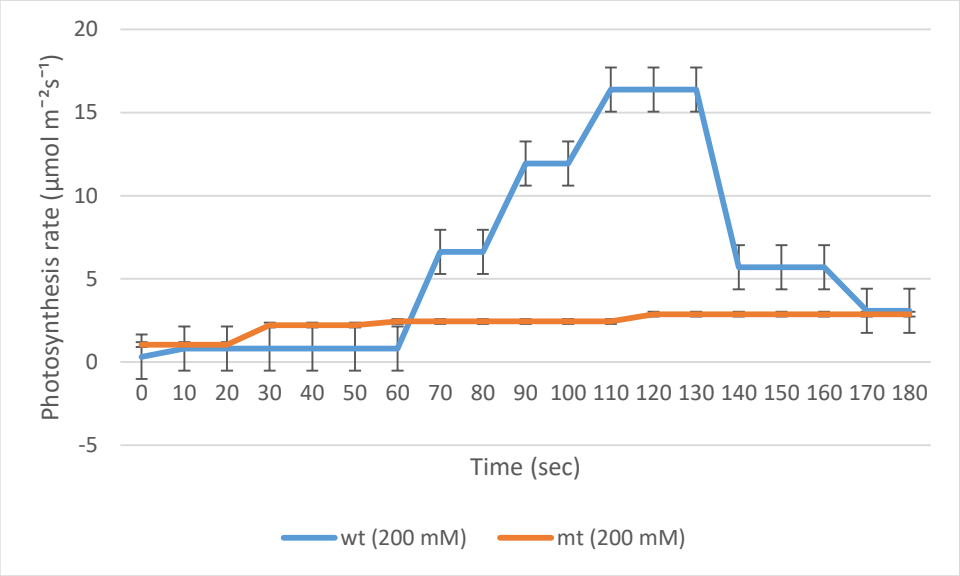

Figure 3.7: Effects of Salt Stress on Rate of Photosynthesis of the wt and mt Maize Plant Lines for the PPR Protein over Time. Total photosynthetic rates for the wt and mt plant lines were measured and recorded after 16 days of salt exposure. All error bars indicate the standard errors of the means (SEM) of nine independent biological replicates for various response values.

3.4.3 Effects of Salt Stress on the Respiration Rate

The effects of salt stress on the rates of respiration in wt and mt maize plant lines for the PPR protein were assessed and recorded as is shown in Appendix B (Table 3.6). After 16 days of salt treatment, the respiration rates were measured. From the results obtained, the rates of the mt lines decreased slightly while those of the wt showed stable high rates from 0 to 60 seconds. The mt lines later sharp inclines from the 70th second followed by temporary constants between the 80th and 90th seconds, then declines at the 100th second. For the next 120 seconds up to the 180th second, both the wt and mt plant lines showed some fluctuating increases, whereby the mt plants showed slight increases while the wt plants showed moderate decreases (Figure 3.8).

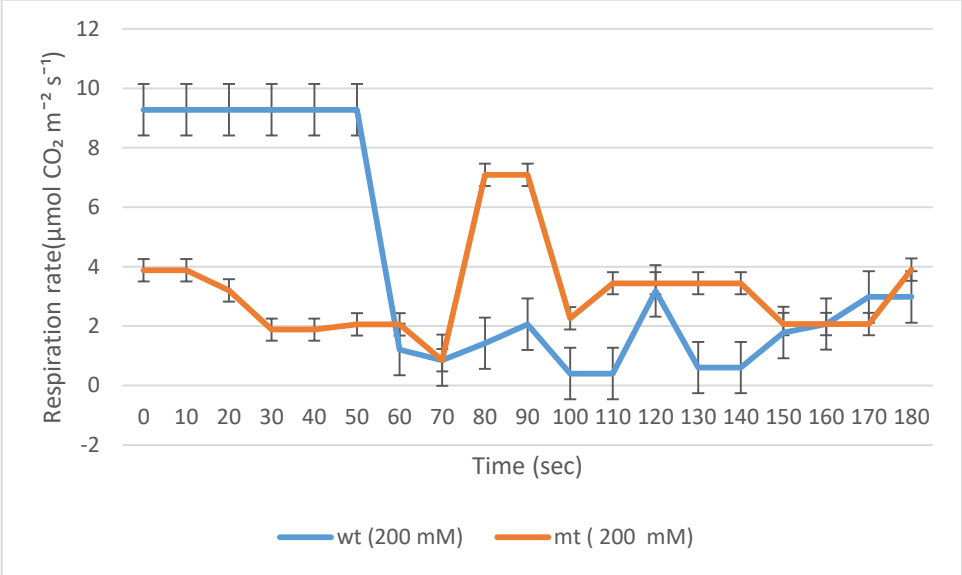

Figure 3.8: Effects of Salt Stress on the Rate of Respiration of the wt and mt Maize Plant Lines for the PPR Protein over Time. Net respiration rates of the wt and mt plant lines were measured and recorded after 16 days of salt exposure. Error bars represent the standard errors of the means (SEM) of nine biological replicates.

3.4.4 Effects of Salt Stress on the Transpiration Rate

The effects of salt stress on the rate of transpiration in wt and mt maize plant lines were assessed and recorded as is shown in Appendix C (Table 3.7). After 16 days of salt treatment, transpiration rates were assessed, whereby some moderate increases were observed for both the wt and mt plant lines during the first 60 seconds and then decreased constantly from the 70^{th} – 80^{th} seconds. Stable increases for the wt lines were observed from the 90^{th} - 140^{th} seconds with steady inclines up until 180 seconds. Furthermore, mt lines showed some constant increases from the 90^{th} – 160^{th} seconds and mild constant decreases at the 180^{th} seconds (Figure 3.9).

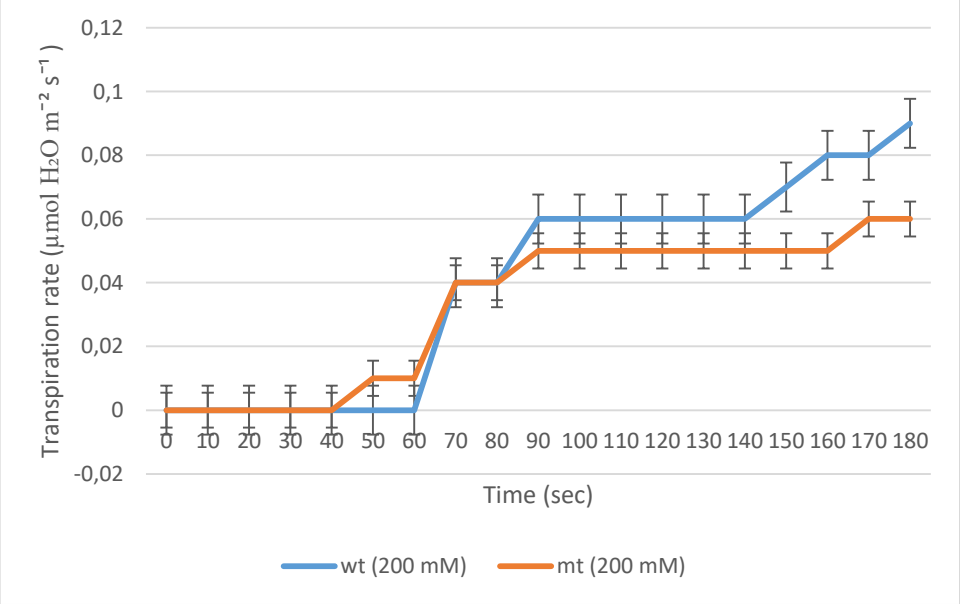

Figure 3.9: Effects of Salt Stress on the Rate of Transpiration of wt and mt Maize Plant Lines for the PPR Protein over Time. Net transpiration rates of the plant lines were measured and recorded after 16 days of salt exposure. Error bars represent the standard errors of the means (SEM) of nine biological replicates.

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1 Discussion

Agricultural production is continually impacted and negatively affected by soil salinity as the number of contributing factors such as global warming, inefficient usage of agricultural lands, lack of rainfall, excessive evaporation and lack of proper drainage systems intensifies. Salinity is a major constraint that affects plant growth and development, which then leads to diverse negative reactions at varying stress levels. Plants are classified as either halophytes or glycophytes due to their ability to grow or not survive in a high salt environment. Maize being a salt-sensitive crop, its production is at risk of diminishing under salt stress. The degree of salt stress and stage of crop growth results in various responses, affecting plant growth and development. Saline conditions reduce the ability of plants to uptake water and nutrients, causing osmotic stress (the lowering of the external water potential), oxidative damage to plant cells by increasing production of reactive oxygen species (ROS) and ion effect in the cytosol. Some of the physiological processes such as stomatal functioning, photosynthesis, respiration and transpiration are adversely affected, leading to massive reduction in plant growth and crop productivity (Cicek and Carkirlar, 2002; Farooq *et al*., 2015).

When plants are exposed to salt stress, they activate signal-transduction pathways that allow them to respond and adapt to the changes that are imposed on them. Sensing salt stress by recognising Ca^{2+}, salinity-induced changes in cellular structures results in reduced turgor pressure. In general, the discovery of the salt overly sensitive (SOS) signalling pathway in plants established the exclusion of Na^{+} from the cytosol to the outside of the plant cell; thus salt exclusion prevents salts from entering into the vascular system and this is common in the roots to prevent ion movement to the aerial parts of the plant. Furthermore, a plant can also eliminate salt from the salt-secreting glands and trichomes by preventing salt accumulation in different organs such as leaves, to keep the concentration at a lower level. On the other hand, glycophytes growth is inhibited and results in plant death whereas halophytes can survive under severe saline conditions by developing resistance mechanisms such as osmotic adjustment to regulate ion solutes (Cheong *et al*., 2007; Jiang *et al*., 2015 Motos *et al*., 2017; Zhao *et al*., 2020).

Understanding, the response mechanisms utilized by plants when exposed to varying environmental stresses has been an area of interest in the plant science community due to its impact in threatening food security worldwide. Thus, the present study investigated the physiological function of a maize pentatricopeptide repeat (PPR) protein under salt stress. This protein was targeted due to its confirmed function as an adenylate cyclase (Ruzvidzo *et al*., 2013), which is known to be involved in important cell signalling and plant development processes (Saha *et al*., 2007) that may facilitate responses to varying environmental stresses (Choi and Xu, 2010; Ito *et al*., 2014; Swiezawska *et al*., 2014; Thomas *et al*., 2013;). In order to assess and evaluate this novel protein candidate, the physiological and morphological responses of maize mutant (mt) and wild type (wt) plant lines for this key protein were studied by allowing their plant lines to grow under salt stress conditions in a greenhouse system for 16 days.

Significant changes in growth and physiological parameters were observed after 16 days of salt stress exposure. A number of studies have reported that salinity stress causes a significant reduction of growth in various crop plants due to specific-ion toxicities and nutritional imbalances (Munns *et al*., 1995; Paul, 2012; Giuffrida *et al*., 2013). In our case, our results indicated that plant morphological parameters such as plant height, shoot and root length, leaf width, shoot and root weight of the wt were always higher when compared to the mt plant lines, which significantly decreased under salinity stress (Figure 3.1A-D and Table 3.1). The effects of salinity on plant height have previously been reported in many plant species for example rice (*Oryza sativa* L), tomato (*Solanum lycopersicum*) and pepper (Capsicum sp.) grown under salt stress (Chatzoulakis *et al*., 2002; Hussain *et al*., 2017; Shahid *et al*., 2020). Furthermore, salinity hindered the elongation of shoot in our mt plants unlike the wt (Figure 3.1B) and this could be as a result of osmotic adjustment imbalance. This trend was also observed in *Vicia faba* (L.) (Qados, 2011).

The root and shoot growth inhibition are the primary response of various plants to salt stress, where in most cases, the root is the first organ to be exposed to salt stress while shoots are more sensitive to salt stress (Munns and Sharp, 1993) and on that note, water is transported from roots to shoots. It is common that salinity with adequate calcium supply supresses shoot growth (Lauchi and Epstein, 1990). A study from a wild type Ailsa Craig tomato genotypes reported that a high salt concentration demonstrated an increase in shoot and root lengths (Tuna *et al*., 2007). Observations made in this study, Figure 3.1B and Figure 3.1C, indicated a significant decrease in shoot and root lengths for the mt plants and similarly, this same aspect was also

observed in studies conducted in radish plant (Jamil *et al*., 2007b) and cow pea (Taffouo *et al*., 2009), whereby high concentration of NaCl led to a decline in lengths of the plants while increased shoot and root lengths were noted for the wt plants. Our results align well with reported studies in tomato and pistachio (*Pistacia vera* L.) cultivar, which showed a significant increase in root to shoot length (Rahneshan *et al*., 2018). Such an observation may be due to the mt plants experiencing an increased osmotic pressure around the roots especially under saline conditions, which then results in stunted root and shoot growths (Sultana *et al*., 1999), while on the other hand, wt plants managed to survive well under salt stress. Furthermore, this may suggest that the PPR protein could be significant for regulating plant shoot and root lengths and other essential growth processes under stress.

In addition to the outlined analysed morphological traits, leaf parameters including leaf width and area were also studied. Plant leaves are the most important organs that have an outer layer termed the epidermis, consisting of an upper (adaxial) and lower (abaxial) epidermis, which are present on either sides of the leaf. The epidermis is essential for the regulation of gaseous exchange, it contains stomata which opens and closes to allow gas exchange. Leaves utilize light energy for photosynthesis to make food and this enables the plant to stay healthy and regulate the photosynthetic capacity needed for growth and development of the whole plant (Kalve *et al*., 2014). Our study demonstrated an increase in leaf width for the wt plants whilst the mt plants showed a slight reduction (Figure 3.1D). These observations were similar to those demonstrated in wheat leaves, where width reduced correspondingly in plants treated with NaCl (Hu *et al*., 2000). Interestingly, the leaf area in wt plants decreased while it significantly increased by 5.26 cm^2 in mt (Figure 3.2 and Table 3.2). The obtained results were similar to those of the previously reported studies in moth bean plant (*Vigna aconitifolia* L.), where salt stress resulted in the reduction of leaf area, which is inversely proportional to salt stress concentration (Bohra and Vyas, 2006). Leaf area demonstrated a marginal decrease in *Solanum tuberosum* and broad bean (green vegetable) exposed to salt stress (Evers, 1998) and also in two maize cultivars subjected to salinity (Cicek and Carkirlar, 2002). This effect on leaf area could be due to the disturbed photosynthesis process, reduction of leaf seedling fresh weight and suppression of leaf expansion (Hussain *et al*., 2013; Negrao *et al*., 2017).

High salinity inhibits root and shoot elongation, and this occurs due to the slowed uptake of water and lowered essential mineral nutrition from the soil by the plant (Neumann, 1997). Shoot and root weights (Figure 3.3, Figure 3.4 and Table 3.3) both showed a decrease in the mt plants and this has been observed in various plants such as rice and wheat compared to the

significant increases observed in wt plants. This phenomenon was also observed from previous studies in salt-tolerant maize (Zahra et al., 2020), and *Brassica campestris* L. (Bohra and Vyas, 2006) varieties. A decreased shoot and root weight may be due to the plant losing a lot of water and making it difficult for salt ions to dissolve hence decreased weight. In addition, another reason may be due to loss of turgor pressure, dehydration of the protoplasm, and reduced cell expansion, contributing to the diminished biomass of shoot and roots (Farooq *et al*., 2015). On that note, our obtained results directly associated the PPR protein to shoot and root development, since it has been shown to be involved in the development of plant organelles, which are crucial for the normal growth and development of plants (Lee *et al*., 2019).

Stomata are the smallest openings or pores formed by specialized cells termed guard cells in plant tissues commonly found on the surfaces of leaves under the epidermis, which are important for gaseous exchange; allowance of the entry of carbon dioxide into the leaf for photosynthesis and water exchange (Willmer and Fricker, 1996). Therefore, in order to assess the structure and functioning of stomata from leaves of the wt and mt maize plant lines, stomata were counted from randomly selected leaves of the two lines. Figure 3.5 shows significant differences between the wt and mt plant lines grown under salt stress conditions. Our study revealed that under salt stress, stomatal count on the upper surfaces of the wt leaves increased in number while their number on the upper surfaces of the mt leaves decreased (Figure 3.5, Table 3.4). The stomatal numbers on the lower surfaces of the wt leaves also showed an increased count whereas the lower surfaces of the mt leaves showed a decrease (Figure 3.5, Table 3.4). Similarly, salinity had a significant effect on the stomatal density (Table 3.4). The results obtained indicated that the upper and lower surfaces of the wt leaves had an increase in stomatal density as compared to the upper and lower surfaces of the mt leaves, which showed a significant reduction in stomatal density (Figure 3.6). It was previously reported that stomatal conductance is highly sensitive to increased salt stress, hence a decreased conductance illustrates a reduction of water loss in plants as a response mechanism to compensate for the salt accumulation and reduced osmotic pressure (Iyengar *et al*., 1996).

Our results thus concurred with a previous research conducted in *Kandelia candel* (L.) Druce seedlings leaf features (stomatal number and density), which were exposed to salinity (Qiu *et al.*, 2007). The increased stomatal count and density observed in the wt leaves could be a way in which plants preserve water under stress condition such as salinity by generating multiple stomata. Therefore, since PPR protein is known to be involved in crucial plant developmental processes such as leaf growth and expansion, which control photosynthesis and carbon dioxide

fixation (Jarvis and López-Juez, 2013), it might be possible that this protein could be responsible for the regulation of stomatal activities under stress and other related environmental conditions.

On the physiological basis, several other parameters including photosynthesis, respiration and transpiration were assessed between the two studied plant lines under salt stress. Photosynthesis, respiration and transpiration play a vital role in plants as they attribute to the biochemical pathways and overall functioning in plants. Rates of these three key processes were assessed from randomly selected leaves of the two plant lines and recorded at 10 seconds intervals. It was observed that the rates of photosynthesis and transpiration were higher for the wt plants as compared to the mt lines (Figures 3.7 & 3.9, Appendices A & C, and Tables 3.5 & 3.7) thus associating the PPR protein to these two key physiological processes. This protein might possibly be involved in the facilitation of acclimation mechanisms to resist osmotic shock in stressed plants. Photosynthesis is a key house for the production of ATP, which provides energy for CO_2 fixation. As stomata is associated with photosynthesis, then its lower count in any plant would definitely lead to a significant decrease in photosynthesis rate. The data represented in Figure 3.7 shows a higher photosynthesis rate for the wt plants compared to the mt plants with a lower photosynthesis rate and this relating so well with the obtained stomatal counts for the two studied plant lines (Figure 3.5).

A study from *Capsicum sp*. (pepper) has reported a lower photosynthesis rate when the entire root is subjected to salinity (Yeo *et al*., 1985). Similarly, a study in rocket (*Eruca sativa)* has also demonstrated a significant decrease in photosynthesis and this could be due to stomatal closure, absence of stomata or both factors (Saibo *et al*., 2009), whilst transpiration rate was reduced by stomatal conductance and accumulation of intracellular sodium ions under salt stress (Hnilickova *et al*., 2017). So salt effect has a whole lot of effects on plants, including these few stated ones.

The data represented in Figure 3.8, Appendices B and Table 3.6 furthermore shows the respiration rates measured from randomly selected leaves in the wt and mt plants. Respiration is an important biological process in which energy flows in the exchange of oxygen and carbon dioxide between living organisms and the environment. It supplies ATP (adenosine triphosphate) as an energy source needed for plants growth (Pholo, 2009), also facilitates acclimation for plant adaptation to adverse conditions (Rakhmankulova *et al*., 2001). Various reports have indicated that the respiration rates tend to increase under salinity from both tolerant and salt sensitive species (Nieman, 1962; Zidan and Eleva, 1995). Furthermore, the

same trend was also noted in *Bruguiera cylindrica* leaf pieces exposed to salt stress (Atreya and Bhargava, 2008). Similarly, our study demonstrated increased photosynthesis and respiration rates in wt plants, meanwhile there were lower photosynthesis and respiration rates in mt plants (Figures 3.7 and 3.8). In our case respiration rate decreased in the absence of our gene this might be as a result of the deterioration of the chloroplast structure affecting the stomatal number and closure.

The results of lower respiration rates are similar to the study on the salt tolerant wheat cultivar Kharchid that demonstrated a lower respiration rate and this may be due to the respiratory energy that is used in ion influx to maintain cell turgor, secretion and or repair of cellular damage caused by the toxicity of salt (Schwarz and Gale, 1981; McGree, 1986; Munns *et al*., 2006; Netondo *et al*., 2007). Reduction in photosynthesis and respiration rates are mainly due to the reduction of water potential as well as high concentration of sodium and/or chloride accumulation in chloroplasts (Iqbal et al., 2020).

Transpiration is an important biological process that regulates plant water levels and leaf energy stability (Pearcy *et al*., 2000). Reduction in transpiration rates may be due to stomatal closure as a way of preserving water status of the leaves. Our results, in the first 90 seconds of analysis corresponded to those of Negrao *et al.,* (2017), which under salt stress, plants are able to tolerate salt by maintaining normal transpiration rates. This might be a way to assisting in assessing salinity tolerance in plants. In the wt plants growth and development was maintained in the presence of the PPR protein hence an increased transpiration rate and the mt plants showed a significant decrease because growth and development of the plants was slowed.

To the best of our knowledge, despite the key importance of PPR protein in plant development and its possible involvement in stress response (Bentolila *et al*., 2002; Desloire *et al*., 2003), its growth responses and physiological function to salinity stress has not been documented, hence the significance and originality of our undertaken study.

4.2 Conclusion

The present study evaluated the morphological and physiological performances of wt and mt maize plant lines for the PPR protein in response to salt stress. Salt stress highly affected the mt plant lines at both the morphological and physiological basis when compared to the wt plants, thus the physiological function of the PPR protein as a mitigating factor in maize under excessive salinity stress conditions was determined through direct inference of this protein to

these processes. On that note, our study has succeeded to associate the PPR protein to key morphological parameters such as (plant height, shoot length, root length, leaf width, leaf area, shoot weight and root weight) and essential physiological processes including stomatal conductance, photosynthesis, respiration and transpiration. Collectively, these mentioned growth parameters and processes are important for the overall growth and functioning of maize as an essential agricultural crop. The results of this study provided a vital background on the PPR protein as a mitigating component on maize crops growing under salt conditions, and can therefore, serve as a viable recommendation for maize or related crop breeding screening programs on salinity tolerance.

REFERENCES

Abah, J., Ishaq, M.N. and Wada, A.C., 2010. The role of biotechnology in ensuring food security and sustainable agriculture. *African Journal of Biotechnology*, *9*(52), pp.8896-8900.

Ashraf, M.H.P.J.C. and Harris, P.J., 2013. Photosynthesis under stressful environments: an overview. *Photosynthetica*, *51*(2), pp.163-190.

Aubourg, S., Boudet, N., Kreis, M. and Lecharny, A., 2000. In *Arabidopsis thaliana*, 1% of the genome codes for a novel protein family unique to plants. *Plant Molecular Biology*, *42*(4), pp.603-613.

Bentolila, S., Alfonso, A.A. and Hanson, M.R., 2002. A pentatricopeptide repeat-containing gene restores fertility to cytoplasmic male-sterile plants. *Proceedings of the National Academy of Sciences*, *99*(16), pp.10887-10892.

Blanco, E., Fortunato, S., Viggiano, L. and de Pinto, M.C., 2020. Cyclic AMP: A polyhedral signalling molecule in plants. *International Journal of Molecular Sciences*, *21*(14), pp.4862.

Bockheim, J.G. and Gennadiyev, A.N., 2000. The role of soil-forming processes in the definition of taxa in soil taxonomy and the world soil reference base. *Geoderma*, *95*(1-2), pp.53-72.

Bray, E.A., 2002. Abscisic acid regulation of gene expression during water-deficit stress in the era of the Arabidopsis genome. *Plant, cell & Environment*, *25*(2), pp.153-161.

Brukhin, V. and Morozova, N., 2011. Plant growth and development-basic knowledge and current views. *Mathematical Modelling of Natural Phenomena*, *6*(2), pp.1-53.

Chen, G., Zou, Y., Hu, J. and Ding, Y., 2018. Genome-wide analysis of the rice PPR gene family and their expression profiles under different stress treatments. *BMC Genomics*, *19*(1), pp.1-14.

Cheong, M.S. and Yun, D.J., 2007. Salt-stress signaling. *Journal of Plant Biology*, *50*(2), pp.148-155.

Choi, Y.E. and Xu, J.R., 2010. The cAMP signaling pathway in Fusarium verticillioides is important for conidiation, plant infection, and stress responses but not fumonisin production. *Molecular Plant-Microbe Interactions*, *23*(4), pp.522-533.

Çiçek, N. and Çakirlar, H., 2002. The effect of salinity on some physiological parameters in two maize cultivars. *Bulgarian Journal of Plant Physiology*, *28*(1-2), pp.66-74.

Davenport, R., James, R.A., Zakrisson-Plogander, A., Tester, M. and Munns, R., 2005. Control of sodium transport in durum wheat. *Plant Physiology*, *137*(3), pp.807-818.

Desloire, S., Gherbi, H., Laloui, W., Marhadour, S., Clouet, V., Cattolico, L., Falentin, C., Giancola, S., Renard, M., Budar, F. and Small, I., 2003. Identification of the fertility restoration locus, Rfo, in radish, as a member of the pentatricopeptide-repeat protein family. *EMBO Reports*, *4*(6), pp.588-594.

Du Plessis, J., 2003. Maize Production, Department of Agriculture, Pretoria, South Africa.

El-Bassiouny, H.M. and Bekheta, M.A., 2004. Role of putrescine on growth, regulation of stomatal aperture, ionic contents and yield by two wheat cultivars under salinity stress. *Egyptian Journal of Physiological Science (Egypt)*, *13*(4), pp.122-153.

Evers D, Hemmer K, Hausman JF., 1998. Salt stress induced biometric and physiological changes in *Solanum tuberosum* L. cv. Bintje grown *in vitro*. *Acta Physiologiae Plantarum*, 20(1), pp.3-7.

Farooq, M., Hussain, M., Wakeel, A. and Siddique, K.H., 2015. Salt stress in maize: effects, resistance mechanisms, and management. A review. *Agronomy for Sustainable Development*, *35*(2), pp.461-481.

Fisk, D.G., Walker, M.B. and Barkan, A., 1999. Molecular cloning of the maize gene crp1 reveals similarity between regulators of mitochondrial and chloroplast gene expression. *The EMBO Journal*, *18*(9), pp.2621-2630.

Gehring, C., 2010. Adenyl cyclases and cAMP in plant signalling-past and present. *Cell Communication and Signalling*, *8*(1), pp.1-5.

Gehring, C. and Turek, I.S., 2017. Cyclic nucleotide monophosphates and their cyclases in plant signaling. *Frontiers in Plant Science*, *8*, pp.1704.

Giuffrida, F., Cassaniti, C. and Leonardi, C., 2013. The influence of rootstock on growth and ion concentrations in pepper (*Capsicum annuum* L.) under saline conditions. *The Journal of Horticultural Science and Biotechnology*, *88*(1), pp.110-116.

Gong, X., Chao, L., Zhou, M., Hong, M., Luo, L., Wang, L., Ying, W., Jingwei, C., Songjie, G. and Fashui, H., 2011. Oxidative damages of maize seedlings caused by exposure to a combination of potassium deficiency and salt stress. *Plant and Soil*, *340*(1), pp.443-452.

Gothandam, K.M., Kim, E.S., Cho, H. and Chung, Y.Y., 2005. OsPPR1, a pentatricopeptide repeat protein of rice is essential for the chloroplast biogenesis. *Plant Molecular Biology*, *58*(3), pp.421-433.

Gucci, R. and Tattini, M., 1997. Salinity tolerance in olive. *Horticultural Reviews*, *21*, pp.177-214.

Gupta, B. and Huang, B., 2014. Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. *International Journal of Genomics*, *20*, pp.14-21.

Hniličková, H., Hnilička, F., Martinkova, J. and Kraus, K., 2017. Effects of salt stress on water status, photosynthesis and chlorophyll fluorescence of rocket. *Plant, Soil and Environment*, *63*(8), pp.362-367.

Holding, D.R. and Streich, A.M., 2013. Plant growth processes: Transpiration, photosynthesis, and respiration. *University of Nebraska Cooperative Extension*.

Horie, T., Karahara, I. and Katsuhara, M., 2012. Salinity tolerance mechanisms in glycophytes: An overview with the central focus on rice plants. *Rice*, *5*(1), pp.1-18.

(https://www.pioneer.com/us/agronomy/staging_corn_growth).

Hu, Y., Schnyder, H. and Schmidhalter, U., 2000. Carbohydrate deposition and partitioning in elongating leaves of wheat under saline soil conditions. *Functional Plant Biology*, *27*(4), pp.363-370.

Hussain, M., Park, H.W., Farooq, M., Jabran, K. and Lee, D.J., 2013. Morphological and physiological basis of salt resistance in different rice genotypes. *International Journal of Agriculture & Biology*, *15*(1), pp. 111-126.

Hussain, M., Ahmad, S., Hussain, S., Lal, R., Ul-Allah, S. and Nawaz, A., 2018. Rice in saline soils: physiology, biochemistry, genetics, and management. *Advances in Agronomy*, *148*, pp.231-287.

Iqbal, S., Hussain, S., Qayyaum, M.A. and Ashraf, M., 2020. The response of maize physiology under salinity stress and its coping strategies. In *Plant Stress Physiology*. IntechOpen.

Ito, M., Takahashi, H., Sawasaki, T., Ohnishi, K., Hikichi, Y. and Kiba, A., 2014. Novel type of adenylyl cyclase participates in tabtoxinine-β-lactam-induced cell death and occurrence of wildfire disease in *Nicotiana benthamiana*. *Plant Signaling & Behavior*, *9*(1), pp.e27420.

Jamil, M., Lee, K.J., Kim, J.M., Kim, H.S. and Rha, E.S., 2007. Salinity reduced growth PS2 photochemistry and chlorophyll content in radish. *Scientia Agricola*, *64*, pp.111-118.

Jarvis, P. and López-Juez, E., 2013. Biogenesis and homeostasis of chloroplasts and other plastids. *Nature Reviews Molecular Cell Biology*, *14*(12), pp.787-802.

Jiang, S.C., Mei, C., Liang, S., Yu, Y.T., Lu, K., Wu, Z., Wang, X.F. and Zhang, D.P., 2015. Crucial roles of the pentatricopeptide repeat protein SOAR1 in Arabidopsis response to drought, salt and cold stresses. *Plant Molecular Biology*, *88*(4), pp.369-385.

Kafi, M., 2009. The effects of salinity and light on photosynthesis, respiration and chlorophyll fluorescence in salt-tolerant and salt-sensitive wheat (*Triticum aestivum* L.) cultivars, *Plant Biotechnology*, pp.21-30.

Kalve, S., De Vos, D. and Beemster, G.T., 2014. Leaf development: a cellular perspective. *Frontiers in Plant Science*, *5*, pp. 362.

Kendall, H.W. and Pimentel, D., 1994. Constraints on the expansion of the global food supply. *Ambio*, pp.198-205.

Kharkwal, M.C., 2012. A brief history of plant mutagenesis. *Plant Mutation Breeding and Biotechnology*, pp.21-30.

Kotagiri, D. and Kolluru, V.C., 2017. Effect of salinity stress on the morphology and physiology of five different Coleus species. *Biomedical and Pharmacology Journal*, *10*(4), pp.1639-1649.

Kumar, B., Karjagi, C.G., Jat, S.L., Parihar, C.M., KR, Y., Singh, V., Hooda, K.S., Dass, A.K., Mukri, G., Sekhar, J.C. and Kumar, R., 2011. Maize Biology: An Introduction.

Kwasek, M., 2012. Threats to food security and common agricultural policy. *Економика пољопривреде*, *59*(4), pp.1-10.

Läuchli, A. and Epstein, E., 1990. Plant responses to saline and sodic conditions. *Agricultural Salinity Assessment and Management*, *71*, pp.113-137.

Lahmy, S., Barnèche, F., Derancourt, J., Filipowicz, W., Delseny, M. and Echeverria, M., 2000. A chloroplastic RNA-binding protein is a new member of the PPR family. *FEBS Letters*, *480*(2-3), pp.255-260.

Lee, S., Costanzo, S. and Jia, Y., 2012. The structure and regulation of genes and consequences of genetic mutations. *Plant Mutation Breeding and Biotechnology*, pp.31-45.

Levin, L.R. and Reed, R.R., 1995. Identification of functional domains of adenylyl cyclase using *in Vivo* Chimeras (∗). *Journal of Biological Chemistry*, *270*(13), pp.7573-7579.

Lichtenthaler, H.K., 1996. Vegetation stress: an introduction to the stress concept in plants. *Journal of Plant Physiology*, *148*(1-2), pp.4-14.

Li, X.J., Zhang, Y.F., Hou, M., Sun, F., Shen, Y., Xiu, Z.H., Wang, X., Chen, Z.L., Sun, S.S., Small, I. and Tan, B.C., 2014. Small kernel 1 encodes a pentatricopeptide repeat protein required for mitochondrial nad7 transcript editing and seed development in maize (*Zea mays*) and rice (*Oryza sativa*). *The Plant Journal*, *79*(5), pp.797-809.

Lurin, C., Andreés, C., Aubourg, S., Bellaoui, M., Bitton, F., Bruyère, C., Caboche, M., Debast, C., Gualberto, J., Hoffmann, B. and Lecharny, A., 2004. Genome-wide analysis of Arabidopsis pentatricopeptide repeat proteins reveals their essential role in organelle biogenesis. *The Plant Cell*, *16*(8), pp.2089-2103.

Manna, S., 2015. An overview of pentatricopeptide repeat proteins and their applications. Biochemistry, *113*, pp.93-99.

Máthé, C., 2012. The plant tissue culture collection at the Department of Botany, University of Debrecen. *Acta Biologica Szegediensis*, *56*(2), pp.179-182.

Mathur, N., Singh, J., Bohra, S., Bohra, A. and Vyas, A., 2006. Biomass production, productivity and physiological changes in moth bean genotypes at different salinity levels. *Am J Plant Physiology*, *1*(2), pp.210-213.

McCree, K.J., 1986. Whole-plant carbon balance during osmotic adjustment to drought and salinity stress. *Functional Plant Biology*, *13*(1), pp.33-43.

Munns, R., Schachtman, D.P. and Condon, A.G., 1995. The significance of a two-phase growth response to salinity in wheat and barley. *Functional Plant Biology*, *22*(4), pp.561-569.

Munns, R., James, R.A. and Läuchli, A., 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *Journal of Experimental Botany*, *57*(5), pp.1025-1043.

Munns, R. and Sharp, R.E., 1993. Involvement of abscisic acid in controlling plant growth in soil of low water potential. *Functional Plant Biology*, *20*(5), pp.425-437.

Murphy, D., 2011. *Plants, Biotechnology and Agriculture*. CABI.

Musa, U.T. and Usman, T.H., 2016. Leaf area determination for maize (*Zea mays* L.), okra (*Abelmoschus esculentus* L.) and cowpea (*Vigna unguiculata* L.) crops using linear measurements. *Journal of Biology, Agriculture and Healthcare*, *6*(4), pp.103-111.

Nandal, M. and Hooda, R., 2013. Salt tolerance and physiological response of plants to salinity: A Review. *International Journal of Scientific & Engineering Research*, *4*(10), pp.44-67.

Negrão, S., Schmöckel, S.M. and Tester, M., 2017. Evaluating physiological responses of plants to salinity stress. *Annals of Botany*, *119*(1), pp.1-11.

Neumann, P., 1997. Salinity resistance and plant growth revisited. *Plant, Cell and Environment*, *20*(9), pp.1193-1198.

Ngara, R., Ndimba, R., Borch-Jensen, J., Jensen, O.N. and Ndimba, B., 2012. Identification and profiling of salinity stress-responsive proteins in *Sorghum bicolor* seedlings. *Journal of Proteomics*, *75*(13), pp.4139-4150.

Nieman, R.H., 1962. Some effects of sodium chloride on growth, photosynthesis, and respiration of twelve crop plants. *Botanical Gazette*, *123*(4), pp.279-285.

Netondo, G.W., Onyango, J.C. and Beck, E., 2004. Sorghum and salinity: II. Gas exchange and chlorophyll fluorescence of sorghum under salt stress. *Crop Science*, *44*(3), pp.806-811.

Oladosu, Y., Rafii, M.Y., Abdullah, N., Hussin, G., Ramli, A., Rahim, H.A., Miah, G. and Usman, M., 2016. Principle and application of plant mutagenesis in crop improvement: a review. *Biotechnology & Biotechnological Equipment*, *30*(1), pp.1-16.

Omoto, E., Taniguchi, M. and Miyake, H., 2012. Adaptation responses in C4 photosynthesis of maize under salinity. *Journal of Plant Physiology*, *169*(5), pp.469-477.

Paul, D., 2013. Osmotic stress adaptations in *Rhizobacteria*. *Journal of Basic Microbiology*, *53*(2), pp.101-110.

Pearcy, R.W., Schulze, E.D. and Zimmermann, R., 2000. Measurement of transpiration and leaf conductance. In *Plant Physiological Ecology*, 7, pp.137-160).

Pingali, P.L., 2001. CIMMYT 1999/2000 World maize facts and trends. Meeting world maize needs: Technological opportunities and priorities for the public sector.

Qados, A.M.A., 2011. Effect of salt stress on plant growth and metabolism of bean plant *Vicia faba* (L.). *Journal of the Saudi Society of Agricultural Sciences*, *10*(1), pp.7-15.

Qiu, N., Chen, M., Guo, J., Bao, H., Ma, X. and Wang, B., 2007. Coordinate up-regulation of V-H^+-ATPase and vacuolar Na^+/H^+ *antiporter as a response to NaCl treatment in a C3 halophyte Suaeda salsa*. *Plant Science*, *172*(6), pp.1218-1225.

Quintero, J.M., Fournier, J.M. and Benlloch, M., 2007. Na+ accumulation in shoot is related to water transport in K^+-starved sunflower plants but not in plants with a normal K^+ status. *Journal of Plant Physiology*, *164*(1), pp.60-67.

Rakhmankulova, Z.F., Ramazanova, G.A., Mustafina, A.R. and Usmanov, I.Y., 2001. Assessment of the respiratory costs of adaptation in plant species that differ in their responses to insufficient and excessive mineral nutrition. *Russian Journal of Plant Physiology*, *48*(5), pp.651-656.

Rahneshan, Z., Nasibi, F. and Moghadam, A.A., 2018. Effects of salinity stress on some growth, physiological, biochemical parameters and nutrients in two pistachio (*Pistacia vera* L.) rootstocks. *Journal of Plant Interactions*, *13*(1), pp.73-82.

Ranum, P., Peña-Rosas, J.P. and Garcia-Casal, M.N., 2014. Global maize production, utilization, and consumption. *Annals of the New York Academy of Sciences*, *1312*(1), pp.105-112.

Ruzvidzo, O., Dikobe, B.T., Kawadza, D.T., Mabadahanye, G.H., Chatukuta, P. and Kwezi, L., 2013. Recombinant expression and functional testing of candidate adenylate cyclase domains. In *Cyclic Nucleotide Signaling in Plants* (pp. 13-25). Humana Press, Totowa, NJ.

Saha, D., Prasad, A.M. and Srinivasan, R., 2007. Pentatricopeptide repeat proteins and their emerging roles in plants. *Plant Physiology and Biochemistry*, *45*(8), pp.521-534.

Saibo, N.J., Lourenço, T. and Oliveira, M.M., 2009. Transcription factors and regulation of photosynthetic and related metabolism under environmental stresses. *Annals of Botany*, *103*(4), pp.609-623.

Schwarz, M. and Gale, J., 1981. Maintenance respiration and carbon balance of plants at low levels of sodium chloride salinity. *Journal of Experimental Botany*, pp.933-941.

Shahid, M.A., Sarkhosh, A., Khan, N., Balal, R.M., Ali, S., Rossi, L., Gómez, C., Mattson, N., Nasim, W. and Garcia-Sanchez, F., 2020. Insights into the physiological and biochemical impacts of salt stress on plant growth and development. *Agronomy*, *10*(7), pp.938.

Small, I.D. and Peeters, N., 2000. The PPR motif–a TPR-related motif prevalent in plant organellar proteins. *Trends in Biochemical Sciences*, *25*(2), pp.45-47.

Sarsu, F., 2020. Contribution of induced mutation in crops to global food security. *ACI Avances en Ciencias e Ingenierías*, *12*(3), pp.10-10.

Świeżawska, B., Jaworski, K., Pawełek, A., Grzegorzewska, W., Szewczuk, P. and Szmidt-Jaworska, A., 2014. Molecular cloning and characterization of a novel adenylyl cyclase gene, HpAC1, involved in stress signaling in *Hippeastrum hybridum*. *Plant Physiology and Biochemistry*, *80*, pp.41-52.

Taffouo, V.D., Kouamou, J.K., Ngalangue, L.M.T., Ndjeudji, B.A.N. and Akoa, A., 2009. Effects of salinity stress on growth, ions partitioning and yield of some cowpea (*Vigna unguiculata* L. Walp.) cultivars. *International Journal of Botany*, *5*(2), pp.135-143.

Thomas, S.C., Frye, S., Gale, N., Garmon, M., Launchbury, R., Machado, N., Melamed, S., Murray, J., Petroff, A. and Winsborough, C., 2013. Biochar mitigates negative effects of salt additions on two herbaceous plant species. *Journal of Environmental Management*, *129*, pp.62-68.

Thorneycroft, D., Sherson, S.M. and Smith, S.M., 2001. Using gene knockouts to investigate plant metabolism. *Journal of Experimental Botany*, *52*(361), pp.1593-1601.

Tripathi, K.K., Warrier, R., Govila, O.P. and Ahuja, V., 2011. Biology of *Zea mays* (Maize). *Department of Biotechnology, Ministry of Science & Technology & Ministry of Environment and Forests, Government of India*.

Tuna, A.L., Kaya, C., Ashraf, M., Altunlu, H., Yokas, I. and Yagmur, B., 2007. The effects of calcium sulphate on growth, membrane stability and nutrient uptake of tomato plants grown under salt stress. *Environmental and Experimental Botany*, *59*(2), pp.173-178.

Türkan, I. and Demiral, T., 2009. Recent developments in understanding salinity tolerance. *Environmental and Experimental Botany*, *67*(1), pp.2-9.

Voleníková, M. and Tichá, I., 2001. Insertion profiles in stomatal density and sizes in *Nicotiana tabacum* L. plantlets. *Biologia Plantarum*, *44*(2), pp.161-165.

Wani, A.A., Sogi, D.S., Singh, P. and Shivhare, U.S., 2011. Characterization and functional properties of watermelon (Citrullus lanatus) seed protein isolates and salt assisted protein concentrates. *Food Science and Biotechnology*, *20*(4), p.877.

Willmer, C. and Fricker, M., 1996. *Stomata* (Vol. 2). Springer Science & Business Media.

Xing, H., Fu, X., Yang, C., Tang, X., Guo, L., Li, C., Xu, C. and Luo, K., 2018. Genome-wide investigation of pentatricopeptide repeat gene family in poplar and their expression analysis in response to biotic and abiotic stresses. *Scientific Reports*, *8*(1), pp.1-9.

Zidan, M.A. and Elewa, M.A., 1995. Effect of salinity on germination, seedling growth and some metabolic changes in four plant species (*Umbelliferae*). *Indian Journal of Plant Physiology*, *38*, pp.57-61.

Zhao, C., Zhang, H., Song, C., Zhu, J.K. and Shabala, S., 2020. Mechanisms of plant responses and adaptation to soil salinity. *The Innovation*, *1*(1), pp.100-117.

Zhu, C., Jin, G., Fang, P., Zhang, Y., Feng, X., Tang, Y., Qi, W. and Song, R., 2019. Maize pentatricopeptide repeat protein DEK41 affects cis-splicing of mitochondrial nad4 intron 3 and is required for normal seed development. *Journal of Experimental Botany*, *70*(15), pp.3795-3808.

Zörb, C., Schmitt, S., Neeb, A., Karl, S., Linder, M. and Schubert, S., 2004. The biochemical reaction of maize (*Zea mays* L.) to salt stress is characterized by a mitigation of symptoms and not by a specific adaptation. *Plant Science*, *167*(1), pp.91-100.

Appendix A

Table 3.5: Photosynthetic rates of the wt and mt maize plant lines for the PPR protein in response to 200 mM NaCl treatment.

Time (sec)	wt (200 mM)	mt (200 mM)
0	0,32	1,05
10	0,81	1,05
20	0,81	1,05
30	0,81	2,22
40	0,81	2,22
50	0,81	2,22
60	0,81	2,44
70	6,62	2,44
80	6,62	2,44
90	11,94	2,44
100	11,94	2,44
110	16,38	2,44
120	16,38	2,88
130	16,38	2,88
140	5,7	2,88
150	5,7	2,88
160	5,7	2,88
170	3,09	2,88
180	3,09	2,88

Appendix B

Table 3.6: Respiration rates of the wt and mt maize plant lines for the PPR protein in response to 200 mM NaCl treatment.

Time (sec)	wt (200 mM)	mt (200 mM)
0	9,28	3,88
10	9,28	3,88
20	9,28	3,2
30	9,28	1,88
40	9,28	1,88
50	9,28	2,06
60	1,21	2,06
70	0,85	0,85
80	1,42	7,09
90	2,06	7,09
100	0,4	2,26
110	0,4	3,44
120	3,18	3,44
130	0,6	3,44
140	0,6	3,44
150	1,78	2,07
160	2,07	2,07
170	2,98	2,07
180	2,98	3,9

Appendix C

Table 3.7: Transpiration rates of the wt and mt maize plant lines for the PPR protein in response to 200 mM NaCl treatment.

Time (sec)	wt (200 mM)	mt (200 mM)
0	0	0
10	0	0
20	0	0
30	0	0
40	0	0
50	0	0,01
60	0	0,01
70	0,04	0,04
80	0,04	0,04
90	0,06	0,05
100	0,06	0,05
110	0,06	0,05
120	0,06	0,05
130	0,06	0,05
140	0,06	0,05
150	0,07	0,05
160	0,08	0,05
170	0,08	0,06
180	0,09	0,06