

T.R.
GEBZE TECHNICAL UNIVERSITY
GRADUATE SCHOOL

**INVESTIGATION OF COMPETING ENDOGENOUS RNA (ceRNA)
NETWORK ROLE IN LEAF GROWTH REGULATION DURING
MAIZE (ZEA MAYS) PLANT AND PATHOGENIC FUNGUS
FUSARIUM VERTICILLIOIDES INTERACTIONS**

ERDEM EMRE DELİGÖZ

**A THESIS OF MASTER OF SCIENCE
DEPARTMENT OF MOLECULAR BIOLOGY AND GENETICS**

ADVISOR: ASSOC. PROF.DR. FATMA AYDINOĞLU

JANUARY 2025

T.R.
GEBZE TECHNICAL UNIVERSITY
GRADUATE SCHOOL

**INVESTIGATION OF COMPETING ENDOGENOUS RNA
(ceRNA) NETWORK ROLE IN LEAF GROWTH REGULATION
DURING MAIZE (ZEA MAYS) PLANT AND PATHOGENIC
FUNGUS FUSARIUM VERTICILLIOIDES INTERACTIONS**

ERDEM EMRE DELİGÖZ

**A THESIS OF MASTER OF SCIENCE
DEPARTMENT OF MOLECULAR BIOLOGY AND
GENETICS**

ADVISOR: ASSOC. PROF.DR. FATMA AYDINOĞLU

JANUARY 2025

T.C.
GEBZE TEKNİK ÜNİVERSİTESİ
LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ

**MISIR (ZEA MAYS) BİTKİSİ VE PATOJENİK FUNGUS
FUSARIUM VERTICILLIODES ETKİLEŞİMİ SIRASINDA
YAPRAK BÜYÜME REGÜLASYONUNDA REKABETÇİ
ENDOJEN RNA (ceRNA) AĞININ ROLÜNÜN ARAŞTIRILMASI**

ERDEM EMRE DELİGÖZ

YÜKSEK LİSANS TEZİ
MOLEKÜLER BİYOLOJİ VE GENETİK
ANABİLİM DALI

DANIŞMAN: DOÇ. DR. FATMA AYDINOĞLU

OCAK 2025

MASTER of SCIENCE JURY APPROVAL FORM

A thesis submitted by Erdem Emre, DELIGOZ, defended on 14/01/2025 before the jury formed with the 30/12/2024 date and 2024/68 numbered decision of the GTU Graduate Administration Board, has been accepted as a MASTER of SCIENCE thesis in the Department of Molecular Biology and Genetics.

JURY

MEMBER

(THESIS ADVISOR) : Assoc. Prof. Dr. Fatma AYDINOĞLU

MEMBER

: Assoc. Prof. Dr. Mine Gül ŞEKER

MEMBER

: Asst. Prof. Dr. Stuart James LUCAS

APPROVAL

Gebze Technical University Graduate Administration Board

...../...../.....date and/..... numbered decision.

SIGNATURE/SEAL

ABSTRACT

Maize (*Zea mays*) is the third most consumed crop plant globally. However, fungal pathogen *Fusarium verticillioides* causes biotic stress, leading to a reduction in maize leaf growth. Leaf growth, driven by processes such as cell division and expansion, is intricately controlled at the genetic level. Emerging evidence highlights the crucial role of plant non-coding RNAs (ncRNAs) in regulating gene expression. These ncRNAs includes long non-coding RNAs (lncRNAs, over 200 nucleotides) and microRNAs (miRNAs, 19–24 nucleotides). Recent studies propose that competing endogenous RNA networks, which involve interactions among miRNAs, mRNAs, and lncRNAs, are key regulators of gene expression. As such, this research aimed to evaluate the effects of *F. verticillioides* infection in maize leaf growth focusing the regulatory role of ceRNA networks. In this regards, tolerant (ADK310, R) and susceptible (ADK1188, S) maize lines were subjected to soil inoculation method and the infected (I) plants in RI and SI groups was observed at morphological, biochemical, physiological and transcriptional levels by comparatively to non-inoculated control (RC and SC) groups at six-leaf stage. Results showed that final length of six leaf (FLL6) decreased by 26% in RI and 16% in SI groups, elongation rate of six leaf (LER6) by 19% in SI groups and mature cell length of six leaf by 9% in the RI and 9% in the SI groups compared to the RC and the SC groups. Biochemical analysis showed varied antioxidant enzyme activities across growth zones of six leaf which are meristem, elongation and mature. As a result, ascorbate peroxidase (APX) activity exhibited a substantial increase of 314% specifically in meristem zone of the RI group compared to the RC group, highlighting a notable enhancement in antioxidant response. Glutathione reductase (GR) activity decreased in the elongation and mature zones of the RI groups, while demonstrating an increase in the corresponding zones of the SI groups when compared to the RC and the SC groups. This differential response suggests a zone-specific regulation of antioxidant enzyme activity influenced by infection. Superoxide dismutase (SOD) activity showed a 49% increase in the elongation zones of the RI groups compared to the RC group. Transcriptional analysis of leaf growth regulatory miRNAs, miR319 and miR396-related ceRNAs showed miR319 was expressed 11-fold and 7-fold more in the meristem zone of the SI group than in the elongation and mature zone of the SI group, correspondingly, and downregulated in the elongation and mature zones of the RI group compared to the meristem zone. TCP38 downregulated in the mature zone of the SI groups. miR396 expressed 24-fold and 17-fold more in the elongation and mature zones of the SI group than in the meristem zone, correspondingly, and was upregulation in the elongation and mature zones. GRF15 was expressed 55-fold and 26-fold more in meristem of RI group than in the elongation and mature zones, correspondingly, and upregulated in the meristem zone. Associated lncRNAs exhibited distinct expression patterns; lnc319 was upregulated in all growth zones of RI and SI groups, while lnc89 was upregulated in the mature zone of both R and S groups.

This study highlights the critical roles of ceRNA networks in regulating maize leaf growth under *F. verticillioides* infection, linking morphological changes with biochemical and molecular responses. By understanding these regulatory mechanisms, this research contributes to developing maize varieties with improved stress tolerance against *F. verticillioides* infection.

Keywords: Maize, *Fusarium verticillioides*, Leaf Growth Zones, Biotic Stress Tolerance, ceRNA Regulatory Network, miRNA, lncRNA

ÖZET

Mısır (*Zea mays*), dünya genelinde en çok tüketilen üçüncü tarım bitkisidir. Ancak fungal bir patojen olan *Fusarium verticillioides*, biyotik strese neden olarak mısır yaprak büyümesini azaltmaktadır. Hücre bölünmesi ve genişlemesi gibi süreçlerle meydana gelen yaprak büyümesi, genetik düzeyde karmaşık bir şekilde kontrol edilir. Giderek artan kanıtlar, bitki kodlamayan RNA'larının gen ekspresyonunun düzenlenmesinde kritik bir rol oynadığını vurgulamaktadır. Bu RNA türleri arasında, 200 nükleotitten uzun olan uzun kodlamayan RNA'lar (lncRNA'lar) ve 19–24 nükleotitten oluşan mikroRNA'lar (miRNA'lar) yer alır. Son çalışmalar, miRNA'lar, mRNA'lar ve lncRNA'lar arasındaki etkileşimleri içeren "rekabetçi endojen RNA (ceRNA) ağlarının" gen ekspresyonunun temel düzenleyicileri olduğunu öne sürmektedir. Bu bağlamda, bu araştırma *F. verticillioides* enfeksiyonunun mısır yaprak büyümesindeki ceRNA ağlarının düzenleyici rolünü incelemeye odaklanmıştır. Bu amaçla, dirençli (ADK310, R) ve hassas (ADK1188, S) mısır hatları toprak inokülasyonu yöntemi ile enfekte edilerek enfekte edilmeyen kontrol R ve S gruplarına kıyasla morfolojik, biyokimyasal ve transkripsiyonel düzeyde analiz edilmişlerdir. Sonuçlar, enfekte gruplarda yaprak uzunluğunun R grubunda %26, S grubunda %16 azaldığını; yaprak uzama oranının S grubunda %19 azaldığını ve olgun hücre uzunluğunun hem R hem de S gruplarında %9 oranında düştüğünü göstermiştir. Biyokimyasal analizler, büyüme bölgeleri (meristem, uzama ve olgun) arasında farklı antioksidan enzim aktiviteleri ortaya koymuştur. Çalışmada, askorbat peroksidaz (APX) aktivitesi özellikle RI grubunun meristem bölgesinde %314 oranında bir artış sergileyerek istatistiksel olarak önemli bir artışı ortaya koymuştur. Buna karşılık, glutatyon redüktaz (GR) aktivitesi RI grubunun uzama ve olgun bölgelerinde azalırken, aynı bölgelerde SI grubunda bir artış göstermiştir. Bu farklı tepkiler, antioksidan enzim aktivitesinin uygulama koşullarına bağlı olarak bölgeye özgü bir düzenlemeye tabi olduğunu göstermektedir. Süperoksit dismutaz aktivitesi ise R grubunun uzama bölgelerinde %49 oranında artış göstermiştir. Yaprak büyümesini düzenleyen miRNA'ların, özellikle miR319 ve miR396 ile ilişkili ceRNA'ların transkripsiyonel analizi, miR319'un SI grubunun meristem bölgesinde uzama ve olgun bölgelere göre sırasıyla 11 kat ve 7 kat daha fazla ifade edildiğini; RI grubunun meristeminde yukarı regüle edilirken, uzama ve olgun bölgelerinde aşağı regüle edildiğini göstermiştir. TCP38, SI grubunun uzama bölgesinde olgun bölgeye göre ifadesi 2 kat artmıştır. miR396, SI grubunun uzama ve olgun bölgelerinde meristem bölgesine göre sırasıyla 24 kat ve 17 kat daha fazla ifade edilmiştir. GRF15, RI grubunun meristem bölgesinde uzama ve olgun bölgelere göre sırasıyla 55 kat ve 26 kat daha fazla ifade edilmiştir. lnc319, enfekte grupların tüm bölgelerinde yukarı regüle edilirken, lnc89, R ve S gruplarının olgun bölgelerinde yukarı regüle edilmiştir. Bu çalışma, *F. verticillioides* enfeksiyonu altında mısır yaprak büyümesinin düzenlenmesinde ceRNA düzenleme ağlarının kritik rollerini vurgulayarak morfolojik değişimleri biyokimyasal ve moleküler tepkilerle ilişkilendirmektedir. Bu düzenleyici mekanizmaların anlaşılması, stres toleransı artırılmış mısır çeşitlerinin geliştirilmesine katkı sağlayacaktır.

Anahtar Kelimeler: Maize, *Fusarium verticillioides*, Leaf Growth Zones, Biotic Stress Tolerance, ceRNA Regulatory Network, miRNA, lncRNA

ACKNOWLEDGEMENTS

I would like to thank my supervisor Assoc. Prof. Dr. Fatma Aydınoğlu for her support, contribution and interest during my master's degree education and thesis study. Thesis committee jury members Assoc. Prof. Dr. Mine Gül Şeker and Asst. Prof. Dr. Stuart James Lucas for their contributions. I would like to thank the members of the Plant Systems Biology Laboratory, my friends, my family and my girlfriend Hasibe Günay for their support and help.

I would like to thank the Science Fellowship and Grant Programmes Directorate (BİDEB) 2210/A National MSc/MA Scholarship Program of the Scientific and Technological Research Council of Türkiye (TÜBİTAK) for supporting me by a scholarship throughout my master science study and for financially supporting this project by Grand number 221O321.



TABLE OF CONTENTS

	<u>Page</u>
ABSTRACT	v
ÖZET	vi
ACKNOWLEDGEMENTS	vii
TABLE OF CONTENTS	viii
LIST OF SYMBOLS AND ABBREVIATIONS	x
LIST OF FIGURES	xii
LIST OF TABLES	xiv
 1. INTRODUCTION	 1
1.1. Aim, Contributions and Content of the Thesis	5
2. GENERAL INFORMATION	7
2.1. General Information of <i>Fusarium verticillioides</i>	7
2.2. Plant-Pathogen Interactions	8
2.3. Plant Growth	10
2.4. Antioxidant Enzymes and Reactive Oxygen Species (ROS) in Plants	12
2.5. Biogenesis and Functions of Plant MicroRNAs	14
2.6. Plant Long Non-Coding RNAs (lncRNAs)	16
2.7. Competitive Endogenous RNA (ceRNA) Network	16
3. MATERIALS and METHODS	18
3.1. Plant Material, Growth Conditions and Inoculation by Pathogenic Fungal Isolate	18
3.2. Pathogenic Fungal Isolate, Culturing Medium and Preparing Fungal Inoculum	18
3.3. Microscopic Analysis	20
3.4. Kinematic Analysis	20
3.4.1. Harvesting Samples from the Sixth Leaf	20
3.4.2. Leaf Length (LL) and Leaf Elongation Rate (LER) Measurements	20
3.4.3. Meristem Cell Length Measurements	21
3.4.4. Cell Length Profile	21
3.4.5. Determination of Growth Parameters at Cellular Level	21
3.5. Enzymatic Analysis	23
3.5.1. Harvesting of Maize Leaf Tissue Samples, Extraction and Determination of Total Protein Amounts	23
3.5.2. Determination of Catalase Enzyme Activity in Sixth Leaf of Maize Lines in Response to <i>F. verticillioides</i>	24
3.5.3. Determination of Superoxide Dismutase (SOD) Enzyme Activity in Sixth Leaf of Maize Lines in Response to <i>F. Verticillioides</i>	25
3.5.4. Determination of Peroxidase (POX) Enzyme Activity in Sixth Leaf of Maize Lines in Response to <i>F. verticillioides</i>	26

3.5.5. Determination of Ascorbate Peroxidase (APX) Enzyme Activity in Sixth Leaf of Maize Lines in Response to <i>F. verticillioides</i>	26
3.5.6. Determination of Glutathione Reductase (GR) Enzyme Activity in Sixth Leaf of Maize Lines in Response to <i>F. verticillioides</i>	27
3.6. <i>In silico</i> ceRNA Analyses	28
3.6.1. <i>In silico</i> Identification of miRNA, lncRNA and Target Genes Associated with Growth	28
3.6.2. Primer Designing	29
3.7. <i>In vitro</i> ceRNA Network Analyses	30
3.7.1. Harvesting of Leaf Tissue Samples	30
3.7.2. RNA Isolation	30
3.7.3. cDNA Synthesis and Stem-loop Reverse Transcription	31
3.7.4. Determination of Transcript Level by qRT-PCR (Quantitative Real-Time-PCR) Method	32
4. RESULTS	34
4.1. Measurements of Leaf Length (LL) and Determination of Leaf Elongation Rate (LER)	34
4.2. Cell Length Measurement in Maize Leaf Growth Zones	37
4.3. Meristem Zone Length Measurement	38
4.4. Kinematic Analysis	39
4.5. Observing <i>F. verticillioides</i> in Maize	42
4.6. <i>In silico</i> Prediction of ceRNA Networks	43
4.7. Antioxidant Enzymatic Activities in Maize Leaf Growth Zones in Response to <i>F. verticillioides</i> Infection	48
4.7.1. Catalase (CAT) Enzyme Activity	48
4.7.2. Peroxidase (POX) Enzyme Activity	49
4.7.3. Ascorbate Peroxidase Enzyme (APX) Activity	50
4.7.4. Superoxide Dismutase (SOD) Enzyme Activity	51
4.7.5. Glutathione Reductase (GR) Enzyme Activity	52
4.8. Transcriptional Analyses of ceRNA Networks in Maize Leaf Growth Zones in Response to <i>F. verticillioides</i> Infection	53
4.8.1. Expression Analysis of ceR319 Network in Maize Leaf Growth Zones in Response to <i>F. verticillioides</i> Infection	53
4.8.2. Expression Analysis of ceR396 Network in Maize Leaf Growth Zones in Response to <i>F. verticillioides</i> Infection	55
5. CONCLUSION and DISCUSSION	58
REFERENCES	66
BIOGRAPHY	74
PUBLICATIONS AND PRESENTATIONS FROM THE THESIS	75

LIST OF SYMBOLS AND ABBREVIATIONS

°C	: Celcius
%	: Percent
µg	: Microgram
µl	: Microliter
AGO	: Argonaute
APX	: Ascorbate peroxidase
BSA	: Bovine serum albumin
CAT	: Catalase
CDK	: Cyclin dependent kinase
cDNA	: complementary DNA
ceRNA	: Competing endogenous RNA
cm	: Centimeter
CP	: Cell Production
DAPI	: 4'-6-diamino-2-phenylindole
DCL	: Dicer-like enzyme
ddH ₂ O	: Double distilled water
DIC	: Differential interference microscope
DNA	: Deoxyribonucleic acid
dNTP	: Deoxynucleotide
EDTA	: Ethylene diamine tetra acetic acid
el	: Elongation zone
EtOH	: Ethyl alcohol
FLL	: Final leaf length
g	: Gram
GR	: Glutathione Reductase
GRF	: Growth regulating factor
GSSG	: Glutathione Disulfide
H ₂ O ₂	: Hydrogen peroxide
Hz	: Hertz
LER	: Leaf elongation rate
LL	: Leaf length
lncRNA	: Long non-coding RNA
lx	: Lux
M	: Molar
ma	: Mature zone
MCL	: Mature cell length
me	: Meristem zone
mg	: Miligram
miRNA	: MicroRNA
ml	: Mililiter
mM	: Milimolar
mRNA	: Messenger RNA
NADPH	: Nicotinamide Adenine Dinucleotide Phosphate
NBT	: Nitroblue tetrazolium
nt	: Nucleotide
POX	: Peroxidase
PVP	: Polyvinylpyrrolidone

q-RT-PCR	: Quantitative Real-Time Polymerase Chain Reaction
RISC	: RNA-induced silencing complex
RNA	: Ribonucleic acid
ROS	: Reactive Oxygen Species
rpm	: Revolutions per minute
SOD	: Superoxide dismutase
SPSS	: Statistical Package for the Social Sciences
TCP	: Teosinte Branched /Cycloidea/Proliferating Cell Factor



LIST OF FIGURES

	<u>Page</u>
Figure 2.1: Fusarium ear rot (FER) symptoms on maize kernels.	7
Figure 4.1: Leaf length (LL) of maize (<i>Z. mays</i>) seedlings infected by the fungal pathogen <i>Fusarium verticillioides</i> .	34
Figure 4.2: Final Leaf length (FLL) of maize (<i>Z. mays</i>) seedlings infected by the fungal pathogen <i>F. verticillioides</i> .	35
Figure 4.3: Leaf elongation rate (LER) of maize (<i>Z. Mays</i>) seedlings infected by the fungal pathogen <i>F. verticillioides</i> .	36
Figure 4.4: Leaf elongation rate (LER) in the logarithmic growth phase of maize (<i>Z. mays</i>) seedlings infected by the fungal pathogen <i>F. verticillioides</i> .	37
Figure 4.5: Cell length (CL) maize (<i>Z. mays</i>) seedlings infected by the Fungal pathogen <i>F. verticillioides</i> .	38
Figure 4.6: Visualization of cell nuclei stained with DAPI under ZOET TM Fluorescent Cell Imager.	39
Figure 4.7: <i>F. verticillioides</i> hyphae on the first leaf of infected under Differential interfering contrast (DIC) microscope at 40X.	42
Figure 4.8: <i>F. verticillioides</i> spores and maize trichomes observed in the first leaf of the infected maize (<i>Z. mays</i>) group under Differential interfering contrast (DIC) microscope at 40X.	43
Figure 4.9: The first leaf of non- <i>F. verticillioides</i> infected control group under Differential interfering contrast (DIC) microscope at 40X.	43
Figure 4.10: Phylogenetic tree of Growth-Regulating Factor (GRF) genes retrieved from Maize Genetics and Genomics Database (MaizeGDB).	44
Figure 4.11: Phylogenetic tree of TEOSINTE BRANCHED/CYCLOIDEA /PCF (TCP) genes retrieved from Maize Genetics and Genomics Database (MaizeGDB).	45
Figure 4.12: Phylogenetic tree of mature zm-miR319 retrieved from the MicroRNA Sequence Database (miRBase).	46
Figure 4.13: Phylogenetic tree of mature zm-miR396 retrieved from the MicroRNA Sequence Database (miRBase).	47
Figure 4.14: <i>In silico</i> prediction of putative leaf growth regulating <i>Z. mays</i> ceR319 network under fungal pathogen <i>F. verticillioides</i> infection.	47
Figure 4.15: <i>In silico</i> prediction of putative leaf growth regulating <i>Z. mays</i> ceR396 network under fungal pathogen <i>F. verticillioides</i> infection.	48
Figure 4.16: Catalase (CAT) enzyme activity in the growth zones of the 6 th leaves of maize lines in response to <i>F. verticillioides</i> infection.	49
Figure 4.17: Peroxidase (POX) enzyme activity in the growth zones of the 6 th leaves of maize lines in response to <i>F. verticillioides</i> infection.	50
Figure 4.18: Ascorbate peroxidase (APX) enzyme activity in the growth zones of the 6 th leaves of maize lines in response to <i>F. verticillioides</i> infection.	51

Figure 4.19: Superoxide dismutase (SOD) enzyme activity in the growth zones of the 6 th leaves of maize lines in response to <i>F. verticillioides</i> infection.	52
Figure 4.20: Glutathione reductase (GR) enzyme activity in growth zones of the 6 th leaves of maize lines in response to <i>F. verticillioides</i> infection.	53
Figure 4.21: The expression analysis of the ceR319 network in the 6 th leaf growth zone of maize seedlings in response to <i>F. verticillioides</i> .	55
Figure 4.22: The expression analysis of the ceR396 in the 6 th leaf growth zone of maize seedlings in response to <i>F. verticillioides</i> .	57



LIST OF TABLES

	<u>Page</u>
Table 3.1: Synthetic nutrient agar (SNA) medium components.	19
Table 3.2: The list of the primers used in the study.	29
Table 4.1: The values of growth parameters as a result of kinematic analysis of the 6 th leaf of maize seedlings grown under <i>F.verticillioides</i> inoculated (I) and control (C) groups of tolerant ADK310 (R) and susceptible ADK1188 (S) lines.	41

1. INTRODUCTION

Maize (*Zea mays L.*) is an annual monocotyledonous plant that is taxonomically classified within the Gramineae order. The diploid maize species exhibit considerable variation in their genome size, which ranges from 2.2 to 2.7 GB. Furthermore, they contain approximately 32,000–42,000 protein-coding genes. However, it is estimated that non-coding sequence accounts for approximately 98% of the maize genome, yet the majority of this remains unexplored. The rapidly expanding global population is exerting significant pressure on the demand for high-calorie foods. Although maize is originally an American product, it is currently most-produced cereal crop in the world, with wheat and rice coming in as the next most produced crops. Indeed, maize feeds 21% of the global population and is included in over 500 basic foods and additives (FAOSTAT data). In light of the repercussions of climate change and the recent loss of agricultural land, there is an imperative to cultivate maize varieties that exhibit high yields and growth potential, while demonstrating resilience to diverse biotic and abiotic stresses within confined areas [Kushanov et al., 2022; Parvathaneni et al., 2020].

Fusarium verticillioides is a highly destructive fungal pathogen that is responsible for various types of fungal diseases, including root, crown, root, ear and stalk rots, as well as seedling blight, in maize. Furthermore, it has been shown to have a considerable effect on crop quality due to the production of fumonisin, a mycotoxin that is toxic to humans and animals [Gelderblom et al., 1988; Shephard, 2011; Marasas, 2001]. This fungus, which belongs to the Ascomycota phylum, primarily infects the roots, stems, and ears of maize. It is notable for its ability to persist in soil through crop residues [Omotayo, 2023; Leslie et al., 1990]. *F. verticillioides* reproduces asexually, producing both macroconidia and microconidia, which facilitate long-distance dispersal and rapid colonization, respectively [Leslie & Summerell, 2006]. Elevated temperatures (30–35°C) and specific maize endosperm compositions have been identified as environmental factors that facilitate its growth and toxin production [Chulze, 2010; Picot, 2011], contributing to its prevalence in temperate and Mediterranean regions [Alberts, 2016].

The plant response to biotic stress is characterized by a complex interaction between the plant and the pathogen, which is essential for the prevention of disease in crops. Plants have evolved sophisticated immune systems to detect and respond to pathogens. This process begins with the recognition of pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRRs), which results in the establishment of PAMP-triggered immunity (PTI) [Doods, 2010; Peng, 2017]. Furthermore, resistance (R) proteins facilitate the recognition of specific pathogen effectors, thereby initiating effector-triggered immunity (ETI) [Gassmann et al., 2012]. *F. verticillioides* is a fungus that commonly infects maize through the aerial conidia it produces on tassels and through the silks. Other possible routes of infection involve seed or soil-borne inocula [Headrick, 1991; Munkvold, 1997; Kedera, 1992]. The fungus produces PAMPs, such as oligogalacturonides, which trigger maize defense responses. This process activates enzymes, including chitinases and β -1,3-glucanases, which are responsible for the degradation of fungal cell walls [Ridley et al., 2001; Lanubile et al., 2012a]. Transcriptomic studies have identified a number of PRRs, including receptor-like kinases (RLKs), receptor-like proteins (RLPs), and serine/threonine kinases (STKs), as part of the plant's defense network against *F. verticillioides* [Boutrot & Zipfel, 2017; Lanubile et al., 2014b]. The recognition of effectors by R genes, particularly within the coiled-coil nucleotide binding-site leucine-rich repeat (CC-NBS-LRR) and nucleotide-binding adaptor shared by APAF-1, R proteins, and CED-4 (NB-ARC) families, serves to reinforce the resistance of maize to this pathogen [Jones & Dangl, 2006].

Plant growth is characterized by a permanent and irreversible increase in overall size, a process that is fundamentally driven by two key cellular activities: cell division, which generates new cells, and cell expansion, which enables the enlargement of these cells to contribute to the plant's overall development and structural formation. [Aydinoglu, 2020]. In meristematic tissues, undifferentiated cells engage in active division, subsequently differentiating into specialized structures that give rise to plant organs [Perianez-Rodriguez et al., 2021]. Maize, a plant species of significant economic importance, displays distinctive growth patterns, particularly in its leaves. The process of cell division, elongation, and differentiation occurs in discrete zones, extending from the leaf base to the tip [Sleewinski, 2012; Nelissen et al., 2016]. Maize is frequently employed by researchers as a model organism for investigating the

regulatory roles of hormones such as auxins and gibberellins in growth processes [Li et al., 2018]. Methods like kinematic analysis enable researchers to accurately measure and analyze the spatial distribution patterns in cell division and elongation. By providing detailed insights into these cellular processes, these techniques significantly contribute to a deeper understanding of the complex dynamics underlying plant growth and development [Nelissen, 2016]. Within the cellular level, growth is regulated by the cell cycle, with phases governed by cyclins and cyclin-dependent kinases (CDKs), which balance cell division and differentiation [Inzé & De Veylder, 2006]. This knowledge facilitates the development of agricultural strategies to optimise crop growth and stress resilience [Nelissen et al., 2016; Dewitte & Murray, 2003].

Reactive oxygen species (ROS) are highly reactive molecules that are essential for plant growth and stress signaling. However, they can cause oxidative damage when present in excess. The most common ROS types are produced in chloroplasts, mitochondria, and peroxisomes and include superoxide anions and hydrogen peroxide. Plants employ a sophisticated antioxidant system, comprising enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), to mitigate oxidative stress by neutralising ROS [Foyer, 2009; Mhamdi, 2010]. In response to stressors such as drought or pathogen attack, ROS levels increase, activating antioxidant defenses that prevent cellular damage and support stress responses [Mittler, 2011; Torres, 2010]. This precisely regulated equilibrium between ROS and antioxidants permits the signaling function of ROS to be fulfilled while the potential for harm is minimised [van Breusegem, 2006].

MicroRNAs (miRNAs) are a group of small, non-coding RNAs, typically ranging from 21 to 24 nucleotides in length, that have been recognized across various organisms as essential regulators of gene expression. These molecules influence a wide-ranging array of vital biological processes, including development, response to stress and disease resistance. Initial research into this field was conducted using plants, with studies focusing on the model species *Arabidopsis thaliana*. It was discovered that plant miRNAs influence growth patterns and stress responses, for example in response to drought or pathogens [Jones-Rhoades, 2006; Chen, 2009].

The biogenesis of plant miRNAs occurs through a complex process in which they are initially produced in the nucleus from MIR genes, forming a primary structure known as a 'primary miRNA'. This primary structure is then subjected to a process of

processing by DICER-LIKE1 (DCL1) to yield the mature miRNAs that are subsequently expressed. After being methylated by HUA ENHANCER1 (HEN1) to protect them from degradation, the resulting miRNAs are then transported into the cytoplasm, where they become part of the RNA-induced silencing complex (RISC). Within RISC complex, the precise function of miRNAs is to bind to target mRNAs, either inducing their degradation or repressing their expression based on complementarity [Park, 2005; Mi et al., 2008].

MiRNAs such as miR319 and miR396 are indispensable for leaf development. miR319 regulates TEOSINTE BRANCHED CYCLOIDEA PROLIFERATING CELL NUCLEAR ANTIGEN FACTOR (TCP) transcription factors, influencing leaf morphology and size, whereas miR396 targets GROWTH REGULATING FACTORS (GRFs), regulating cell division to ensure balanced growth [Aydinoglu and Lucas 2019]. These pathways demonstrate the pivotal role of miRNAs in plant morphology and adaptation [Palatnik, 2003; Rodriguez, 2010].

Recent developments in high-throughput sequencing methods have enabled the discovery of long non-coding RNAs (lncRNAs), which are RNA molecules over 200 nucleotides in length and do not code for proteins. This development has significantly impacted our understanding of gene regulation in plants. High-throughput sequencing has uncovered a large number of lncRNAs that are involved in diverse biological processes, including plant growth, stress responses, and the regulation of epigenetic mechanisms [Zhao et al., 2022; Cui et al., 2023].

Plant lncRNAs are transcribed by RNA polymerase II and undergo modifications. The transcription process is regulated by epigenetic changes and alternative splicing. They regulate genes by acting as decoys, scaffolds, or guides, for example, by recruiting chromatin modifiers or binding transcription factors. To illustrate, the LAIR lncRNA affects leaf polarity and morphology, thereby influencing plant structure [Zhang et al., 2016].

lncRNAs are also crucial for the plant's ability to respond to stress. In response to drought stress, they orchestrate gene networks for stress tolerance and can function as target mimics to modulate miRNA activity, thereby regulating stress responses [Jha et al., 2023].

The competitive endogenous RNA (ceRNA) hypothesis explains how RNA molecules, such as messenger RNAs (mRNAs) and lncRNAs, regulate gene expression by competing for binding to shared miRNAs through common miRNA response elements (MREs). In this interaction, miRNAs typically suppress their target mRNAs by binding to complementary sequences, inhibiting translation. However, ceRNAs can act as "sponges" that sequester miRNAs, reducing their availability to bind mRNAs, and thereby promoting mRNA translation. While extensively studied in animal systems, ceRNA networks have also been shown to play vital roles in regulating key processes in plants [Salmena et al., 2011; Franco-Zorrilla et al., 2007]. For example, in *A. thaliana*, the long non-coding RNA induced by phosphate starvation1 (IPS1) binds to miR399, a microRNA that typically targets Phosphate Overaccumulator (PHO2), a gene that regulates phosphate levels. The sequestration of miR399 by IPS1 permits the expression of PHO2, thereby stabilising phosphate homeostasis [Franco-Zorrilla et al., 2007].

Although ceRNA networks have been observed to modulate leaf morphology in response to environmental changes, as shown in *Morus alba*, their role in leaf growth under biotic stress remains understudied [Shi et al., 2023]. In this study, we investigate the role of ceRNA networks in maize leaf growth regulation, specifically in response to *F. verticillioides* infection. By exploring this regulatory mechanism, we aim to clarify how ceRNA interactions contribute to leaf growth processes during pathogen challenges, highlighting their role in modulating gene expression dynamics essential for sustaining growth under biotic stress conditions.

1.1. Aim, Contributions and Content of the Thesis

It is crucial to comprehend the leaf growth responses of maize to sustain larger leaves and more efficient photosynthesis to gain biomass. However, the pathogenic fungus *F. verticillioides* infest maize plants and causes biotic stress in plants which results in growth inhibition. Leaf growth occurred via cell division and cell expansion mechanism which are highly regulated by molecular level. Plant ncRNAs are shown the main regulators of the expression of many genes in many biological process. Recently, miRNA and lncRNA and mRNA molecules have been hypothesized as a ceRNA network that plays a distinctive role in gene expression regulation. Therefore, this study aims to investigate ceR319 and ceR396 regulatory networks in maize growth

zones under *F. verticillioides* infection compared to non-infected control plants. Moreover, it is essential to ascertain the specific cell division and cell expansion mechanisms that are influenced by these interactions. It is hypothesized that ceR319 and ceR396 regulatory networks regulate maize leaf growth by differentially expressed in the leaf growth zones which are meristem, elongation, and mature zones -in response to *F. verticillioides* infection. This study aims to elucidate the molecular mechanisms of maize leaf growth regulation through ceRNAs involvement in response to the pathogenic fungus *F. verticillioides*. The targets of this thesis is to investigate the morphology, biochemistry and molecular response in the leaf growth zones of maize plants infected by the pathogenic fungus *F. verticillioides* and to determine the role of ceRNA networks during leaf growth and developmental stages.

2. GENERAL INFORMATION

2.1. General Information of *Fusarium verticillioides*

A number of fungal species have the potential to infect the developing ears of maize and kernels, resulting in the manifestation of diseases that are categorised as ear rot. Furthermore, these fungi often produce mycotoxins, which exert a deleterious effect on the quality and market value of maize. *Fusarium verticillioides* (Sacc.) Nirenberg (formerly designated as *F. moniliforme* Sheldon, teleomorph *Gibberella moniliformis* Wineland), an important pathogen that is responsible for both stalk and ear rot in maize, is frequently encountered in maize fields during harvest time (Bottalico, 1998; Battilani, 2008). It is widely documented that fumonisin, a potent secondary metabolite produced by *F. verticillioides*, is the principal causative agent of Fusarium ear rot (FER) (Figure 2.1) (Logrieco et al., 2002; Folcher et al., 2009). Following the discovery of its capacity to produce fumonisin, there was a notable increase in research interest surrounding this fungus (Gelderblom et al., 1988). There are documented cases that demonstrate fumonisins to be a significant hazard to human and animal health, with a causal relationship have been demonstrated between their ingestion and the development of maladies such as esophageal cancer and leukoencephalomalacia in animals, and esophageal cancer in humans [Shephard, 2011; Marasas, 2001; Giannitti, 2011].

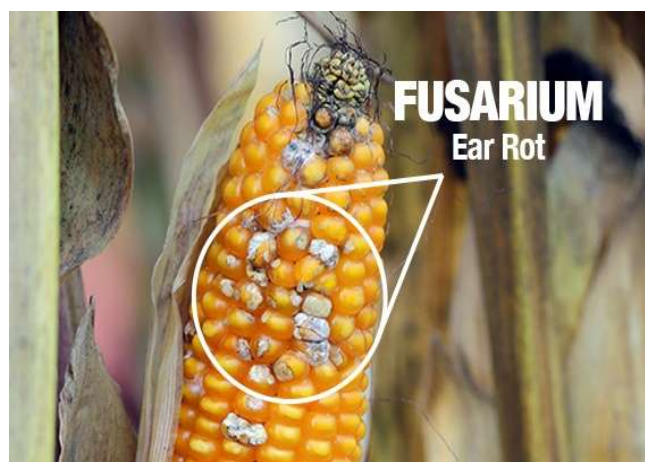


Figure 2.1: Fusarium ear rot (FER) symptoms on maize kernels.

The fungus *F. verticillioides* is taxonomically classified within the Kingdom Fungi, the phylum Ascomycota, and the family Nectriaceae. Its evolutionary origins are estimated to be around 7.3 million years old, a period coinciding with the divergence of the African and Asian clades within the *Fusarium fujikuroi* species complex. It is classified as part of the African clade, distinguished by the production of a variety of secondary metabolites. The fungus has an association with maize, infecting the roots, stems, and ears of the plant, and causing diseases such as Fusarium ear rot and stalk rot. Notably, the fungus demonstrates a remarkable capacity for persistence within the soil environment, frequently thriving in the presence of crop residue, thereby ensuring its consistent occurrence in maize fields [Omotayo OP, 2023; Leslie et al., 1990].

In the event of unfavourable environmental conditions, the fungus is also capable of forming chlamydospores, which are thick-walled resting spores that assist in its survival during periods of stress. While sexual reproduction is less prevalent, it can occur under specific environmental circumstances and contribute to genetic diversity [Leslie and Summerell, 2006]. The growth and fumonisin production of *F. verticillioides* are influenced by environmental factors, including high temperatures (30–35°C) and the composition of the endosperm of the maize kernel [Chulze, 2010; Picot et al., 2011; Alberts et al., 2016; Omotayo OP, 2023]. These factors, in conjunction with favourable climatic conditions, contribute to the prevalence of this fungus in temperate and Mediterranean regions. Optimal conditions during maize growth, particularly during the flowering stage, elevate the probability of toxin accumulation [Kamle et al., 2019; Omotayo OP, 2023]. Both macroconidia and microconidia can be readily dispersed by wind, water, or human activities such as the utilisation of contaminated agricultural machinery. The aerial dispersal of conidia enables the rapid dissemination of *F. verticillioides* in maize fields, where it can infect plants through natural openings or wounds [Munkvold & Desjardins, 1997]. This pervasive dispersal mechanism plays a pivotal role in the incidence of disease in maize crops.

2.2. Plant-Pathogen Interactions

The response of plants to biotic stresses is a complex phenomenon, involving the interaction of two living organisms: the plant and the pathogen. A comprehensive understanding of plant-pathogen interactions is essential for the prevention of disease

in plants. From a co-evolutionary perspective, plants progress through a series of stages in the process of recognising and responding to pathogens. Typically, plants initiate defense mechanisms by developing a multilayered immune system that prevents colonization by the majority of potential pathogens [Zhang et al., 2019]. This defense mechanism is initiated when immune receptors detect pathogen-associated molecular patterns (PAMPs), which are recognised by pattern recognition receptors (PRRs) [Doods et al., 2010]; Peng et al., 2017]. Secreted effector proteins from invading pathogens suppress PTI. Plants develop specific resistance (R) proteins that are capable of recognising pathogen effectors and triggering a defense response known as effector-triggered immunity (ETI) [Gassmann et al., 2012]. Subsequently, plants initiate acquired resistance while pathogens endeavour to circumvent these defenses [Campos et al., 2021].

F. verticillioides infects maize via several routes, with the most common being the infection of maize tassels by aerial conidia [Headrick et al., 1991; Munkvold et al., 1997]. Once introduced via the silks, the fungus continues to invade the kernels, but typically only a small proportion of infected kernels exhibit symptoms [Munkvold et al., 1997]. An additional potential route of infection is via the seeds themselves [Foley, 1962]. This occurs when conidia or mycelia present within or on the surface of the seeds invade the young plant, migrate from the roots to the stem, and eventually reach the cobs and kernels. A number of studies have demonstrated that the fungus migrates from infected seeds to the stem and kernels of the plant. Kedera and others [Kedera et al., 1992] employed vegetative compatibility groups as markers and substantiated the hypothesis that the fungus had disseminated from seeds to kernels, recovering the inoculated strain from approximately 10% of kernels. Similarly, Munkvold and colleagues observed the movement of the fungus and concluded that although seed infection may contribute to kernel infection, the primary route of infection was via silks [Munkvold et al., 1997]. It is also possible for systemic infections to arise from surviving inoculum in soil and crop residues. However, the relative importance of soil-borne and seed-borne inoculum in causing systemic infections remains unclear [Leslie et al., 1990; Rheeder et al., 1998].

The initial line of defense for plants is the recognition of conserved microbial molecules, known as pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs). Fungal enzymes that degrade plant cell walls produce oligogalacturonides,

a type of PAMP that triggers plant defense responses [Ridley et al., 2001]. Well-characterised enzymes such as β -1,3-glucanases and chitinases in maize [Lanubile et al., 2012a] may play a role in the degradation of the cell walls of *F. verticillioides*, resulting in the release of PAMP-derived cell wall fragments.

The recognition of PAMPs by plasma membrane-localised pattern recognition receptors (PRRs), including receptor-like kinases (RLKs) and receptor-like proteins (RLPs), initiates PAMP-triggered immunity (PTI), thereby strengthening the plant's defense system [Boutrot and Zipfel, 2017; Zhang et al., 2017]. A number of PRRs have been identified through transcriptomic studies, including cysteine-rich receptor-like kinase (CRRK), leucine-rich receptor-like kinase (LRRK), RLK, serine-threonine kinase (STK), and BRASSINOSTEROID INSENSITIVE 1-related receptor kinase 1 (BAK1) [Lanubile et al., 2014b; Wang et al., 2016].

A second layer of plant defense is initiated when specific effectors are recognised by resistance (R) gene products, resulting in effector-triggered immunity (ETI) [Jones and Dangl, 2006; Pel and Pieterse, 2013]. R genes involved in maize-*F. verticillioides* interactions have been identified as belonging to the coiled coil-nucleotide binding site-leucine-rich repeat (CC-NBS-LRR), NBS-LRR, and nucleotide binding adaptor shared by the APAF-1, R proteins, and CED-4 (NB-ARC) families [Lanubile et al., 2014b; Wang et al., 2016].

2.3. Plant Growth

Plant growth is defined as an irreversible increase in size driven by two primary cellular processes: cell division and cell expansion. Cell division occurs via mitosis, which increases the number of cells, while elongation allows individual cells to increase in size [Nelissen et al., 2016].

At the cellular level, plant growth originates in meristematic tissues, where undifferentiated cells actively divide and differentiate into specialized structures that form the roots, stems, and leaves [Perianez-Rodriguez et al., 2021]. This growth is organized into distinct zones, where cells undergo division and then elongation, propelling the overall increase in plant size.

Maize (*Zea mays*) is widely studied as a model organism for plant growth due to its economic importance and the large size of its organs, which facilitate detailed analysis

[Sleewinski, 2012]. In maize leaves, growth follows a clear pattern of cell division, elongation, and differentiation. At the base of the leaf, cells divide rapidly in the meristematic zone, contributing to leaf expansion. As these cells move away from the base, they transition to a phase of elongation before eventually entering a mature, non-growing state [Nelissen et al., 2016].

The structured growth of maize leaves makes them an excellent system for investigating the molecular mechanisms behind cell division and expansion. Researchers often use maize to study how hormones such as auxins and gibberellins interact with the cellular and genetic machinery governing these processes [Li et al., 2018].

Plant scientists employ growth analysis to examine differences in growth across genotypes or in response to environmental factors. This includes measuring plant size, organ weight, and growth rates to uncover the mechanisms underlying these variations. The two primary cellular drivers of organ growth, cell division and expansion, are quantified to provide insights into how genetic and environmental factors influence overall growth [Kutschera & Niklas, 2013].

A more detailed analysis of plant growth can be achieved through kinematic analysis, a technique particularly suited to linear growth systems like maize leaves. This method allows researchers to measure spatial patterns of cell division and elongation along the length of the leaf. By quantifying cell numbers and sizes at different positions, scientists gain a clearer understanding of how these processes contribute to leaf growth dynamics [Nelissen et al., 2016].

At the heart of these processes lies the cell cycle, which regulates the timing of cell division and significantly impacts growth rates. The cell cycle consists of several phases: G1 (cell growth), S (DNA replication), G2 (preparation for mitosis), and M (mitosis). Progression through these phases is tightly controlled by proteins such as cyclins and cyclin-dependent kinases (CDKs), ensuring a balance between cell division and differentiation [Inzé & De Veylder, 2006].

Understanding how the cell cycle is regulated opens up possibilities for agricultural innovations, such as manipulating growth patterns to enhance crop yields or improve resilience to environmental stresses [Nelissen et al., 2016; Dewitte & Murray, 2003].

2.4. Antioxidant Enzymes and Reactive Oxygen Species (ROS) in Plants

Reactive oxygen species (ROS) are exceptionally reactive molecules that play a dual role in plant physiology. While ROS are indispensable for typical cellular activities such as growth and signaling, they can also result in oxidative stress when present in excess. As sedentary organisms, plants are exposed to a multitude of environmental stressors that predispose them to ROS accumulation. Consequently, plants have evolved sophisticated antioxidant defense mechanisms to neutralise ROS and preserve cellular homeostasis [Mansoor et al., 2022; Dumanović et al., 2021]. ROS are by-products of normal metabolic processes and are primarily produced in chloroplasts, mitochondria, and peroxisomes during photosynthesis and respiration. The principal ROS species in plants are superoxide anions, hydrogen peroxide (H_2O_2), hydroxyl radicals ($\text{OH}^\cdot$), and singlet oxygen [Apel, 2004]. Low concentrations of ROS act as signaling molecules that regulate plant development and stress responses. However, high levels can cause oxidative damage to lipids, proteins, and nucleic acids. For example, the peroxidation of lipids induced by ROS compromises cell membrane integrity, leading to cellular dysfunction and death [Gill, 2010].

Plants have evolved sophisticated antioxidant defense mechanisms to regulate ROS levels, comprising both enzymatic and non-enzymatic components. Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and peroxidases (POX). SOD represents the initial line of defense against ROS, catalyzing the conversion of superoxide radicals to hydrogen peroxide and oxygen [Foyer, 2009]. SOD is present in a number of cellular compartments, including chloroplasts, mitochondria and the cytoplasm. This underscores the crucial function of SOD in safeguarding against ROS generated during photosynthesis and respiration [Mittler, 2002]. CAT is responsible for the conversion of hydrogen peroxide, a relatively less reactive ROS, into water and oxygen. CAT is particularly abundant in peroxisomes, where hydrogen peroxide is produced during photorespiration [Mhamdi, 2010]. APX plays a significant role in the ascorbate-glutathione cycle, whereby hydrogen peroxide is reduced to water through the use of ascorbate as a specific electron donor. APX is located in various cellular compartments, including chloroplasts, mitochondria and peroxisomes, which enables the localized detoxification of H_2O_2 [Foyer, 2011]. It is of critical importance in the

protection of chloroplasts, where hydrogen peroxide is produced during photosynthesis. GR plays a role in the regeneration of reduced glutathione (GSH) from oxidized glutathione (GSSG) during the ascorbate-glutathione cycle. This enzyme is responsible for maintaining a consistent supply of reduced glutathione, which is essential for the detoxification of ROS by enzymes such as APX [Noctor, 1998]. The primary localization of GR is in chloroplasts, the cytosol, and mitochondria, thereby contributing to the overall cellular redox balance. POX reduces H_2O_2 using a variety of electron donors, including phenolic compounds. These enzymes are widely distributed in plant tissues and play a pivotal role in lignification, pathogen defense and the oxidative stress response [Gupta, 2010].

A precisely regulated network comprising ROS and antioxidants in plants is responsible for maintaining cellular homeostasis. In conditions that are optimal for the plant, the production and scavenging of ROS are in equilibrium. This allows ROS to function as signaling molecules that regulate gene expression, cell differentiation and defense mechanisms [Mittler, 2011]. However, in the presence of stress, the equilibrium may shift towards an overproduction of ROS, which can result in oxidative damage. Crosstalk between antioxidant enzymes, such as SOD and CAT, is essential for effective ROS detoxification. H_2O_2 produced by SOD is rapidly converted to water by CAT and APX [Gupta, 2010]. The equilibrium between ROS production and scavenging can be disrupted by a variety of abiotic and biotic stresses. For example, drought stress has been demonstrated to result in stomatal closure, which in turn reduces CO_2 availability and increases ROS production due to impaired photosynthesis [Zhang, 1996]. Similarly, salt stress induces ionic imbalances and osmotic stress in plant cells, which in turn leads to an accumulation of ROS [Mittler, 2006]. In such circumstances, plants typically enhance the expression and activity of antioxidant enzymes, including SOD, CAT, APX, and GR, to mitigate oxidative damage [Asada, 1999]. In addition, biotic stressors, such as pathogen attacks, also elicit ROS production as part of the plant's defense mechanism. The oxidative burst, which is characterised by the rapid accumulation of ROS, serves as a signaling event to activate downstream defense responses, including the hypersensitive response (HR) [Torres, 2010]. However, it is essential to ensure that excessive ROS levels are tightly controlled by antioxidants in order to prevent collateral damage to healthy tissues [van Breusegem, 2006].

2.5. Biogenesis and Functions of Plant MicroRNAs

MiRNAs (micro-RNAs), a group of non-coding RNA molecules, are characterised by their typically 21–24 nucleotide length. These molecules have been indicated to play a fundamental role in the post-transcriptional regulation of gene expression in both plants and animals. In plants, miRNA as a regulatory element was first found in *A. thaliana*, where they were found to influence a variety of developmental processes, including leaf morphogenesis, root formation, and flower patterning [Jones-Rhoades, 2006]. Beyond development, plant miRNAs have been demonstrated to play a critical role in responses to various environmental stresses, including biotic and abiotic stresses such as pathogen infections, drought, and nutrient deficiencies [Chen, 2009]. In recent years, the study of plant miRNAs has gained significant attention, as these small RNAs have been associated with various regulatory networks crucial for plant growth, adaptation, and defense [Axtell, 2008]. Given their highly conserved nature across plant species, understanding miRNA biogenesis and functions provides valuable insights not only into fundamental plant biology but also into agricultural biotechnology, where miRNAs could be leveraged to enhance crop yield, stress tolerance, and disease resistance [Park, 2002].

The biogenesis of plant miRNAs is a complex, multi-step process that begins in the nucleus and is tightly regulated to ensure the accurate expression of miRNAs in response to developmental and environmental cues. Plant miRNAs are transcribed as primary miRNAs (pri-miRNAs) by RNA polymerase II from MIR genes, which are often located in intergenic regions or introns [Reinhart, 2002]. These pri-miRNAs are characterized by a stem-loop or hairpin structure, which is crucial for their subsequent processing into mature miRNAs. Pri-miRNAs can vary significantly in length, ranging from several hundred to a few thousand nucleotides, and their transcription is regulated by various transcription factors, depending on the developmental stage or stress conditions [Rogers, 2013]. After transcription, the pri-miRNAs undergo sequential cleavage events to become precursor miRNAs (pre-miRNAs). This processing is mediated by DICER-LIKE1 (DCL1), a highly conserved ribonuclease III enzyme [Park, 2005]. DCL1 binds to the stem-loop structure of the pri-miRNA and makes two precise cuts: the first near the base of the stem and the second near the terminal loop. This generates the shorter, double-stranded pre-miRNA, which still retains the hairpin

structure [Kurihara, 2004]. The pre-miRNA is further processed within the nucleus by DCL1 to produce a mature miRNA/miRNA* duplex, which consists of a guide strand (miRNA) and a passenger strand (miRNA*). The guide strand is the functional miRNA that will be incorporated into the RNA-induced silencing complex (RISC), while the miRNA* strand is often degraded [Mi et al., 2008]. Before export to the cytoplasm, the miRNA duplex is methylated by HUA ENHANCER1 (HEN1), a methyltransferase that adds a 2'-O-methyl group to the 3' end of the miRNA, protecting it from degradation. Once processed and methylated, the miRNA/miRNA* duplex is transported from the nucleus to the cytoplasm by HASTY, a plant homolog of Exportin-5, where it becomes part of the RNA-induced silencing complex (RISC) [Park, 2002]. Within RISC, the guide strand of the miRNA directs the complex to target mRNAs by binding to complementary sequences, typically in the 3' untranslated region (UTR) of the target mRNA. This binding results in either the cleavage of the target mRNA or the repression of its translation, depending on the level of complementarity between the miRNA and its target.

One of the most well-studied functions of miRNAs in plant growth is their role in leaf development. miR319, for example, is crucial for controlling the growth and differentiation of leaf cells. It regulates the expression of several TCP (TEOSINTE BRANCHED/CYCLOIDEA/PCF) transcription factors, which promote cell proliferation in young leaves [Palatnik et al., 2003; Aydinoglu & Lucas, 2019]. Overexpression of miR319 leads to larger, broader leaves due to the suppression of TCP activity, resulting in increased cell division [Ori et al., 2007]. On the other hand, the loss of miR319 function leads to narrow, serrated leaves, indicating its importance in shaping leaf morphology [Wang et al., 2005]. Similarly, miR396 regulates leaf size by targeting GROWTH REGULATING FACTORS (GRFs), a family of transcription factors involved in cell proliferation. miR396-mediated downregulation of GRFs restricts excessive cell division, ensuring that leaves reach their proper size and shape [Rodriguez et al., 2010; Aydinoglu & Lucas, 2019]. By carefully controlling these transcription factors, miRNAs ensure that leaves develop optimally in terms of both size and structure, contributing to overall plant fitness.

2.6. Plant Long Non-Coding RNAs (lncRNAs)

The discovery of non-coding RNAs (ncRNAs) has significantly altered our understanding of gene regulation in plants. lncRNAs, typically over 200 nucleotides in length, play crucial roles in regulating gene expression, often without encoding proteins. Recent advancements in high-throughput sequencing have identified thousands of lncRNAs, revealing their critical functions in plant development, stress responses, and epigenetic regulation [Zhao et al., 2022; Cui et al., 2023].

Plant lncRNAs are transcribed by RNA polymerase II and undergo various post-transcriptional modifications. The regulation of lncRNA transcription often involves epigenetic modifications, including histone alterations, which can impact chromatin structure and gene expression. Alternative splicing has also been identified as a mechanism generating various lncRNA isoforms [Cui et al., 2023].

lncRNAs influence gene expression by acting as molecular decoys, scaffolds, or guides. For instance, they can recruit chromatin-modifying complexes or bind directly to transcription factors, altering the expression of target genes. lncRNAs contribute to leaf growth by regulating cell division and expansion. For instance, the LAIR lncRNA plays a critical role in leaf polarity and morphogenesis [Zhao et al., 2022]. By interacting with chromatin-modifying proteins, these lncRNAs influence the growth and development of leaf tissues, thereby impacting overall plant architecture [Zhang et al., 2016]. lncRNAs play vital roles in plant responses to both biotic and abiotic stresses. During drought stress, for example, lncRNAs regulate gene networks that enhance stress tolerance by interacting with transcription factors and small RNAs. Furthermore, some lncRNAs act as endogenous target mimics, modulating miRNA activity to fine-tune stress responses [Jha et al., 2023].

2.7. Competitive Endogenous RNA (ceRNA) Network

The concept of competitive endogenous RNA (ceRNA) describes how RNA molecules such as messenger RNAs (mRNAs) and lncRNAs, can regulate gene expression by competing for shared miRNAs through common miRNA response elements (MREs). miRNAs typically bind to target mRNAs and inhibit their translation or induce degradation, but ceRNAs "sponge" these miRNAs, preventing

them from binding to their targets, thereby allowing mRNAs to be translated into proteins. This regulatory mechanism has been well-established in animals, and recent research suggests that plants also utilize ceRNA networks to regulate important physiological processes [Salmena et al., 2011; Franco-Zorrilla et al., 2007].

In plants, ceRNA networks have been found to regulate gene expression through interactions among lncRNAs, mRNAs, and miRNAs. lncRNAs act as decoys for miRNAs, preventing them from targeting specific mRNAs. For example, in *Arabidopsis thaliana*, the lncRNA IPS1 binds to miR399, a miRNA that typically targets PHO2, a gene involved in phosphate regulation. By sequestering miR399, IPS1 allows PHO2 mRNA to be translated, thereby maintaining phosphate homeostasis [Franco-Zorrilla et al., 2007].

Gao et al. (2023) investigated plant responses to sugarcane mosaic virus (SCMV) infection in resistant and susceptible maize lines. Using circRNA/lncRNA-vsiRNA-mRNA ceRNA networks, the researchers identified different regulation patterns of SCMV between resistant and susceptible lines. Li et. al. (2019) investigated ceRNA-miRNA-target gene regulatory networks during anther development of maize. In the study, many miRNAs, ceRNAs and target genes were identified by transcriptome analyses and their roles in anther development were elucidated. The research showed that genes associated with hormone signaling, cell cycle regulation and sterility are critical in this process. These findings provide new molecular targets for understanding the mechanisms of genetic regulation in maize male reproductive organ development.

3. MATERIALS and METHODS

3.1. Plant Material, Growth Conditions and Inoculation by Pathogenic Fungal Isolate

In this study, the plant material was maize (*Zea mays*) lines, ADK310 (R) and ADK1188 (S) which are tolerant and susceptible to the pathogenic fungus *Fusarium verticillioides*, respectively. Maize seeds of R and S lines were obtained Maize Research Institute (Sakarya/TURKEY).

The control plants (ADK310, tolerant to *F. verticillioides*; R and ADK1188, susceptible to *F. verticillioides*; S) were cultivated under optimal conditions within a plant growth chamber. The growth conditions for both groups were as follows: The plants were subjected to a 16-hour light period and an 8-hour dark period per 24-hour cycle. The relative humidity was maintained at 70%, the light intensity at 18,000 lux, and the temperature was set at 25°C during the day and 20°C at night.

Prior to the germination phase, the seeds were subjected to a surface sterilisation protocol. This involved a three-minute, one-minute rinse in 70% ethanol and a subsequent five-minute immersion in a 5% sodium hypochlorite solution. The latter was subsequently removed by rinsing the seeds with autoclaved distilled water. Once sterilised, the seeds were placed between filter papers within glass petri dishes and left to germinate within the plant growth chamber. After germination, seedlings were transferred to pots containing turf and grown in infected (I) and control (C) groups until the six leaves fully expanded. I and C groups were subjected to similar conditions as mentioned above except the RI and SI groups were grown in *F. verticillioides* inoculated soil which was explained under title 3.3.

3.2. Pathogenic Fungal Isolate, Culturing Medium and Preparing Fungal Inoculum

The fungal pathogen *Fusarium verticillioides* was obtained from the Maize Research Institute (Sakarya/TURKEY). *F. verticillioides* were cultured in a synthetic nutrient agar (SNA) medium in order to proliferate the fungal spores (conidia). The fungus was cultivated in Petri dishes for a period of two weeks at a temperature of 24-28°C, which

is optimal for its growth. SNA was prepared as in the table according to Correll et al. (1987) and autoclaved at 121°C for 20 minutes.

Table 3.1: Synthetic nutrient agar (SNA) medium components.

Medium Components	Amount (Weight/Volume)
KH ₂ PO ₄	1.0 g/L
KNO ₃	1.0 g/L
MgSO ₄ 7H ₂ O	0.5 g/L
KCl	0.5 g/L
Glucose	0.2 g/L
Sucrose	0.2 g/L
NaOH (1.0 M)	0.6 g/L
Agar	13.2 g/L

Synthetic nutrient agar (SNA) was prepared in accordance with the quantities indicated in table 3.1 and subjected to autoclaving. The fungus *F. verticillioides* was grown on Petri dishes containing SNA medium for two weeks at 25±2°C to prepare soil inoculum following the methodology described by Correll et al. (1987). Then, A 1000 ml glass jar was filled with sand (180 g), water (40 ml) and maize flour (20 g) in a 9:2:1 ratio, respectively. The solids were introduced initially and blended thoroughly, after which the water was incorporated. Subsequently, the mixture was subjected to autoclaving and sterilisation. The culture grown in SNA was divided into 32 pieces with the back of a pipette tip. Of these, half were placed in a jar and removed with the pipette tip, and half were dropped into jars.

Subsequently, the inoculum was cultivated at 25±2°C for a period of one week. Following the incubation period, the inoculum was combined with 600 grams of autoclaved soil and incubated for one week under the same conditions.

3.3. Microscopic Analysis

The first and second leaves of the plants grown under infected and control condition were harvested for observation of any fungal growth under microscopic. The leaves harvested were cut into 3 cm fragments and each fragment were placed in 100% ethanol (Merck, USA) for chlorophyll removal. Subsequently, the leaf fragments were immersed in a lactic acid (ISOLAB, Germany) solution until observation. To visualise the fungal spore or hyphae in/on the leaf fragments, a 0.04% trypan blue staining was applied [Boedijn, 1956]. The leaf fragments were immersed in trypan blue solution for 45 minutes. Following the incubation period, the samples were imaged at 4X, 10X and 40X magnifications using a differential interference contrast (DIC) microscope (Leica DM2500).

3.4. Kinematic Analysis

3.4.1. Harvesting Samples from The Sixth Leaf

The kinematic analysis was conducted in accordance with the methodologies established by Sprangers et al. (2016) and Fiorani (2001). For this end, the first 12 cm of maize leaves from inoculated RI and SI and non-inoculated RC and SC groups were harvested at 48 hours after the sixth leaf had emerged and divided into 1 cm fragments. For tissue fixation and chlorophyll removal, 1-4 cm fragments were placed in a 3:1 Ethanol (Merck, USA):acetic acid (Merck, USA) (v/v) solution and stored at +4°C. Fragments between 5-12 cm were kept in 100% Ethanol solution for clearing and then stored in lactic acid at room temperature.

3.4.2. Leaf Length (LL) and Leaf Elongation Rate (LER) Measurements

The length of the sixth leaves (LL6) of R and S maize lines grown under infected and control conditions was measured from their appearance until full maturation from the soil level to the tip of the leaf daily using a ruler. The elongation rate of the sixth leaf (LER6) was calculated as derivative of LL6 by using consecutive measurements with the formula 3.1. x_1 is the measurement of the first day, x_2 is the measurement of the second day, t_1 and t_2 indicate the time difference between the two measurements in

hour units. The significance of the difference between the groups was calculated by one-way analysis of variance (ANOVA) followed by Duncan's test using SPSS software (v27.0.1).

$$LER = \frac{x_2 - x_1}{t_2 - t_1} \quad (3.1)$$

3.4.3. Meristem Cell Length Measurements

In order to determine the length of meristem cell length in the meristematic zone, 1st, 2nd, 3rd and 4th cm of the sixth leaf were stained with 4',6-diamidino-2-phenylindole (DAPI) after chlorophyll removal and visualised under ZOETM Fluorescent Cell Imager (Bio-Rad, USA). Firstly, a rinsing buffer was prepared, containing 50 mM sodium chloride (NaCl), 5 mM ethylenediaminetetraacetic acid (EDTA) and 10 mM Tris (hydroxymethyl) aminomethane-hydrochloric acid (TRIS-HCl; pH 7). During the interval preceding the application of the DAPI, 1 µg/ml was prepared using the rinsing buffer, and the samples were treated with DAPI and maintained for 2 to 5 minutes. All of the aforementioned procedures were conducted on ice and in a dark environment. Subsequently, the mitotic figures were examined under a ZOETM Fluorescent Cell Imager.

3.4.4. Cell Length Profile

In order to ascertain the cell profile in the all growth zone (12 cm in length from the leaf base to the tip), fragments measuring 1 cm were harvested from the sixth leaf of maize plants grown under both infected and control conditions. The fragments were then placed on a slide, lactic acid was dripped onto them, and they were imaged by means of a DIC microscope under 10X, 20X and 40X magnification. Subsequent to this, the lengths of the cells in the images were measured utilizing the ImageJ program (<https://imagej.nih.gov>).

3.4.5. Determination of Growth Parameters at Cellular Level

Growth parameters such as cell production, cell division rate, cell cycle duration, meristem length were calculated according to the data obtained from microscopic observations (section 3.4.3 and 3.4.4) and final leaf length [Fiorani, 2001].

Mature cell length (l_{mat} (μm)) was determined based on the length measured from the 9th and 12th cm fragments in the ImageJ program. Elongation rate of six leaves (LER) was calculated as shown in the formula 3.1.

In the meristem zone, the cells that have undergone cell division are pushed upward by the cells that have formed as a result of cell divisions that continue at the base of the meristem. Cell production per cell row (P; cells produced per hour) was determined by dividing the leaf elongation rate (LER) by the mature cell length (l_{mat}). As shown in the formula 3.2:

$$P(cells/h) = \frac{LER}{l_{mat}} \quad (3.2)$$

In a time period characterised by steady-state growth, the number of cells that depart from the growth zone per unit of time is equivalent to the number of cells that enter this zone from the base of the elongation zone. Once the number of cells in that zone has been determined, the average rate of cell division in the meristem (D; the rate of cells per cell per unit time) can be calculated using the following formula (Beemster and Baskin, 1998). The quantity P is the cells produced per hour.

$$D (cells/cell.h) = \frac{P}{N_{mez}} \quad (3.3)$$

As Granier and Tardieu (1998) describe, in a number of cells undergoing simultaneous division, the duration of the cell cycle is defined by the time that is needed for an average cell to complete the cycle. This is equivalent to the time that is needed to double the size of the population. According to this principle, if we assume that the normal nature of the cell cycle process is such that twice as many cells are produced each time as the number of cells present, we can assume that, on average, the time taken for the cell cycle (T_c ; hours) is as follows (Fiorani, 2001).

$$T_c(h) = \ln 2 \frac{1}{D} \quad (3.4)$$

The number of cells in the growth zone (N_{gz}) was found from the sum of the number of cells in the elongation (N_{ez}) and meristem (N_{mez}). To determine the number of cells in the elongation and meristem zones, the length of each zone (L_{ez} , L_{mez}) was divided by the cell length of that zone.

$$\begin{aligned}
N_{gz} &= N_{ez} + N_{mez} \\
L_{gz} &= L_{mez} + L_{ez} \\
L_{ez} &= L_{gz} - L_{mez}
\end{aligned}
\tag{3.5}$$

Growth parameters were calculated using Microsoft Office Excel program and the statistical significance between the groups was determined by (ANOVA) followed by Duncan's test using SPSS software (v27.0.1).

3.5. Enzymatic Analysis

3.5.1. Harvesting of Maize Leaf Tissue Samples, Extraction and Determination of Total Protein Amounts

The sixth leaf of ADK310 (R) and ADK1188 (S) plants grown under *F. verticillioides*-inoculated infected group and non-*F. verticillioides*-inoculated control conditions were harvested at 48 hours after their emergence. These growth zones of leaves were samples as followed: meristem zone (first 1.5 cm), elongation zone (4-6 cm) and mature zone (10-12 cm). Samples were placed in 2 ml Eppendorf tubes including a 5 cm diameter bead and immediately placed into liquid nitrogen for fixation. They were then utilize for kept at -80°C until used for total protein isolation.

Total protein isolation was conducted in accordance with the methodology outlined by Kayihan et al. (2016). Samples kept at -80°C were transferred to liquid nitrogen and subsequently placed in homogenization blocks. The homogenizer (Retsch, LB MM400) was then operated at a frequency of 25 Hz for a period of 30 seconds. Thereafter, 1 ml of extraction buffer was added, comprising 50 mM KH₂PO₄ (pH 7.8), 1 mM EDTA and 2% polyvinylpyrrolidone (PVP). The tubes were then vortexed. The tubes were then subjected to a centrifugal process at 10,000 rpm and 4°C for 30 minutes. The resultant clear upper layers were then carefully removed without contact with the beads and transferred to clean Eppendorf tubes.

The determination of the total protein content was conducted in accordance with the methodology delineated by Bradford (1976). To this end, a 2 mg/ml BSA stock solution was prepared, and a series of diluted standard solutions were produced from this stock solution at concentrations of 0.0 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1 mg/ml, 1.5 mg/ml, and 2 mg/ml, respectively, in 2 ml Eppendorf

tubes. The spectrophotometric values of the solutions were recorded and a standard curve was generated using the Microsoft Excel programme.

This was then used as a reference to determine the protein amounts in the samples. To this end, a 96-well plate (TPP, Switzerland) was employed, with 5 μ l of each BSA solution and 250 μ l of Bradford reagent (SIGMA) added to one well. The measurements were conducted in triplicate. To establish a control sample, 255 μ l of Bradford reagent was added. For the analysis of protein samples, 5 μ l of the protein solution and 250 μ l of the Bradford reagent were added. The wells were then meticulously and accurately filled with the Bradford reagent using a pipette. The samples were then subjected to incubation in the dark at room temperature for a period of 10 minutes. Subsequently, a spectral scan was conducted at 595 nm on a multi-well reader (Thermo Scientific Varioskan Flash). The total protein content of the samples was then quantitatively determined in milligrams per milliliter (mg/ml) by employing the standard curve function in the Microsoft Office Excel programme. The extracted protein samples were then utilised for the determination of antioxidant enzyme activities.

3.5.2. Determination of Catalase (CAT) Enzyme Activity in the Growth Zones of Sixth Leaf of Maize Lines in Response to *F. verticillioides*

The concentrations employed in the experimental procedure for the measurement of catalase enzyme activity were as follows: 900 μ l of 50 mM KH_2PO_4 (pH: 7.8), 50 μ l of 200 mM H_2O_2 , and 50 μ l of protein samples. The blank was prepared with 950 μ l of 50 mM KH_2PO_4 (pH:7.8) and 50 μ l of H_2O_2 . Measurements were conducted using a 1.5 ml quartz cuvette and a Shimadzu UV1280 spectrophotometer. The spectrophotometer was calibrated to a wavelength of 240 nm for the purpose of measurement. The blank and protein samples were measured in the quartz cuvette for 90 seconds, and the decrease in absorbance was recorded in accordance with the methodology outlined by Chance and Maehly (1995).

The catalase enzyme is responsible for the reduction of hydrogen peroxide (H_2O_2) to water and oxygen, thus preventing oxidative damage to the cell. The concept of a "unit of catalase activity" is defined as the quantity of enzyme necessary to decompose 1 M H_2O_2 into hydrogen and oxygen over a 60-second interval. The activity of the catalase

enzyme was determined in accordance with the methodology proposed by Aebi (1984). The experiment was calculated as the mean of three technical replicates and three biological replicates, with standard error bars employed to indicate the degree of variation. The statistical significance of the observed differences between the groups was ascertained by one-way analysis of variance (ANOVA) followed by Duncan's test using SPSS software (v27.0.1).

3.5.3. Determination of Superoxide Dismutase (SOD) Enzyme Activity in the Growth Zones of Sixth Leaf of Maize Lines in Response to *F. verticillioides*

In order to ascertain the level of SOD enzyme activity, it is first necessary to prepare a solution in accordance with the methodology outlined by Banowertz (2004). The solution was formulated as follows: 44 µl of 250 mM KH_2PO_4 (pH 7.8), 20 µl of 75 mM nitroblue tetrazolium (NBT), 26 µl of 100 mM methionine, 90 µl of riboflavin, and 20 µl of protein (5-10 mg/ml). This solution was added to a 96-well TPP cell culture plate. In the case of the blank, the aforementioned solution was prepared with the following components: 44 µl 250 mM KH_2PO_4 (pH: 7.8) : 5 mM EDTA, 20 µl 75 mM NBT, 26 µl 100 mM methionine, 90 µl riboflavin. The 96-well plate (TPP, Switzerland) was then placed in a Varioskan spectrophotometer (Thermo Scientific Varioskan Flash) and shaken for one minute prior to measurement. Subsequent to this, the absorbances were measured at a wavelength of 560 nm. Following the completion of the measurement, the plate was then subjected to an incubation period under fluorescent light for a duration of 10 minutes. The plate was then returned to the device, and the samples' absorbances were determined once more at 560 nm, following an additional minute of shaking.

In the measurement of SOD enzyme activity, riboflavin present in the samples acts as a cofactor in the enzyme measurement. Riboflavin is activated in the presence of light and reacts with triplet oxygen to form superoxide radicals. The superoxide radicals formed are supported by methionine and increase the radical level in the system. These are then neutralised by SOD in the presence of light. Superoxide radicals react with NBT to form a blue-coloured product. In the presence of SOD, the NBT reaction is inhibited by converting superoxide radicals to O_2 and H_2O_2 . The absorbance change is recorded as a measure of SOD activity. The calculation of SOD enzyme activity was carried out in accordance with the methodology determined by Kono (1978).

Accordingly, one unit of enzyme was determined as the amount of enzyme required to reduce the absorbance difference caused by the change in the amount of NBT at 560 nm by 50% in one minute. SOD enzyme activity was expressed as units per milligram. The experiment was conducted with three technical and three biological replicates. The mean and standard deviation of the replicates were calculated. The statistical significance of the difference between the groups was ascertained by one-way analysis of variance (ANOVA) followed by Duncan's test using SPSS software (v27.0.1).

3.5.4. Determination Of Peroxidase (POX) Enzyme Activity in the Growth Zones of Sixth Leaf of Maize Lines in Response to *F. verticillioides*

Peroxidase enzyme activity was performed in accordance with the methodology established by Quesada (1990). To quantify peroxidase enzyme activity, a solution comprising 850 μ l of 50 mM KH_2PO_4 (pH 7.8), 50 μ l of 0.02% o-dianisidine, 50 μ l of 10 mM H_2O_2 , and 50 μ l of protein samples were utilized. The blank solution was prepared with 900 μ l of 50 mM KH_2PO_4 (pH:7.8), 50 μ l of 10 mM H_2O_2 , and 50 μ l of 0.02% o-dianisidine. Measurements were conducted using a 1.5 ml quartz cuvette and a Shimadzu UV-1280 spectrophotometer. The spectrophotometer was calibrated to a wavelength of 460 nm for measurement. The change in absorbance of the blank and protein samples in the quartz cuvette was recorded for a period of 90 seconds. An increase in absorbance and a corresponding change in colour were observed.

The concept of a "unit of enzyme" is defined as the quantity of enzyme capable of degrading 1 μ M hydrogen peroxide over the course of 1 minute. The experiment was repeated three times, with three replicates each, and the resulting data were averaged. The standard deviation was then calculated. The statistical significance of the difference between the groups was ascertained by one-way analysis of variance (ANOVA) followed by Duncan's test using SPSS software (v27.0.1).

3.5.5. Determination of Ascorbate Peroxidase (APX) Enzyme Activity in the Growth Zones of Sixth Leaf of Maize Lines in Response to *F. verticillioides*

The assay of ascorbate peroxidase enzyme activity was conducted according to the methodology established by Nakano and Asada (1981). To quantify ascorbate peroxidase enzyme activity, a solution comprising 850 μ l of 50 mM KH_2PO_4 (pH 7.0),

50 µl of 5 mM ascorbic acid, 50 µl of 20 mM H₂O₂, and 50 µl of protein samples were utilized. The blank was prepared with 900 µl of 50 mM KH₂PO₄ (pH: 7.0), 50 µl of 5 mM ascorbic acid, and 50 µl of 20 mM H₂O₂. The measurements were conducted using a 1.5 ml quartz cuvette and a spectrophotometer (Shimadzu, UV-1280). The decrease in absorbance was measured at 290 nm over a period of 90 seconds using a spectrophotometer.

Ascorbate peroxidase is the primary antioxidant enzyme responsible for the removal of hydrogen peroxide in peroxisomes, mitochondria, the cytosol and chloroplasts. Ascorbate peroxidase catalyzes the reduction of hydrogen peroxide to water and dehydroascorbic acid (DHA) by utilizing the reducing property of ascorbic acid (AA) [Das et al., 2014]. The term "unit of enzyme" is defined as the quantity of enzyme capable of degrading 1 µM ascorbic acid over the course of 1 minute. In Experiment 3, technical replicates were conducted in triplicate. The mean and standard deviation of the biological replicate were subsequently calculated. The statistical significance of the difference between the groups was ascertained by one-way analysis of variance (ANOVA) followed by Duncan's test using SPSS software (v27.0.1).

3.5.6. Determination of Glutathione Reductase (GR) Enzyme Activity in the Growth Zones of Sixth Leaf of Maize Lines in Response to *F. verticillioides*

The GR enzyme activity assay was conducted in accordance with the methodology established by Çakmak et al. (2006). To measure GR enzyme activity, a solution of 850 µl 50 mM KH₂PO₄ (pH:7.8) : 0.1 mM EDTA, 50 µl 0.5 mM GSSG, 50 µl 0.12 mM NADPH and 50 µl protein samples were prepared. The blank was prepared with 900 µl of 50 mM potassium phosphate (KH₂PO₄) (pH: 7.8) : 0.1 mM EDTA, 0.5 mM GSSG, and 50 µl of 0.12 mM NADPH. The measurements were recorded by monitoring the decrease in absorbance at 340 nm for 90 seconds in a spectrophotometer (Shimadzu, UV-1280) using a 1.5 ml quartz cuvette. GR enzyme activity is defined as the quantity of enzyme that is capable of reducing 1 µmol of GSSG (oxidised glutathione) to GSH (glutathione) at 25°C over the course of 1 minute. The experiment was conducted in triplicate, with three technical and three biological replicates. The mean and standard deviation of the replicates were calculated. The statistical significance of the difference between the groups was ascertained by one-

way analysis of variance (ANOVA) followed by Duncan's test using SPSS software (v27.0.1).

3.6. *In silico* ceRNA Analyses

3.6.1. *In silico* Identification of miRNA, lncRNA and Target Genes Associated with Growth

Leaf growth regulatory miRNAs and their target genes were selected from the study conducted by Aydinoglu & Lucas (2019). In this regard, miR319 and miR396 were selected as miRNAs, and Teosinte branched Cycloidea PCF (TCP) and Growth-Regulating Factor (GRF) genes were selected as the targets of these miRNAs. Subsequently, the sequences of miR319 and miR396 were obtained from the MicroRNA Sequence Database (miRBase) (www.mirbase.org). All variants of the TCP and GRF genes were obtained from the Maize Genetics and Genomics Database (www.maizegdb.org). The complete genome sequence of Zm-B73-Reference-NAM-5.0 was obtained from MaizeGDB and lncRNA sequences were got the transcriptomic data of *F. verticillioides* infected maize plants conducted in our lab (non-published). Phylogenetic trees were constructed for all variants of the miRNA319, miRNA396, GRF and TCP genes using Molecular Evolutionary Genetics Analysis (MEGA) 11 software (see Figures 4.8, 9, 10 and 11).

To determine putative *F. verticillioides* responsive ceRNA regulating maize leaf growth during infection, the RNAHybrid tool and the psRNATarget web service (A Plant Small RNA Target Analysis Server) were used. The parameters of the RNAhybrid program were set as follows; -p: 0.05, -e: -25, -v: 4, u: 2, -m: 80000. parameters used in psRNATarget web service: max. expect value=3, hspsize=20; target accessibility=25, flanking length=17bp upstream and 13bp downstream and central mismatch=9-11 nt [Dai and Zhao, 2011]. The parameters for miRNA-mRNA and miRNA-lncRNA target predictions were employed, respectively, to make a prediction. Subsequently, the results obtained from RNAHybrid and psRNATarget were compared, and common Gene IDs were identified. The common Gene IDs were then compared with the Gene IDs of the variants of TCP and GRF genes, and the common ones were identified. Based on the common TCP, GRF and lncRNA genes identified from the psRNATarget web service and RNAHybrid tool targeted by

miR319 and miR396, a ceRNA network was created with Cytoscape (v3.10.2) software (Figure 4.12 and 4.13).

3.6.2. Primer Designing

Primer design for mRNAs and lncRNAs in ceRNA networks was performed using SCiTools OligoAnalyzer 3.1. The specificity of the primers was checked using the BLASTn search engine (Altschul et al., 1990). Primer design for cDNA synthesis of miRNAs was performed according to Varkonyi-Gasic et al., (2007). Primers and their sequences are shown in table 3.2.

Table 3.2: The list of the primers used in the study.

mRNA		Sequence (5'→3')
TCP38 Zm00001eb328570	qF	CAGCCTCACCACGCAATC
	qR	AGGCACGCATGCTGAGA
GRF15 Zm00001eb113950	qF	CGGTGCTCTAGAGATGCTGTC
	qR	TTGCCTTTTCGGCCTTCCA
miRNA		
Zma-miR396	Stem Loop RT	GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACC AGT TC
	F	TGC GTT CCA CAG GCT TTC TT
	Universal R	GTG CAG GGT CCG AGG T
Zma-miR319	Stem Loop RT	GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACG GGA GC
	F	GCG ACT TGG ACT GAA GGG T
	Universal R	GTG CAG GGT CCG AGG T
lncRNA		
Zm-lnc89 TCONS_00089187	qF	ATGTCCTCCTCTGACCTCTTT
	qR	TCTAGTCATGGCAGTTGACATTC
Zm-lnc319 TCONS_00028828	qF	AGAGCGTCCTTCAGTCCAC
	qR	TTTGCTTGGCATGTGGGTG
Housekeeping Gene		
β-TUB (Beta-Tubulin) NP_001105457	qF	CTACCTCACGGCATCTGCTATGT
	qR	GTCACACACACTCGACTTCACG

3.7. *In vitro* ceRNA Network Analyses

3.7.1. Harvesting of Leaf Tissue Samples

48 hours after the emergence of the sixth leaf of maize seedlings grown in inoculated RI and SI and non-inoculated RC and SC groups, samples from different growth zones were harvested. These growth zones are meristem zone (first 1.5 cm), elongation zone (4-6 cm) and mature zone (10-12 cm). The samples were placed in 2 ml Eppendorf tubes including a 5 cm diameter bead and immediately frozen in liquid nitrogen for fixation. They were then stored at -80 C until used for RNA isolation.

3.7.2. RNA Isolation

Following the acquisition of samples from R and S maize lines inoculated with *F. verticillioides* and cultivated under control conditions, the samples were placed in liquid nitrogen. Homogenization of the tissue samples was conducted using a homogenizer in an Eppendorf tube containing a 5 mm diameter metal bead. The homogenizer was set to operate for 30 seconds at a frequency of 25 Hz. The isolation of RNA was conducted in accordance with the TRIzol method. The samples were promptly removed from the homogenizer blocks and returned to liquid nitrogen. Subsequently, 1 ml of TRIzol (Invitrogen) was added to the samples taken from the liquid nitrogen sequentially and the Eppendorf tubes were vortexed. Subsequently, the samples were vortexed and incubated for 10 minutes at room temperature. Following the incubation period, 200 µl of chloroform was added to the 1 ml TRIzol samples. The tubes were inverted for 15 seconds to facilitate the mixing of the chloroform. The samples were then incubated at room temperature for a period of 2–3 minutes. Subsequently, the samples were subjected to centrifugation in a pre-set centrifuge at 11,000 rpm and 4 °C for 15 minutes, until the incubation period had elapsed. Subsequently, the supernatant was extracted from the tube without disturbing the pellet or the beads and transferred to a new 1.5 ml tube.

In order to precipitate RNA, 450 µl isopropanol was added to the tubes containing the supernatant for 1ml TRIzol, and the samples were incubated for 10 minutes at room temperature. Subsequently, the samples were subjected to centrifugation at 11,000 rpm and 4°C for a period of 10 minutes. Subsequently, the supernatant was removed in

order to facilitate the washing of the precipitated RNA. Subsequently, the pellet was washed with 1 ml of 75% ethanol for 1 ml of TRIzol reagent. Subsequently, the samples were subjected to centrifugation at 11,000 rpm and 4 °C for a period of five minutes. The supernatant in the Eppendorf tubes was discarded in a manner that ensured the pellet was not lost. The pellets were then permitted to dry, with the objective of eliminating the ethanol present in the tubes. Following this process, 25 µl of nuclease-free water was introduced to the tubes. The samples were subsequently stored at -20°C. The concentration and purity of the RNA samples were determined using a NanoDrop spectrophotometer (Thermo Scientific, NanoDrop Lite). The purity of the RNA was evaluated through measurement of the A260/A280 nm ratio. The samples exhibiting the highest degree of purity, approaching a value of 2.0, were selected for cDNA synthesis.

3.7.3. cDNA Synthesis and Stem-loop Reverse Transcription

cDNA synthesis for mRNAs and lncRNAs was conducted using the High Capacity cDNA Reverse Transcription kit (ThermoFisher), following the methodology outlined in the accompanying protocol. The reaction volume was set to 20 µl. For each reaction, the following components were utilized: 2.0 µl of 10x RT buffer, 0.8 µl of dNTP mix (100 mM), 2.0 µl of random primer, and 1.0 µl of multiscribe reverse transcriptase. The quantity of total RNA employed was calculated in accordance with the RNA concentrations of the samples and introduced in accordance with the protocol outlined in the cDNA kit utilized. It was adjusted to 1.0 µg in 20 µl, and the remaining volume was introduced using nuclease-free water, thus completing the 20 µl reaction volume. The tubes were placed in a thermal cycler and the conditions were set as follows: 10 minutes at 25°C, 120 minutes at 37°C, 5 minutes at 85°C and infinite cycling at 4°C. Upon completion of the reaction, the cDNA samples were retrieved and stored at -20 °C for subsequent use in RT-qPCR.

In the synthesis of cDNA for microRNA, the High Capacity cDNA Reverse Transcription kit (ThermoFisher) was employed in accordance with the methodology outlined by Varkonyi-Gasic et al. (2007). A total reaction volume of 20 µl was prepared. For each reaction, the following components were added until the total volume reached 20 µl: 2 µl of 10x RT buffer, 0.5 µl of dNTP mix (100 mM), 0.25 µl of reverse transcriptase, 1 µl of stem-loop RT primer for each of miR319 and miR396,

and 2 µg of template. The final volume was completed with nuclease-free water. The tubes were placed in a thermal cycler (Biometra, Analytik Jena) and pulsed reverse transcription was performed. The cycling conditions of the device were set as follows: an incubation period of 30 minutes at 16°C, 30 seconds at 30°C, 30 seconds at 42°C and 1 second at 50°C for 60 cycles, and an incubation period of 5 minutes at 85°C for reverse transcriptase inactivation. Upon completion of the reaction, the samples were removed and stored at -20°C until they were utilized for quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis.

3.7.4. Determination of Transcript Level by qRT-PCR (Quantitative Reverse Transcriptase- PCR) Method

The transcript amounts of microRNA (miRNA), long non-coding RNA (lncRNA) and messenger RNA (mRNA) from total RNA samples after translation into cDNA were determined by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analysis. The Aidlab SYBR® Green qPCR kit and the accompanying protocol were employed for the purpose of conducting a quantitative real-time polymerase chain reaction (qRT-PCR). For cDNA samples of miRNA, a total of 7.5 µl of 2X Syber Green, 1.5 µl of cDNA, 0.6 µl of universal reverse primer (10 mM), 0.6 µl of forward primer (10 mM) and 4.8 µl of nuclease-free water were added per reaction. For mRNA and lncRNA, a total of 7.5 µl of 2X Syber Green, 1.5 µl of cDNA, 0.6 µl of reverse primer (10 mM), 0.6 µl of forward primer (10 mM) and 4.8 µl of nuclease-free water were added per reaction. The tubes were placed in the Real-Time PCR System (Biometra, Analytik Jena) and the device cycling conditions for mRNA and lncRNA were set as follows: an initial incubation period of 3 minutes at 95°C, followed by 5 seconds at 95°C, 20 seconds at 60°C, 15 seconds at 72°C for 40 cycles, 15 seconds at 95°C, 1 minute at 60°C and 15 seconds at 95°C for the melting curve. For miRNA, an initial incubation period of 3 minutes at 95°C, followed by 5 seconds at 95°C, 20 seconds at 60°C, 15 seconds at 72°C for 45 cycles, 15 seconds at 95°C, 1 minute at 60°C and 15 seconds at 95°C for the melting curve.

The relative transcript amounts of mRNA, miRNA and lncRNA in ceRNA regulatory networks were determined by employing the delta-delta Ct ($2^{-\Delta\Delta C_t}$) method [Pfaffl, 2001]. The cycle threshold (Ct) values of the mRNA, miRNA and lncRNA were normalised to β -tubulin, which was used as a reference gene. The transcript amounts

were calculated by averaging three biological and two technical replicates, and standard error bars were employed to represent the associated error. The statistical significance of the observed difference between the groups was determined by one-way analysis of variance (ANOVA) followed by Duncan's test using SPSS software (v27.0.1).



4. RESULTS

4.1. Measurement of Leaf Length (LL) and Determination of Leaf Elongation Rate (LER)

After the sixth leaf emerged, measurements showed that the inoculated (I) groups grew more slowly and stopped elongation sooner than the control (C) groups in both tolerant ADK310 (R) and susceptible ADK1188 (S) lines (Figure 4.1). Moreover, when the final leaf length of the six leaves (FLL6) were compared, a statistically significant difference was found between I and C groups, FLL6 of the infected groups decreased by 26% and 16% in RI and SI, respectively, compared to the control groups ($p < 0.01$) (Figure 4.2).

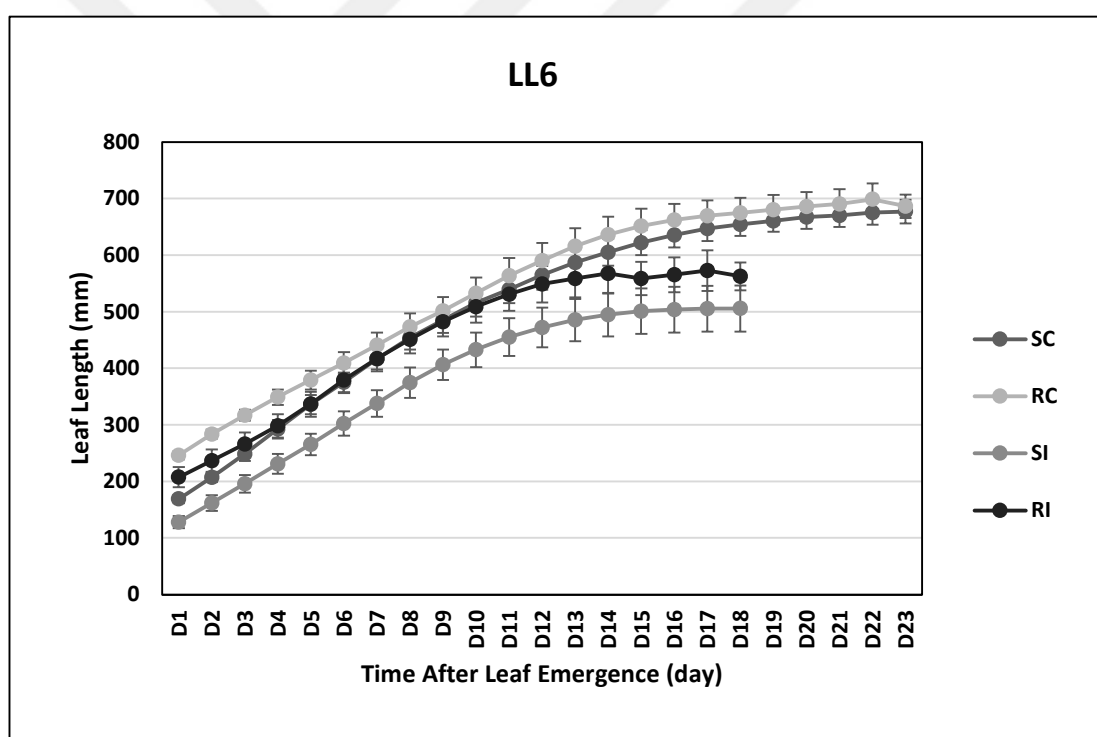


Figure 4.1: Leaf length (LL) of maize (*Z. mays*) seedlings infected by the fungal pathogen *F. verticillioides*. The measurements were recorded from the time of emergence of the sixth leaf of maize seedlings until full maturity. The x-axis is the time scale and is presented in days. Y-axis is leaf length in mm. Measurements were calculated by averaging the measurements of 6 maize lines ADK310 (Tolerant) and ADK1188 (Susceptible). RC=ADK310 line control; SC=ADK1188 line control; RI; ADK310 line *F. verticillioides* inoculated; SI=ADK1188 line *F. verticillioides* inoculated. Error bars indicate standard deviation. $n=3$, $\text{ave} \pm \text{sd}$.

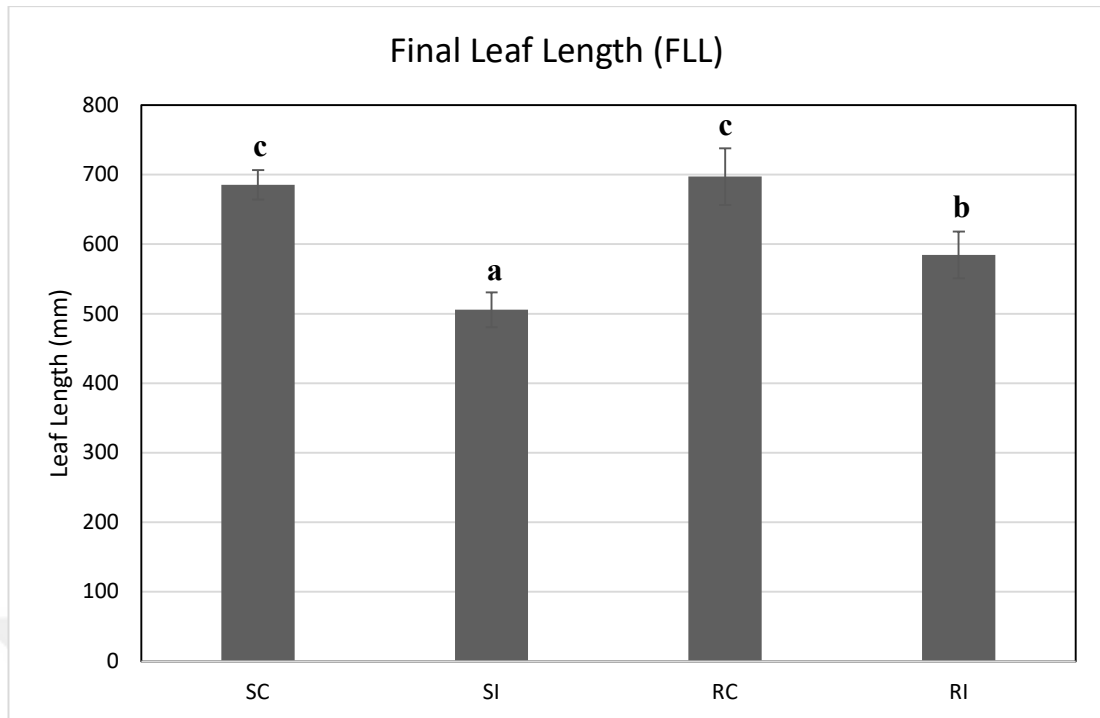


Figure 4.2: Final leaf length (FLL) of maize (*Z. mays*) seedlings infected by the fungal pathogen *F. verticillioides*. RC=ADK310 line control; SC=ADK1188 line control; RI=ADK310 line *F. verticillioides*-inoculated; SI=ADK1188 line *F. verticillioides*-inoculated. Error bars indicate standard deviation. n=3, mean±sd. Different letters indicate significant differences as determined by one-way analysis of variance (ANOVA) followed by Duncan's test ($P<0.05$).

In figure 4.3, the LER graph was formed as a result of the measurements obtained on a daily basis. When the rates in the logarithmic growth phase are compared, tolerant ADK310 *F. verticillioides* infected (RI) groups increased by 8% according to the tolerant ADK310 control (RC) group, but it was not statistically significant ($p>0.05$). Susceptible ADK1188 *F. verticillioides* infected (SI) groups decreased by 19% according to the susceptible ADK1188 control (SC) group and it was a statistically significant difference ($p<0.05$) (Figure 4.4).

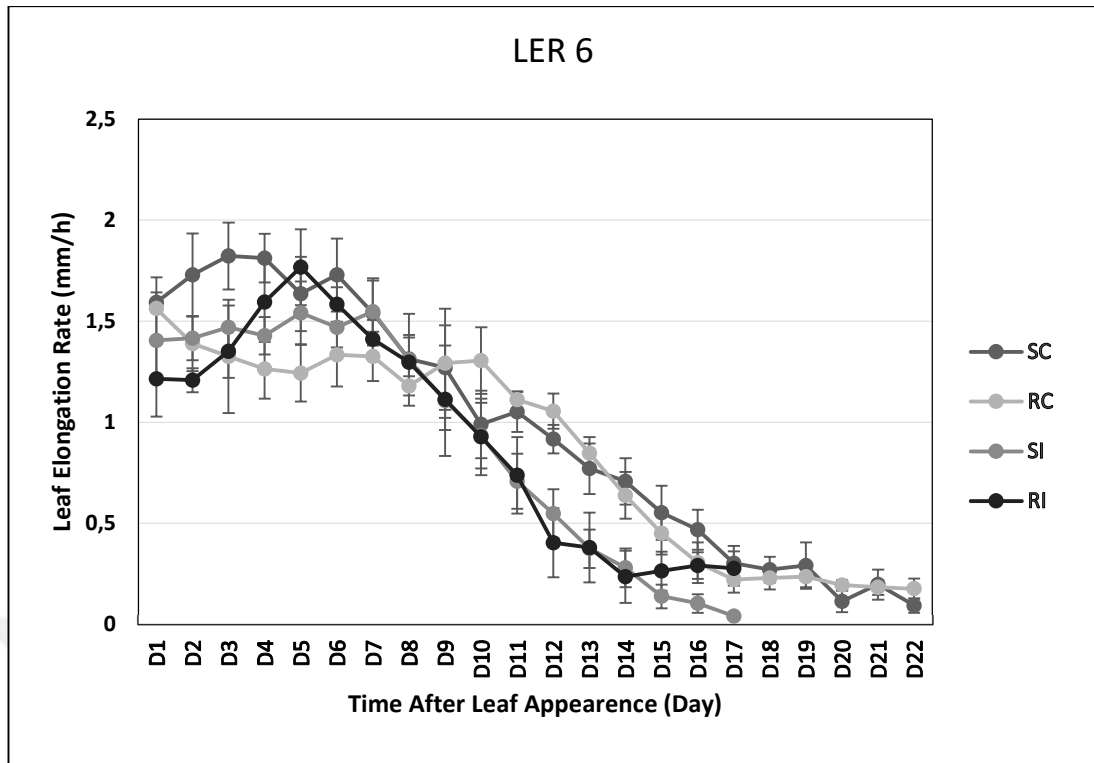


Figure 4.3: Leaf elongation rate (LER) of maize (*Z. mays*) seedlings infected by the fungal pathogen *F. verticillioides*. The LER was calculated as a derivative of two consecutive leaf length measurements from the emergence of the sixth leaf till full maturity. LER (mm/h^{-1}) plotted against time period. RC=ADK310 line control; SC=ADK1188 line control; RI=ADK310 line *F. verticillioides*-inoculated; SI=ADK1188 line *F. verticillioides*-inoculated. Error bars indicate standard deviation. $n=3$, $\text{ave} \pm \text{sd}$.

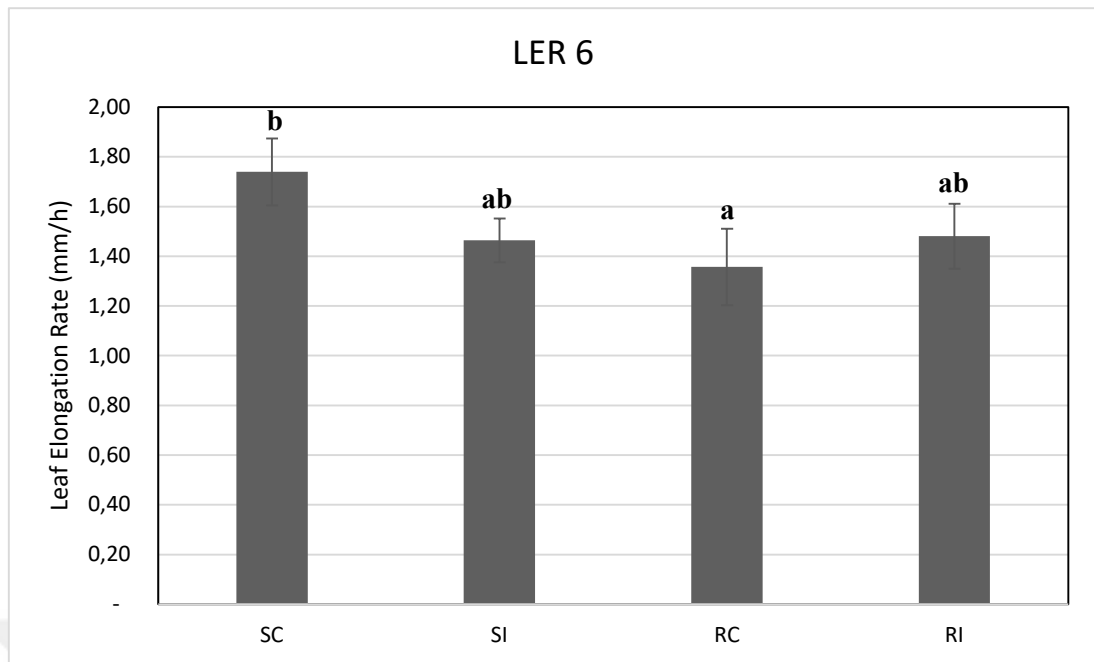


Figure 4.4: Leaf elongation rate (LER) in the logarithmic growth phase of maize (*Z. mays*) seedlings infected by the fungal pathogen *F. verticillioides*. LER measurements were based on growth rates up to the uppermost plateau in the growth phase. RC=ADK310 line control; SC=ADK1188 line control; RI=ADK310 line *F. verticillioides*-inoculated; SI=ADK1188 line *F. verticillioides*-inoculated. Error bars indicate standard deviation. n=3, mean±sd. Different letters indicate significant differences as determined by one-way analysis of variance (ANOVA) followed by Duncan's test (P<0.05).

4.2. Cell Length Measurement in Maize Leaf Growth Zones

CL6 in the all growth zone (12 cm in length leaf base to tip) of the sixth leaf of ADK310 (R) and ADK1188 (S) grown under non-*Fusarium verticillioides* infected control and *Fusarium verticillioides* infected were conditions was ascertained by microscopic observation. Both control and infected maize leaves demonstrated similar CL profiles in the growth zone (Figure 4.5). The cell length in the mer zone was significantly shorter (P<0.01) in comparison with the el and mat zones. The length of meristematic cells was 17.7 µm in the RC group and 15.5 µm in the RI group, a 15% decrease) in comparison with the control (p>0.05). The mer zone was 16 µm in the SC group and 13.2 µm in the SI group with a 22% decrease in comparison with the control (p<0.01). The length of the cells in the el zone was 94.4 µm in the RC group and 99.9 µm in the RI group with a 6% increase in comparison with the control (p>0.05). The el zone was 89.8 µm in the SC group and 93.1 µm in the SI group with

an increase of 4% in comparison with the control ($p>0.05$). The length of the cells in the mat zone was 122 μm in the RC group and 116.4 μm in the RI group with a 5% decrease in comparison with the control ($p<0.01$). In the SC group, the mat zone was 128.2 μm , while in the SI group it was 120.1 μm with a 7% decrease in comparison with the control ($p>0.05$).

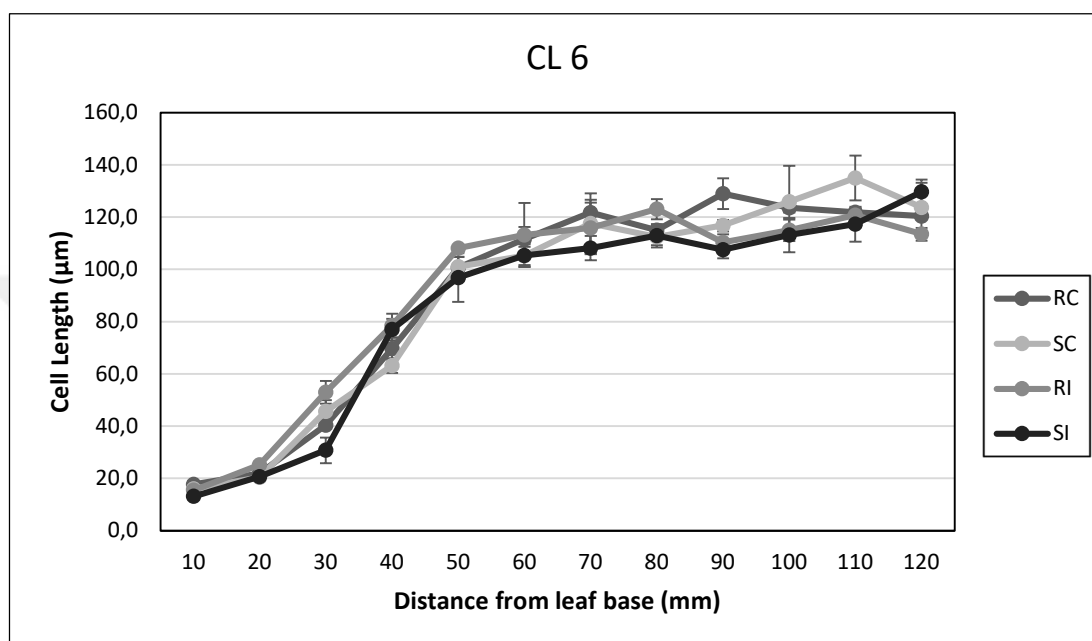


Figure 4.5: Cell length (CL) of maize (*Z. mays*) seedlings infected by the fungal pathogen *F. verticillioides*. Cells adjacent to the stomatal row were measured in the abaxial epidermis of the leaf 120 mm from the base to the tip of the sixth leaf. CL (μm) plotted against the distance from leaf base to tip (mm). RC=ADK310 line control; SC=ADK1188 line control; RI=ADK310 line *F. verticillioides*-inoculated; SI=ADK1188 line *F. verticillioides*-inoculated. Error bars indicate standard deviation. $n=3$, $\text{ave}\pm\text{sd}$.

4.3. Meristem Zone Length Measurement

The nuclei of the plant cells were visualized by ZOE fluorescence microscopy with DAPI (4',6-diamidino-2-phenylindole) staining applied to the 1st and 2nd cm of the samples harvested from the 6th leaf and stored in 3:1 acetic acid:Ethanol (v/v) solution and the length of mitotic figures in the meristematic zone was determined. These measurements were then used for kinematic analysis calculations. (Table 4.1).

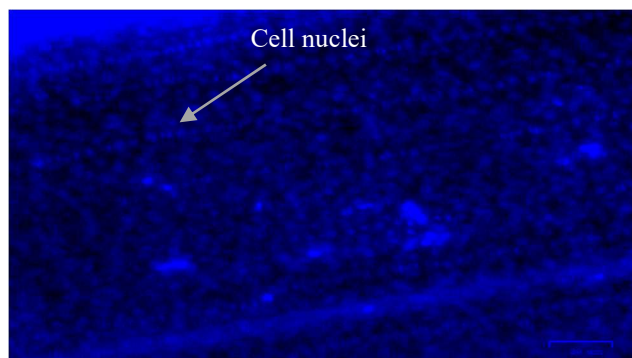


Figure 4.6: Visualization of cell nuclei stained with DAPI under ZOETTM Fluorescent Cell Imager.

4.4. Kinematic Analyses

The effect of tolerant (ADK310, R) and susceptible (ADK1188, S) maize lines in response to *Fusarium verticillioides* under *F. verticillioides*-infected (RI, SI) and non-*F. verticillioides*-inoculated control (RC, SC) conditions were studied by kinematic analysis approach. A significant decrease of 9.3% ($p>0.05$) in mature cell length (L_{ma}) was observed in the RI group in comparison with the RC group. There was a 9.1% decrease in the SI group in comparison with the SC group ($p>0.05$). Meristem cell length (L_{me}) showed a non-significant decrease of 12.4% in the RI group in comparison with the RC group ($p>0.05$) and a decrease of 17.5% in the SI group in comparison with the SC group ($p>0.05$). Cell production (P) showed an increase of 14.4% in the RI group in comparison with the RC group ($p>0.05$) and a decrease of 10.3% in the SI group in comparison with the SC group ($p>0.05$). Cell Division Rate (D) showed a 7.1% increase in the RI group in comparison with the RC group ($p>0.05$), while a 31.3% decrease was significant in the SI group in comparison with the SC group ($p<0.05$). Cell Cycle Duration (T_c) showed a non-significant decrease of 7.5% in the RI group in comparison with the RC group ($p>0.05$) and a significant increase of 42.4% in the SI group in comparison with the SC group ($p<0.05$). Meristem Zone Length (MZL) showed a decrease of 9.1% in the RI group in comparison with the RC group ($p>0.05$) and an increase of 4.8% in the SI group in comparison with the SC group ($p>0.05$). Growth Zone Length (GZL) was increased by 4.1% in the RI group in comparison with the RC group ($p>0.05$) and decreased by 0.4% in the SI group in comparison with the SC group ($p>0.05$). Elongation Zone Length (EZL), 7.3%

increase ($p>0.05$) in elongation zone length in the RI group in comparison with the RC group and 1.6% decrease ($p>0.05$) in the SI group in comparison with the SC group. The number of cells in the meristem (N_{me}) increased by 7.4% ($p>0.05$) in the RI group in comparison with the RC group. A 27.9% increase was found to be significant in the SI group in comparison with the SC group ($p<0.05$). The number of cells in the elongation zone (N_{el}) was increased by 0.6% in the RI group in comparison with the RC group ($p>0.05$) and significantly decreased by 7.5% in the SI group in comparison with the SC group ($p<0.05$). The number of cells in the growth zone (N_{ma}) was increased by 4.4% in the RI group in comparison with the RC group ($p>0.05$) and by 12.8% in the SI group in comparison with the SC group ($p>0.05$). Duration of cell expansion (T_d) showed a 12.8% decrease in the RI group in comparison with the RC group ($p<0.05$). In SI group, 3.1% increase in comparison with the SC ($p>0.05$).

Table 4.1: The values of growth parameters as a result of kinematic analysis of the 6th leaf of maize seedlings grown under *F.verticillioides* inoculated (I) and control (C) groups of tolerant ADK310 (R) and susceptible ADK1188 (S) lines.

Growth Parameters	Samples	% Difference
Mature Cell Length (L_{ma}) (μm)	RC 124,6 $\pm$ 3,8 a SC 121,4 $\pm$ 10,7 a RI 114,6 $\pm$ 1,0 a SI 110,4 $\pm$ 4,0 a	RI vs RC (-) 9,3 SI vs SC (-) 9,1
Meristem Cell Length (L_{me}) (μm)	RC 17,7 $\pm$ 2,2 b SC 16,0 $\pm$ 0,5 ab RI 15,5 $\pm$ 2,7 ab SI 13,2 $\pm$ 0,7 a	RI vs RC (-) 12,4 SI vs SC (-) 17,5
Cell Production (P) (cells/h)	RC 11,1 $\pm$ 2,2 a SC 13,6 $\pm$ 1,5 b RI 12,7 $\pm$ 2,0 ab SI 12,2 $\pm$ 1,3 ab	RI vs RC (+) 14,4 SI vs SC (-) 10,3
Cell Division Rate (D) (cells/cell h)	RC 0,014 $\pm$ 0,003 b SC 0,016 $\pm$ 0,002 b RI 0,015 $\pm$ 0,002 b SI 0,011 $\pm$ 0,001 a	RI vs RC (+) 7,1 SI vs SC (-) 31,3
Cell Cycle Duration (T_c) (h)	RC 51,9 $\pm$ 9,2 a SC 45,0 $\pm$ 5,0 a RI 48,0 $\pm$ 7,7 a SI 64,1 $\pm$ 7,5 b	RI vs RC (-) 7,5 SI vs SC (+) 42,4
Meristem Zone Length (MZL) (μm)	RC 14,7 $\pm$ 0,6 a SC 14 $\pm$ 1 a RI 13,3 $\pm$ 0,6 a SI 14,7 $\pm$ 0,6 a	RI vs RC (-) 9,1 SI vs SC (+) 4,8
Growth Zone Length (GZL) (μm)	RC 74,9 $\pm$ 1,1 a SC 72,7 $\pm$ 3,6 a RI 77,8 $\pm$ 4,5 a SI 72,4 $\pm$ 6,1 a	RI vs RC (+) 4,1 SI vs SC (-) 0,4
Elongation Zone Length (EZL) (μm)	RC 60,1 $\pm$ 0,5 a SC 58,7 $\pm$ 2,4 a RI 64,5 $\pm$ 2,4 a SI 57,7 $\pm$ 3,6 a	RI vs RC (+) 7,3 SI vs SC (-) 1,6
Number of Cells in the Meristem (N_{me})	RC 818,5 $\pm$ 71,7 a SC 875,1 $\pm$ 52,0 a RI 879,2 $\pm$ 84,3 a SI 1119,1 $\pm$ 59,6 b	RI vs RC (+) 7,4 SI vs SC (+) 27,9
Number of Cells in the Elongation (N_{el})	RC 643,6 $\pm$ 19,8 ab SC 653,0 $\pm$ 23,4 b RI 647,3 $\pm$ 29,6 b SI 604,2 $\pm$ 12,4 a	RI vs RC (+) 0,6 SI vs SC (-) 7,5
Number of Cells in the Growth Zone (N_{ma})	RC 1462,2 $\pm$ 106,3 a SC 1528,1 $\pm$ 92,1 ab RI 1526,5 $\pm$ 162,9 ab SI 1723,3 $\pm$ 91,2 b	RI vs RC (+) 4,4 SI vs SC (+) 12,8
Duration of Cell Expansion (T_d)	RC 59,6 $\pm$ 10,0 b SC 48,6 $\pm$ 5,1 a RI 52,0 $\pm$ 8,2 a SI 50,1 $\pm$ 5,6 a	RI vs RC (-) 12,8 SI vs SC (+) 3,1

I: *Fusarium verticillioides*-inoculated; C: Control; R: Tolerant (ADK310); S: Susceptible (ADK1188); +: Percent increase in I groups compared to C groups; -: Percent reduction in I groups compared to C groups. Different letters indicate significant differences as determined by one-way analysis of variance (ANOVA) followed by Duncan's test ($P < 0.05$). $n=3$, $\text{ave} \pm \text{sd}$.

4.5. Observing *F. verticillioides* in Maize

Among the cultivated maize plants, it was observed that 3 out of 10 plants in the SI group and 1 out of 10 plants in the RI group failed to survive due *F. verticillioides* infection. *F. verticillioides* was observed under the microscope. First and second leaves of *F. verticillioides*-inoculated and non-*F. verticillioides*-inoculated control ADK310 and ADK1188 maize lines were examined under DIC microscope. When inoculated maize leaves were compared with control maize leaves (Figure 4.9), hyphae and spores were observed to have colonized and settled on the leaves of inoculated maize plants (Figure 4.7 and Figure 4.8).

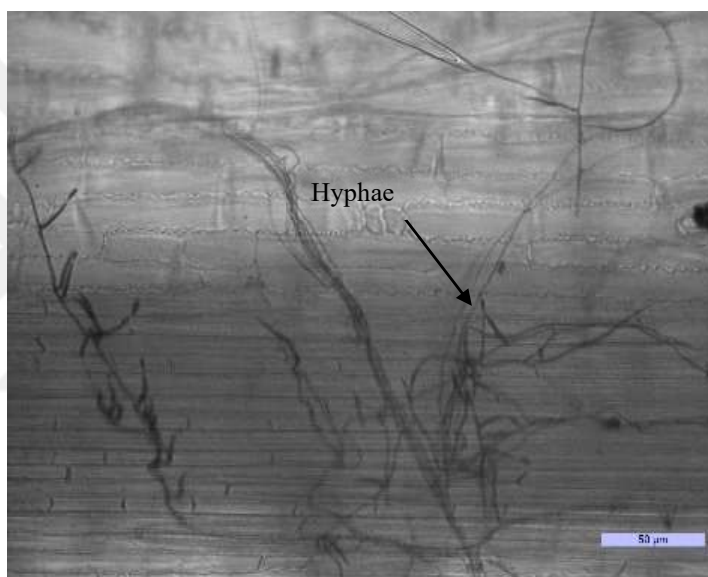


Figure 4.7: *F. verticillioides* hyphae on the first leaf of infected maize (*Z. mays*) under Differential interfering contrast (DIC) microscope at 40X.

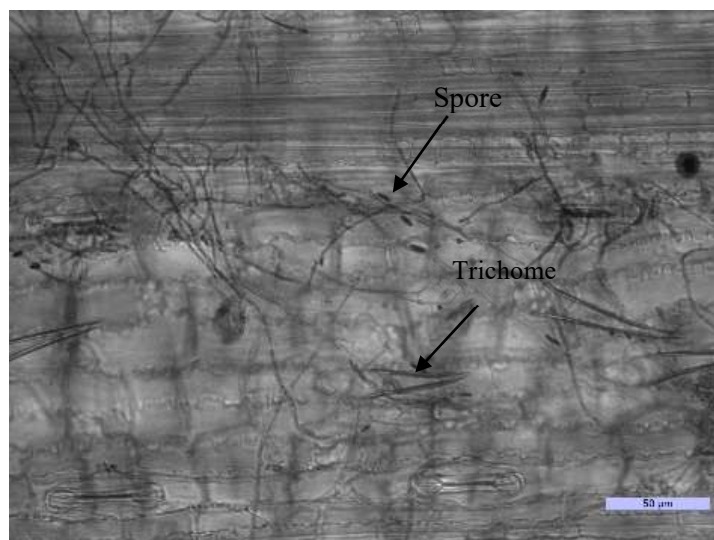


Figure 4.8: *F. verticillioides* spores and maize trichomes observed in the first leaf of infected maize (*Z. mays*) group under Differential interfering contrast (DIC) microscope at 40X.



Figure 4.9: The first leaf of the non-*F. verticillioides* infected control group under Differential interfering contrast (DIC) microscope at 40X.

4.6. *In silico* Prediction of ceRNA Networks

A phylogenetic tree of TEOSINTE BRANCHED/CYCLOIDEA/PCF (TCP) and Growth Regulating Factor (GRF) gene variants from Maize Genetics and Genomics Database and miR319 and miR396 miRNAs from the MicroRNA Sequence Database (miRBase) was constructed using Molecular Evolutionary Genetics Analysis (MEGA)

11 software. Based on target prediction using the RNAHybrid and psRNATarget tools, competing endogenous RNA networks from miRNAs, mRNAs and lncRNAs, lncRNAs from a previous study in our lab (non-published), were constructed using Cytoscape (v3.10.2).

According to the phylogenetic tree, the Growth Regulating Factor 15 (GRF15) gene is in the same clade as GRF17 and GRF14 and shares a common evolutionary history with these genes. The proximity of GRF15 and GRF17 reflects their genetic similarity with short branches. However, this branching is supported by low bootstrap values (31% and 37%), suggesting that the relationship between these genes is statistically less certain (Figure 4.10).

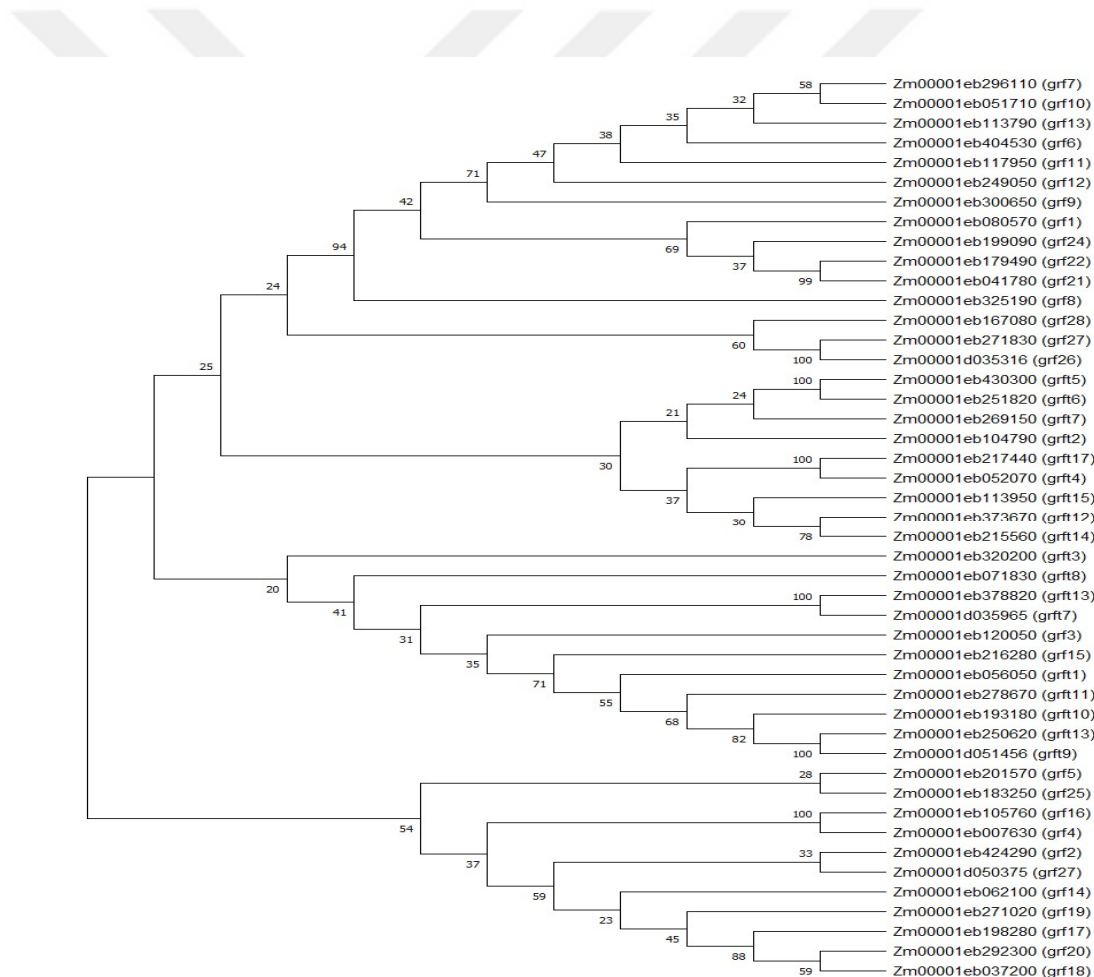


Figure 4.10: Phylogenetic tree of Growth-Regulating Factor (GRF) genes retrieved from Maize Genetics and Genomics Database (MaizeGDB).

According to the analysis of the phylogenetic tree, the TEOSINTE BRANCHED/CYCLOIDEA/PCF 38 (TCP38) gene shows evolutionary relationships

with other TCP gene family members. TCP38 is in the same group as TCP30 and these two genes are supported by a moderate bootstrap value (57%) (Figure 4.11).

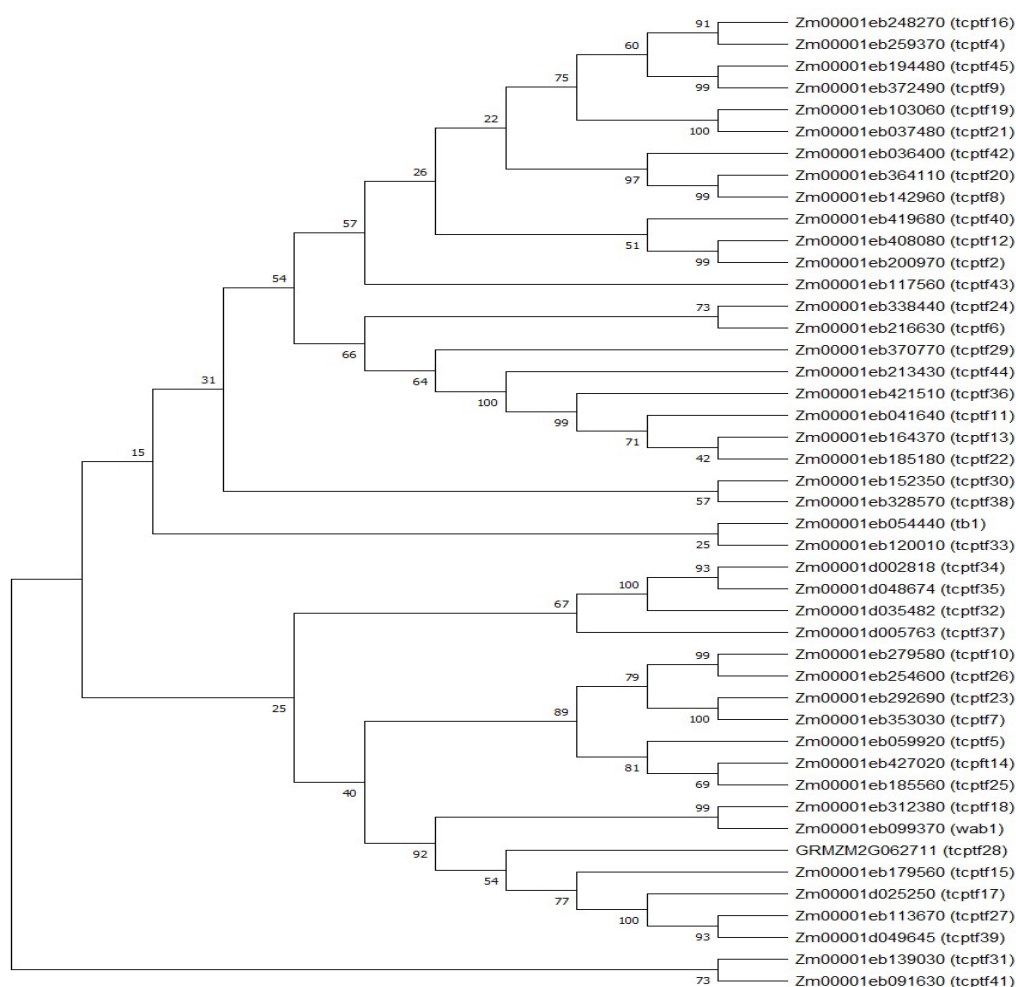


Figure 4.11: Phylogenetic tree of TCP genes retrieved from Maize Genetics and Genomics Database (MaizeGDB).

According to the analysis of the phylogenetic tree, miR319a-5p, miR319b-5p, miR319d-5p and miR319c-5p were supported by a very high bootstrap value (100%), forming a group on the same branch. This strong support suggests that miR319c-5p has high genetic similarity with these miRNAs. miR319b-5p is in the same group as miR319d-5p, and this association was supported by a bootstrap value of 77%. This indicates that these two miRNAs are evolutionarily close to each other. 3p and 5p members of the miR319 family were phylogenetically divided into two main groups. The 5p group (miR319a-5p, miR319b-5p, miR319c-5p and miR319d-5p) is more tightly grouped, while lower bootstrap values are observed among the 3p group (miR319c-3p, miR319d-3p, miR319b-3p and miR319a-3p). In particular, the

bootstrap value of miR319a-3p and miR319b-3p is 89%, indicating that these two members share a close relationship (Figure 4.12).

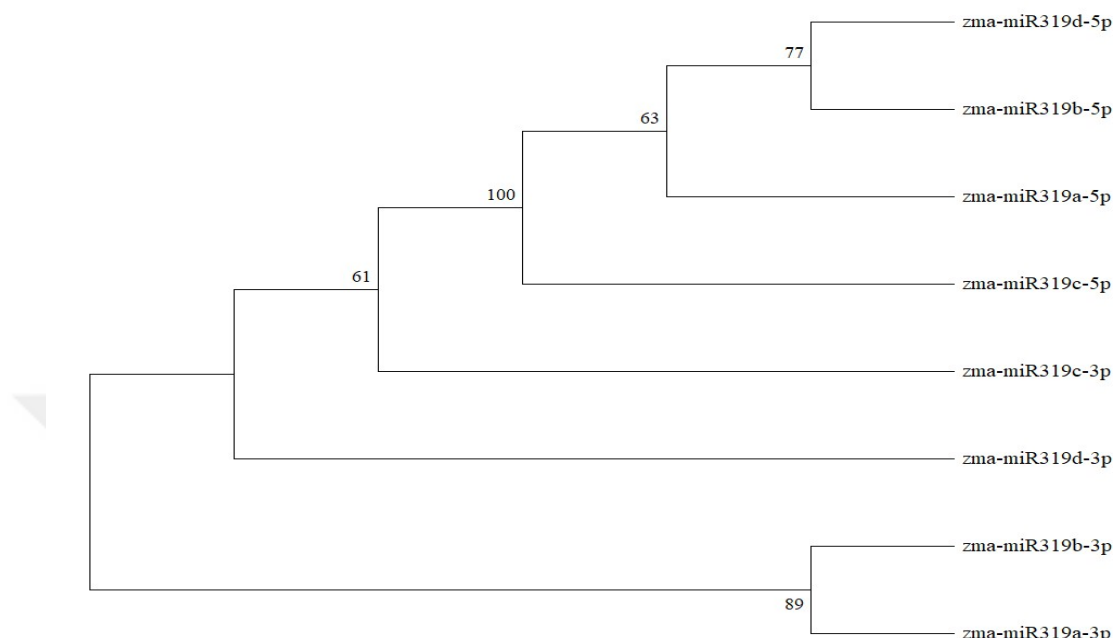


Figure 4.12: Phylogenetic tree of mature zm-miR319 retrieved from the MicroRNA Sequence Database (miRBase).

According to the analysis of the phylogenetic tree miR396e-3p and miR396f-3p are grouped in the same branch and their association is supported by a high bootstrap value (90%). miR396g-3p, miR396a-3p and miR396b-3p are in the same group and the bootstrap values of this branch range from 61% to 99%. miR396h is in a separate group from other members of the miR396 family and the bootstrap value of this branch is 73%. In addition, the -5p members of the miR396 family (miR396-5p group) are located close to each other and the bootstrap value of this branch varies between 46% and 59%. miR396d and miR396c are grouped in the same branch and the bootstrap value is 54%. (Figure 4.13).

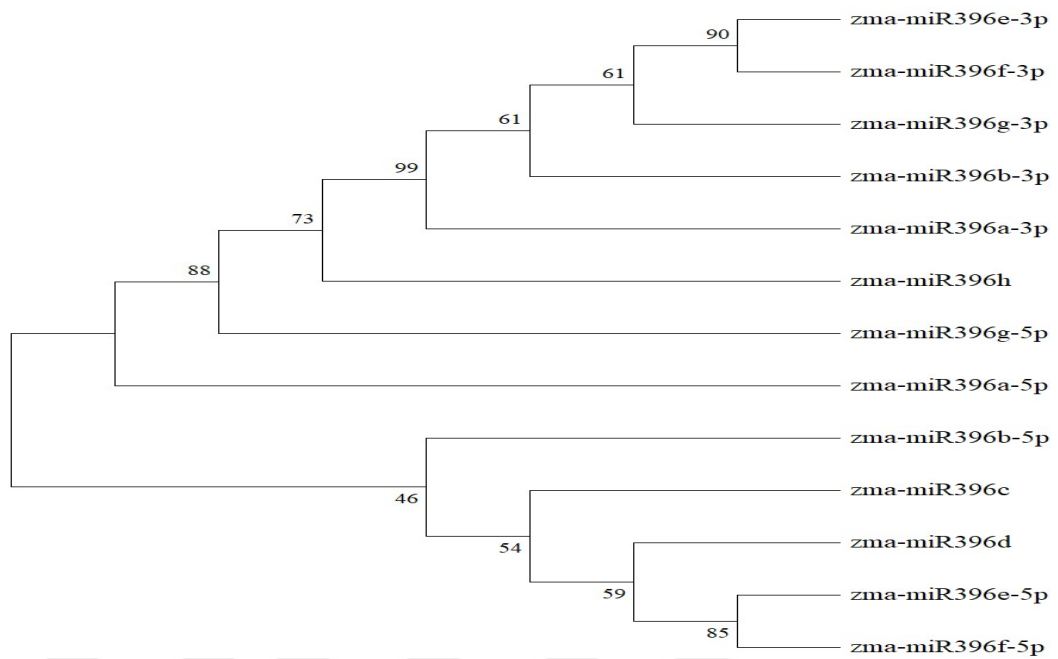


Figure 4.13: Phylogenetic tree of mature zm-miR396 retrieved from the MicroRNA Sequence Database (miRBase).

According to *in silico* analysis results, miR319 targeted 6 TCP genes and 6 lncRNAs, while miR396 targeted 12 GRF genes and 7 lncRNAs (Figure 4.14 and Figure 4.15).

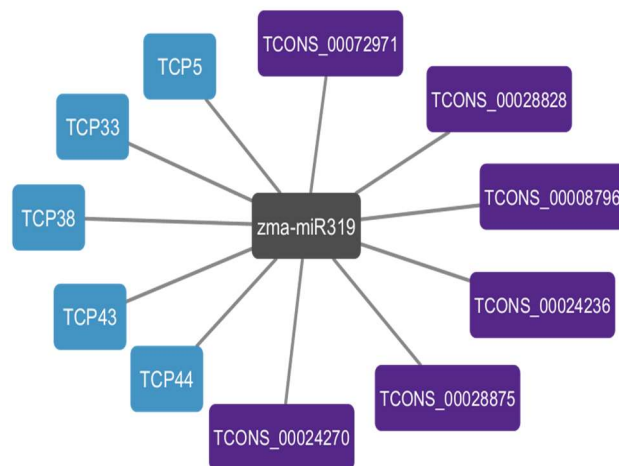


Figure 4.14: *In silico* prediction of putative leaf growth regulating *Zea mays* ceR319 network under fungal pathogen *Fusarium verticillioides* infection. Blue Boxes: mRNA; Purple boxes: lncRNA.

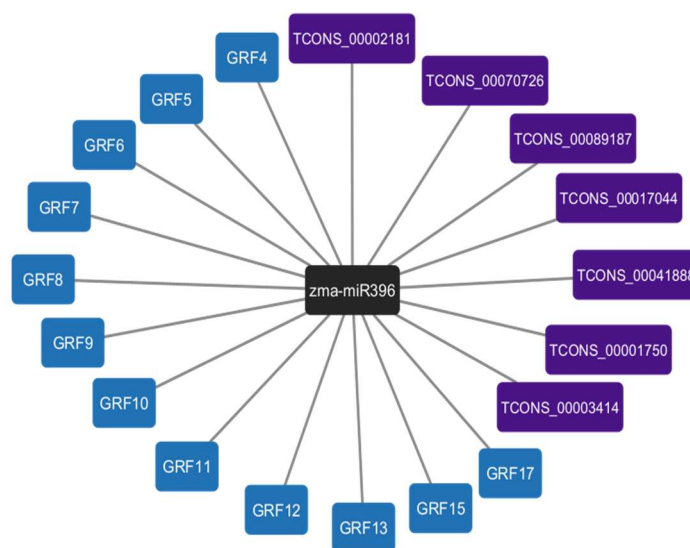


Figure 4.15: *In silico* prediction of putative leaf growth regulating *Zea mays* ceR396 network under fungal pathogen *Fusarium verticillioides* infection. Blue Boxes: mRNA; Purple boxes: lncRNA.

4.7. Antioxidant Enzymatic Activities in Maize Leaf Growth Zones in Response to *F. verticillioides* Infection

4.7.1. Catalase (CAT) Enzyme Activity

Catalase (CAT) enzyme activity of samples taken from different growth zones (meristem, elongation and mature zones) of the sixth leaf of control and *F. verticillioides*-inoculated ADK310 (R) and ADK1188 (S) maize seedlings is given in the graph (Figure 4.16). CAT enzyme activity increased by 280% in the elongation zone of the RI maize seedling compared to the RC maize seedling ($p>0.05$). In addition, CAT enzyme activity increased by 20% and 21% in the mature zone of the RI compared to the RC and in the elongation zone of the SI compared to the SC, respectively ($p>0.05$).

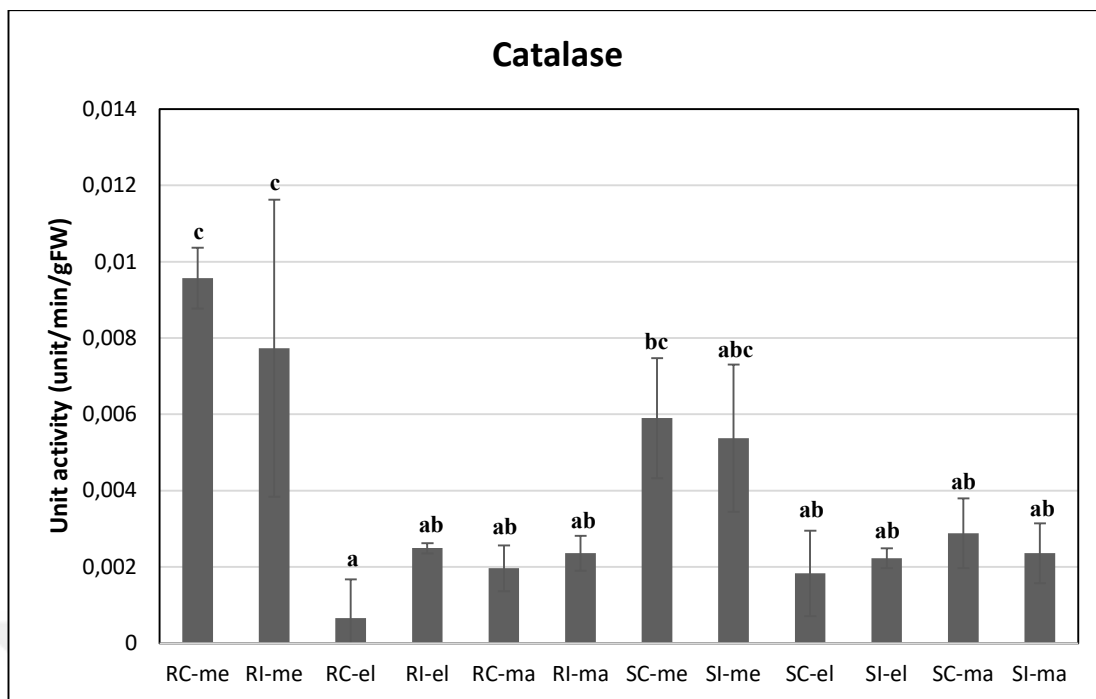


Figure 4.16: Catalase (CAT) enzyme activity in the growth zones of the 6th leaves of maize lines in response to *Fusarium verticillioides* infection. I: *F. verticillioides*-inoculated; C: Control; R: Tolerant line (ADK310); S: Susceptible line (ADK1188); me: Meristem zone; el: Elongation zone; ma: Mature zone. Different letters indicate significant differences as determined by one-way analysis of variance (ANOVA) followed by Duncan's test ($P < 0.05$). $n=3$, $\text{ave} \pm \text{sd}$

4.7.2. Peroxidase (POX) Enzyme Activity

Peroxidase (POX) enzyme activity of samples harvested from different growth zones (meristem, elongation and mature zones) of the sixth leaf of control and *F. verticillioides*-inoculated ADK310 (R) and ADK1188 (S) maize seedlings were given in the graph (Figure 4.17). In the mature zone of the R groups, POX enzyme activity decreased by 69% in the RI groups compared to the RC ($p > 0.05$). Also in the elongation zone of the S groups, POX enzyme activity decreased by 55% in the SI groups compared to the SC ($p < 0.05$).

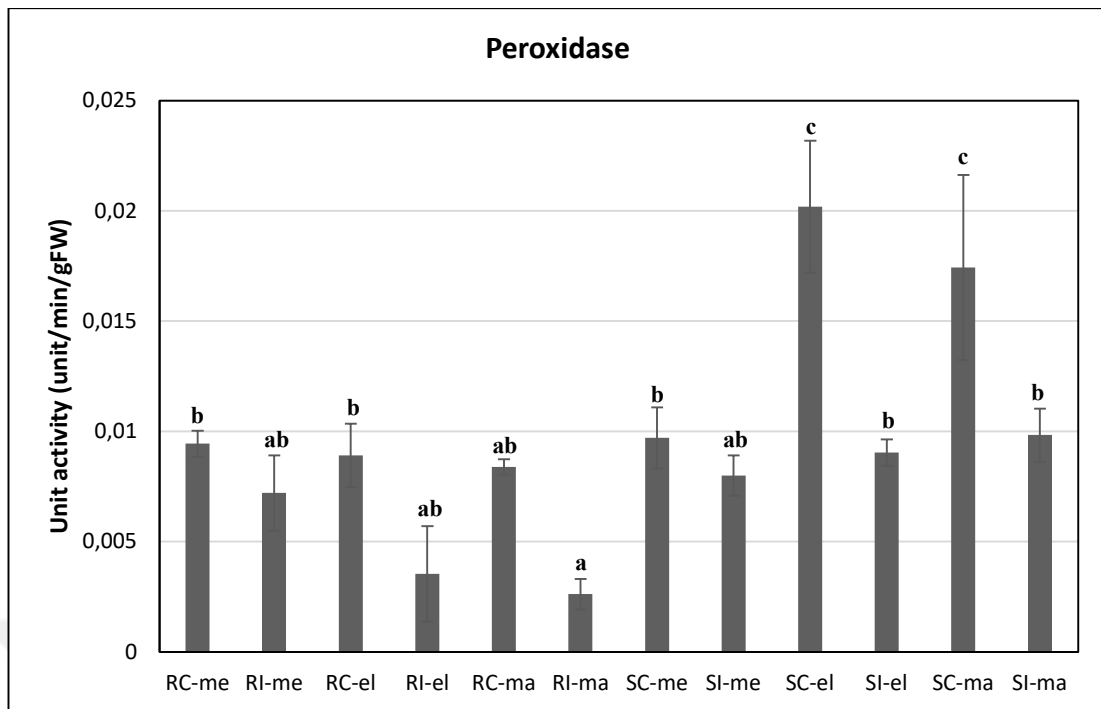


Figure 4.17: Peroxidase (POX) enzyme activity in the growth zones of the 6th leaves of maize lines in response to *F. verticillioides* infection. I: *F. verticillioides*-inoculated; C: Control; R: Tolerant line (ADK310); S: Susceptible line (ADK1188); me: Meristem zone; el: Elongation zone; ma: Mature zone. Different letters indicate significant differences as determined by one-way analysis of variance (ANOVA) followed by Duncan's test ($P < 0.05$). $n=3$, $\text{ave} \pm \text{sd}$

4.7.3. Ascorbate Peroxidase (APX) Enzyme Activity

The ascorbate peroxidase (APX) enzyme activity of samples harvested from different growth zones (meristem, elongation and mature zones) of the sixth leaf of control and *F. verticillioides*-inoculated ADK310 (R) and ADK1188 (S) maize seedlings were given in the graph (Figure 4.18). APX enzyme activity increased by 314%, 262% and 6700% in the growth zones of the RI groups compared to the RC groups, respectively, but a statistical difference was found in the meristem ($p < 0.05$). In addition, APX enzyme activity increased by 114% in the meristem zone of the SI groups compared to the SC group ($p < 0.05$).

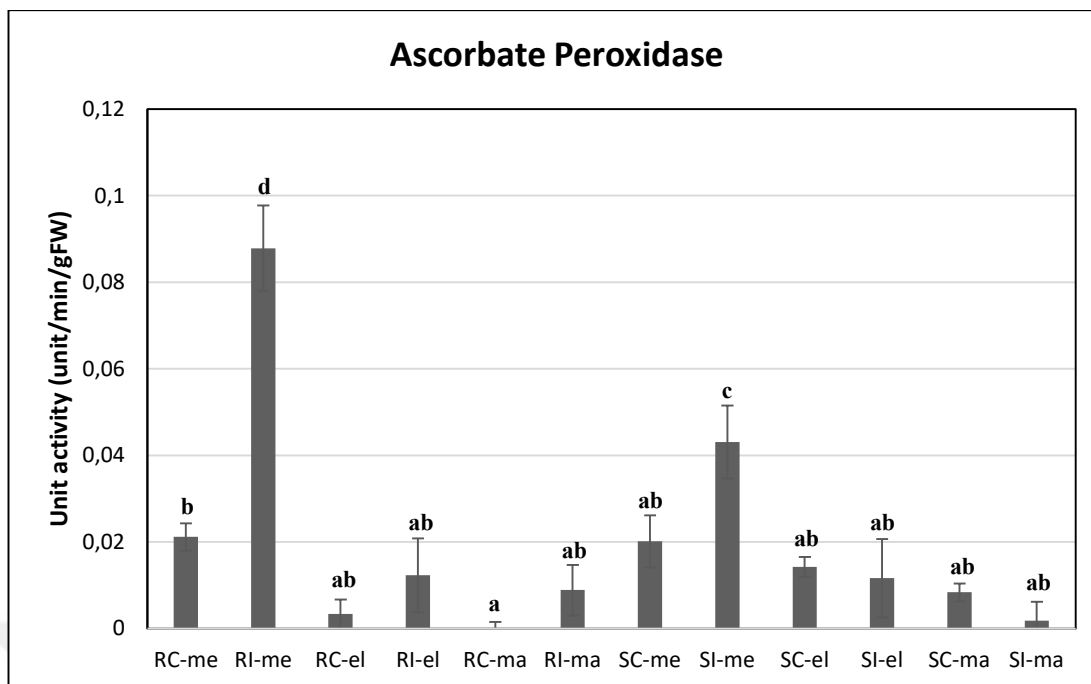


Figure 4.18: Ascorbate peroxidase (APX) enzyme activity in the growth zones of the 6th leaves of maize lines in response to *F. verticillioides* infection. I: *F. verticillioides*-inoculated; C: Control; R: Tolerant line (ADK310); S: Susceptible line (ADK1188); me: Meristem zone; el: Elongation zone; ma: Mature zone. Different letters indicate significant differences as determined by one-way analysis of variance (ANOVA) followed by Duncan's test ($P < 0.05$). $n=3$, $\text{ave} \pm \text{sd}$.

4.7.4. Superoxide Dismutase (SOD) Enzyme Activity

Superoxide dismutase (SOD) enzyme activity of samples harvested from different growth zones (meristem, elongation and mature zones) of the sixth leaf of control and *F. verticillioides*-inoculated ADK310 (R) and ADK1188 (S) maize seedlings were given in the graph (Figure 4.19). SOD enzyme activity increased by 49% in the elongation zone of the RI groups compared to the RC groups ($p > 0.05$). SOD enzyme activity decreased by 46% and 44% in the meristem and the mature zones of the RI groups compared to the RC groups, respectively ($p < 0.05$). SOD enzyme activity decreased by 60%, 34% and 39% in the growth zones of the SI groups compared to the SC groups, respectively ($p < 0.05$).

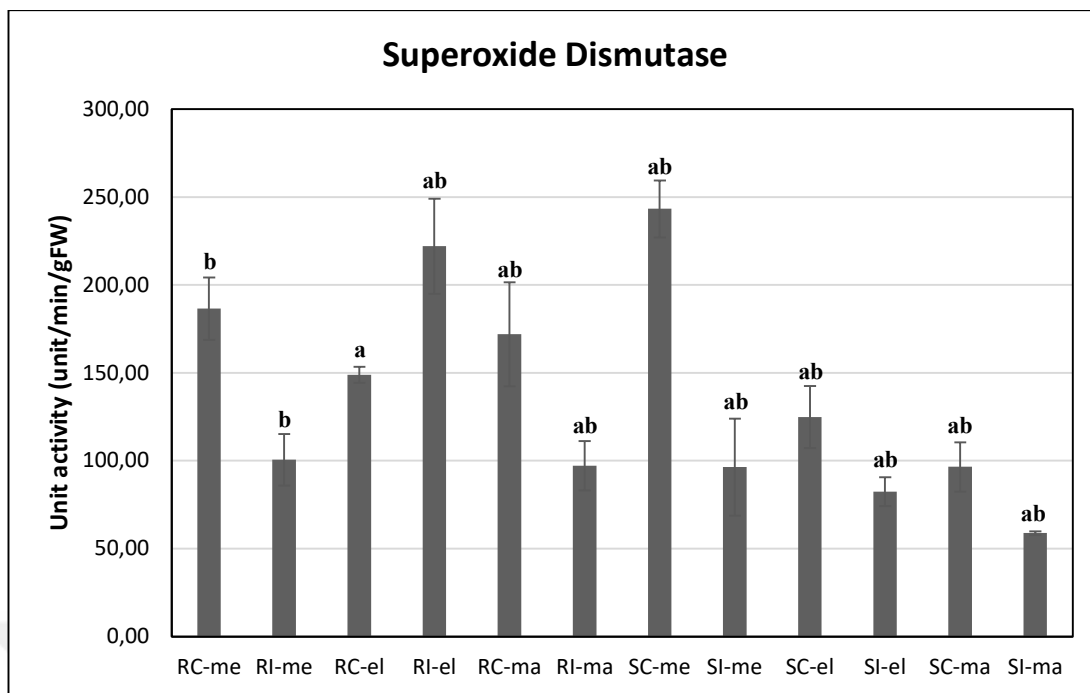


Figure 4.19: Superoxide dismutase (SOD) enzyme activity in the growth zones of the 6th leaves of maize lines in response to *F. verticillioides* infection. I: *F. verticillioides*-inoculated; C: Control; R: Tolerant line (ADK310); S: Susceptible line (ADK1188); me: Meristem zone; el: Elongation zone; ma: Mature zone. Different letters indicate significant differences as determined by one-way analysis of variance (ANOVA) followed by Duncan's test ($P < 0.05$). $n=3$, $\text{ave} \pm \text{sd}$

4.7.5. Glutathione Reductase (GR) Enzyme Activity

Glutathione reductase (GR) enzyme activity of samples harvested from different growth zones (meristem, elongation and mature zones) of the sixth leaf of control and *F. verticillioides*-inoculated ADK310 (R) and ADK1188 (S) maize seedlings were given in the graph (Figure 4.20). In the elongation and the mature zones of the R line, GR activity decreased in the RI group by 17% and 46% compared to the RC group ($p < 0.01$). GR activity increased by 4% and 15% in the elongation and the mature zones of the SI group compared to the SC group ($p > 0.05$), respectively, and decreased by 21% in the meristem zone ($p > 0.05$).

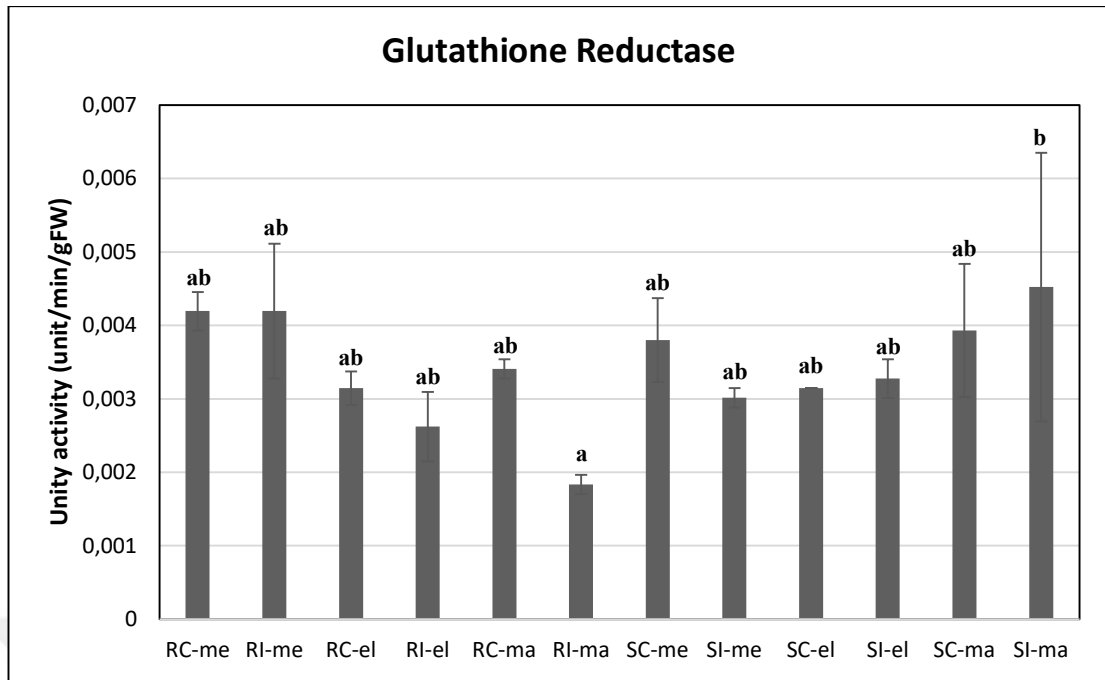


Figure 4.20: Glutathione reductase (GR) enzyme activity in growth zones of the 6th leaves of maize lines in response to *F. verticillioides* infection. I: *F. verticillioides*-inoculated; C: Control; R: Tolerant line (ADK310); S: Susceptible line (ADK1188); me: Meristem zone; el: Elongation zone; ma: Mature zone. Different letters indicate significant differences as determined by one-way analysis of variance (ANOVA) followed by Duncan's test ($P < 0.05$). $n=3$, $\text{ave} \pm \text{sd}$

4.8. Transcriptional Analyses of ceRNA Networks in Maize Leaf Growth Zones in Response to *F. verticillioides* Infection

4.8.1. Expression Analysis of ceR319 Network in Maize Leaf Growth Zones in Response to *F. verticillioides* Infection

miR319 was up-regulated in the meristem zone and down-regulated in the elongation zone of the ADK1188 non-*F. verticillioides* infected group (SC) compared to the meristem zone of SC group. The expression of miR319 was 11-fold and 7-fold higher in the elongation and mature zones of the ADK1188 *F. verticillioides* infected (SI) group than in the meristem zone, respectively ($p < 0.05$). It was up-regulated in both zones of the SI groups compared to the meristem zone of the SI group. When SI and SC groups were compared, miR319 expression increased by 2524% ($p < 0.05$) and 626% ($p < 0.05$) in the SI elongation and mature zones of the SI group in comparison with the SC group, respectively. (Figure 4.21).

When tolerant ADK310 maize lines (R) were analyzed, miR319 was up-regulated in the meristem zone and down-regulated in the elongation and the mature zones of both RI and RC groups compared to the meristem zone in the RI and RC groups (Figure 4.21).

TCP38 was down-regulated in the mature zone of SC group in comparison with the meristem zone in the SC group. In all growth zones of SI group, TCP38 was down-regulated. When SI and SC groups were compared, TCP38 expression increased by 778% and 1233% in the elongation and the mature zones of the SC group compared to the SI group, correspondingly ($p < 0.05$) (Figure 4.21).

In the elongation and the mature zones of the RC group, TCP38 was down-regulated in comparison with meristem zone. In the elongation zone of the RI group, TCP38 was downregulated compared to the meristem zone (Figure 4.21).

lnc319 was down-regulated in the elongation zone of the SC group and up-regulated in the mature zone of the SC group in comparison with meristem zone. lnc319 was up-regulated in the elongation and the mature zone of the SI group in comparison with meristem zones (Figure 4.21).

lnc319 was down-regulated in elongation zone of RC group in comparison with the meristem zone. lnc319 was up-regulated in the elongation and the mature zone of the RI group in comparison with meristem zones (Figure 4.21).

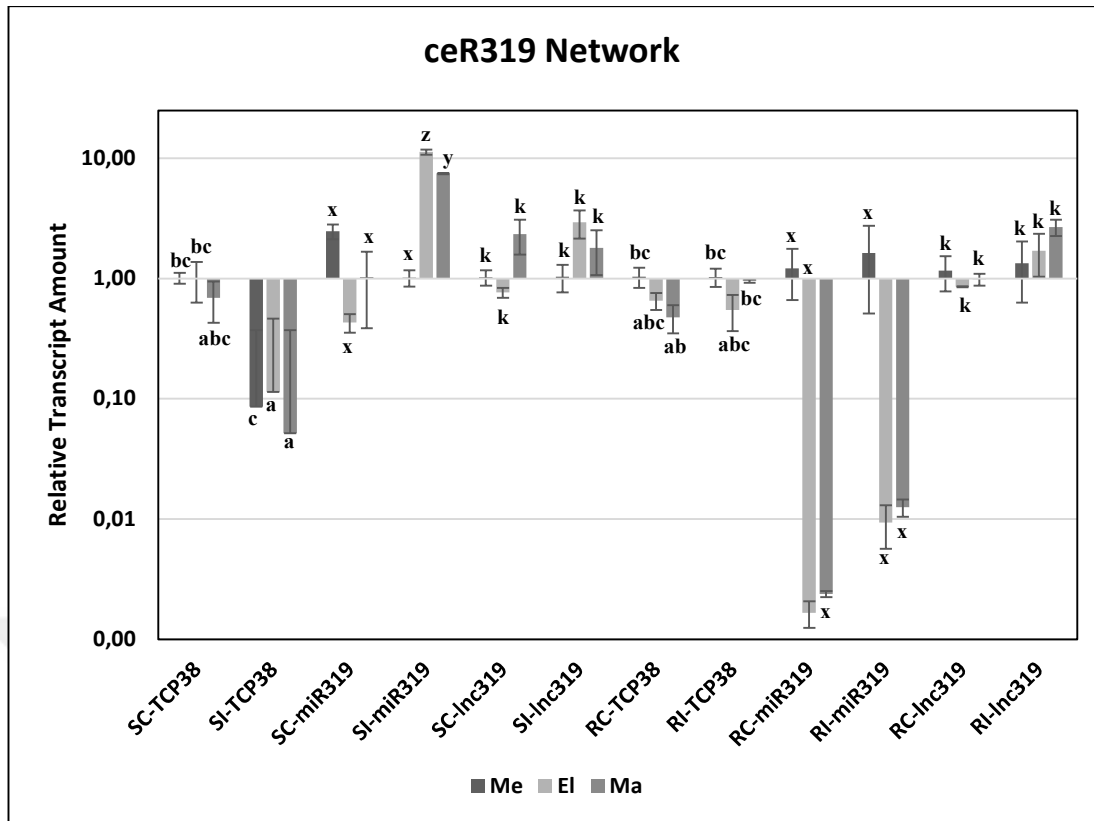


Figure 4.21: The expression analysis of ceR319 network in 6th leaf growth zone of maize seedlings in response to *Fusarium verticillioides*. I: Inoculated; C: Control; R: Tolerant line (ADK310); S: Susceptible line (ADK1188); me: Meristem zone; el: Elongation zone; ma: Mature zone. TCP: Teosinte branched cycloidea proliferating cell factor. miR: microRNA. lnc: long non-coding RNA. Different letters indicate significant differences as determined by one-way analysis of variance (ANOVA) followed by Duncan's test ($P < 0.05$). Three biological and two technical replicates were used for each expression. The error bars indicate the standard error and the Y-axis is logarithmic.

4.8.2. Expression Analysis of ceR396 Network in Maize Leaf growth zones in Response to *F. verticillioides* Infection

miR396 was expressed 52-fold more in the elongation zones of the SC group than in the meristem zone ($p < 0.05$). miR396 was up-regulated in the elongation and the mature zones of the SC group in comparison with the meristem zone. miR396 was expressed 24-fold more in the elongation zones of the SI group than in the meristem zone ($p < 0.05$). In the elongation and the mature zones of the SI group, miR396 were up-regulated in comparison with the meristem zone. When SI and SC groups were compared, expression of miR396 decreased by 132% in the elongation zones of SI group compared to the elongation zone of the SC group ($p < 0.05$) (Figure 4.22).

miR396 was up-regulated in the meristem zones of the RC group compared to the elongation and the mature zones. miR396 was down-regulated in the elongation and the mature zones of the RI group in comparison with meristem zone (Figure 4.22).

The expression of growth regulating factor 15 (GRF15) increased by 34-fold and 16-fold more in the meristem zone of the SC group than in the elongation and the mature zone respectively ($p<0.05$). GRF15 was down-regulated in the elongation and the mature zones of the SC group in comparison with meristem zone. GRF15 was expressed 26-fold and 24-fold more in meristem zone of the SI group than in the elongation and the mature zone respectively ($p<0.05$). GRF15 was down-regulated in the elongation and the mature zones of the SI group in comparison with meristem zone (Figure 4.22).

GRF15 was expressed 72-fold and 26-fold more in the meristem zone of the RC group than in the elongation and the mature zones, correspondingly ($p<0.05$). GRF15 was down-regulated in the elongation and the mature zones of the RC group in comparison with the meristem zone. GRF15 was expressed 55-fold and 26-fold more in the meristem zone of the RI group than in the elongation and the mature zones, correspondingly ($p<0.05$). GRF15 was down-regulated in the elongation and the mature zones of the RI group in comparison with the meristem zone (Figure 4.22).

lnc89 was downregulated in the elongation and the mature zones of the SC group comparison with the meristem. While lnc89 was down-regulated in the elongation zone of the SI group in comparison with the meristem zone, lnc89 was up-regulated in the mature zone of SI group compared to the meristem zone (Figure 4.22).

lnc89 was downregulated in the elongation and the mature zones of the RC group in comparison with the meristem zone. While lnc89 was downregulated in the elongation zone of RI group in comparison with meristem zone, it was upregulated in the mature zone of RI group compared to the meristem zone. The expression of lnc89 increased by 3-fold and 2-fold in the mature zone of the RI group than in the elongation and the meristem zones of the RI group correspondingly ($p<0.05$). When RI and RC groups were compared, expression of lnc89 in the mature zones of the RI group increased by 343% compared to the mature zone of the RC group ($p<0.05$) (Figure 4.22).

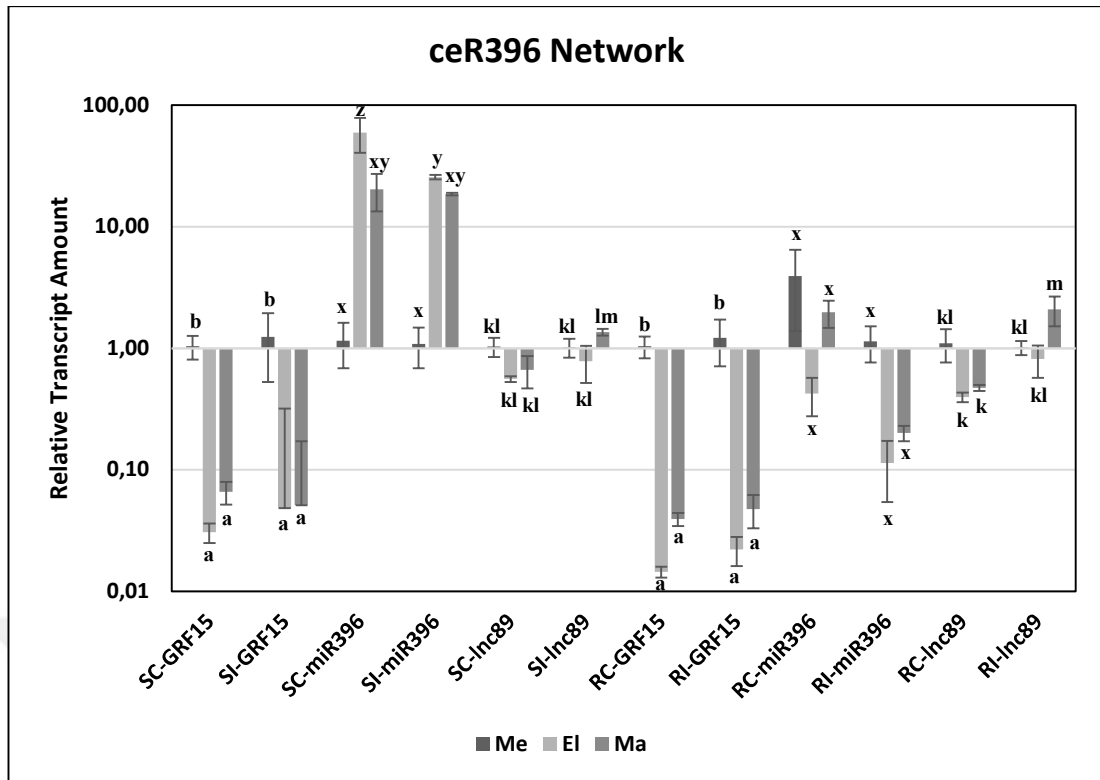


Figure 4.22: The expression analysis of ceR396 in 6th leaf growth zone of maize seedlings in response to *F. verticillioides*. I: Inoculated; C: Control; R: Tolerant line (ADK310); S: Susceptible line (ADK1188); me: Meristem zone; el: Elongation zone; ma: Mature zone. GRF: Growth regulating factor 15. miR: microRNA. lnc: long non-coding RNA. Different letters indicate significant differences as determined by one-way analysis of variance (ANOVA) followed by Duncan's test ($P < 0.05$). Three biological and two technical replicates were used for each expression. The error bars indicate the standard error and the Y-axis is logarithmic.

5. CONCLUSION and DISCUSSION

In this study, the interaction between the fungal pathogen *Fusarium verticillioides* and maize (*Zea mays*) plant was analyzed, focusing on leaf growth response by means of regulation ceRNA network of the maize plant. For this aim, the maize seedlings treated to soil inoculation method and microscopic examinations revealed the presence of *F. verticillioides* spores and hyphae on the first leaves. Consistent with findings by Bacon et al. (2008), the fungus was observed to enter the plant through the epidermis of the root or through crevices formed by lateral roots, utilizing spores present in the soil. Following entry, *F. verticillioides* can spread throughout the plant, colonizing roots, stems, leaves, and grains. The infection process involves both endophytic (symptomless) and pathogenic phases, highlighting the fungus's dual nature. Hyphae were observed to move in parallel within intercellular spaces between plant tissues. Under stress conditions, such as drought or wounding, the hyphae exhibit increased metabolic activity, leading to the production of mycotoxins like fumonisin. Spores were primarily observed on leaf surfaces and at the terminal ends of hyphae in wounded areas. Additionally, wounded maize grains showed intense hyphae growth and spore clusters. These findings are significant for understanding the complex interactions between *F. verticillioides* and maize. They provide insights into the life cycle of the fungus, which alternates between pathogenic and endophytic states depending on environmental and host factors. This knowledge is critical for developing effective management strategies to mitigate *F. verticillioides* infections and associated mycotoxin production in maize.

Kinematic analysis and some morphological parameters such as final leaf length (FLL), leaf elongation rate (LER), and cell length (CL) were used to study the effects of *F. verticillioides* on growth parameters at the morphological and cellular level in maize sixth leaf growth. The inoculation of *F. verticillioides* resulted in a notable reduction in the final leaf length (FLL) in both tolerant ADK310 (R) and susceptible ADK1188 (S) groups. These reductions were pronounced in ADK1188 *F. verticillioides*-infected (SI) group by 16% compared to non-*F. verticillioides* infected (SC) control group ($p < 0.05$), and were pronounced in ADK310 *F. verticillioides*-infected (RI) group by 26% reduction compared to non-*F. verticillioides* infected (RC)

control group ($p < 0.05$). As reported by Sprangers and colleagues (2016), maize leaf growth is dependent on cell division and expansion, with biotic stress caused by pathogens inhibiting these processes and reducing leaf length. This finding corroborates the hypothesis that the decrease in final leaf length is the result of changes occurring at the cellular level. Additionally, Rymen and colleagues (2010) demonstrated that pathogenic stress suppresses growth zones, consequently limiting leaf elongation.

The cell length (CL6) profile in maize sixth leaves under *F. verticillioides* infection showed significant zone-specific variations. In the meristem zone, cell length decreased by 15% in the RI ($p > 0.05$) compared to the RC and decreased by 22% in the SI ($p < 0.01$) compared to the SC. In the elongation zone, cell length increased by 6% in the RI group compared to the RC group ($p > 0.05$) and increased by 4% in the SI group ($p > 0.05$) compared to the SC group. In the mature zone, cell length decreased by 5% in the RI group ($p < 0.01$) compared to the RC group and decreased by 7% in the SI group ($p > 0.05$) compared to the SC group. These results highlight that Fusarium infection affects cell length differently across zones.

The leaf elongation rate of the sixth leaf (LER6) of maize lines was found to be significantly reduced in the SI group ($p < 0.05$), but not in R groups ($p > 0.05$). A difference of 8% was observed in R groups, compared to S groups that displayed a 19% difference in LER6. Inoculation resulted in statistically significant differences in mature cell length (MCL) in R groups ($p < 0.05$) while the differences were not significant in S groups ($p > 0.05$). Mature cell length was decreased by 9% in both RI and SI groups compared to the RC and SC groups. Inoculation with *F. verticillioides* resulted in a significant reduction by 31% in cell division rate in the SI groups compared to the SC group ($p < 0.05$). Meristem cell length decreased by 18% and 12% in the SI and RI groups compared to the SC and RC groups, respectively but the reductions were not significant ($p > 0.05$). Number of cells in the elongation decreased by 8% the SI group compared to the SC group, this reduction was statistically significant ($p < 0.05$). Duration of cell expansion decreased by 13% in the RI group compared to the RC group ($p < 0.05$). Nelissen et al. (2013) reported that leaf elongation in maize and other plant species was directly linked to cell division rates and expansion. They also demonstrated that these processes are suppressed under biotic stress. Aydinoglu (2020) demonstrated that environmental stress conditions,

such as cold stress, influence leaf growth in plants, and that these stresses impede cell expansion and division. It can be hypothesised that the inoculation of *Fusarium* may have produced a similar suppression, which is indicative of the general negative effect of pathogenic stress on plants. The results indicate that *F. verticillioides* impacts the cellular expansion processes in maize leaves. This reduction in cell expansion is directly correlated with the deceleration of leaf elongation.

A 280% increase in catalase activity was observed in the elongation zone of the sixth leaf of tolerant (R) maize seedlings inoculated with *F. verticillioides* (RI) compared to non-infected control group (RC). However, this difference was not statistically significant ($p>0.05$), indicating that further investigation may be required to ascertain whether this observed increase is indeed statistically significant. Furthermore, the activity in the mature zone increased by 20% in the RI group compared to the RC group, while the activity in the elongation zone increased by 21% in the SI group compared to the SC group. However, these differences were not statistically significant ($p>0.05$). Cacique et al. (2020) have previously reported that defence responses induced against *F. verticillioides* may result in an increase in the activity of ROS scavenging enzymes. As demonstrated by Lanubile et al. (2014), the presence of *F. verticillioides* has been observed to elevate the activity of antioxidant enzymes, thereby triggering oxidative stress responses in maize. These findings are consistent with the increases observed in this study and may represent a biochemical defence mechanism against *F. verticillioides*.

The peroxidase (POX) activity observed in the control groups was found to be higher than that of the inoculated groups. In the mature zone of the RI group, the activity demonstrated a 69% decrease compared to the RC group. In the SI group, the activity in the elongation and mature zones exhibited a statistically significant decrease of 55% compared to the SC group. In a study by Lanubile et al. (2012), differences in POX activity against *Fusarium* were associated with effective defence mechanisms against pathogen attack. The results of this thesis demonstrate that *F. verticillioides* is associated with a reduction in POX activity, in plants that are susceptible and tolerant to infection. Such decreases may be linked to the inadequacy of antioxidant enzymes in combating pathogen attacks.

In plants exhibiting RI group, APX activity increased by 314% in the meristematic zone, 262% in the elongation zone, and 6700% in the mature zone compared to the RC

group. However, only the increase observed in the meristematic zone reached a statistically significant level ($p < 0.05$). In the SI group, an increase of 114% was observed in the meristem zone compared to the SC group, which was found to be statistically significant ($p < 0.05$). APX enzyme activity in the RI group exhibited a 104% increase in comparison with the SI group. This difference may be due to the genotype of the tolerant group, which demonstrated a higher degree of tolerance to *F. verticillioides*, thus resulting in a more effective response in comparison with the susceptible group. In their 2014 study, Lanubile and colleagues (2014) highlighted the antioxidant function of APX, emphasising its crucial role in defending against *F. verticillioides* infection. The increased APX activity observed may represent a crucial factor in the reduction of oxidative stress, a finding that aligns with the conclusions of Lanubile et al. (2014).

In the elongation zone of the RI group, superoxide dismutase (SOD) activity increased by 49% compared to the RC group, although this difference was not statistically significant ($p > 0.05$). In the meristem and the mature zones of the RI group, the activity was observed to decrease by 46% and 44% compared to the RC group, respectively ($p > 0.05$). In S groups, superoxide dismutase (SOD) activity exhibited a decline, reaching 60%, 34%, and 39% reductions in the meristem, elongation, and mature zones of the SI group compared to the SC group, respectively ($p > 0.05$). Cacique et al. (2020) reported a reduction in SOD activity during *Fusarium* infection, which was associated with an ineffective management of ROS accumulation in plants. This is consistent with the findings of the present study. The decrease in SOD activity, particularly in susceptible plants, may indicate an insufficient scavenging of ROS.

A 46% reduction in GR activity was observed in the mature zone of the RI group compared to the RC group ($p > 0.05$). In S groups, there was a 4% and 15% increase in activity in the elongation and mature zones of the SI group compared to the SC group, respectively, while a 21% decrease was observed in the meristem zone of the SI group. However, these differences were not statistically significant ($p > 0.05$). GR plays a pivotal role in reducing oxidative stress. Lanubile et al. (2012) have reported that GR is activated at different levels in the defense response to *Fusarium* infection. This finding may prove significant in elucidating the manner by which alterations in GR activity in the present study influence plant responses to *Fusarium*.

These findings indicate that the plant defense mechanisms against *F. verticillioides* are highly complex. In particular, the variations in antioxidant enzyme activities observed in different growth zones provide insight into how plants respond to Fusarium attacks. It is hypothesised that the activity of antioxidant enzymes will increase under biotic stress conditions. In a study by Avramova et al. (2015), it was observed that hydrogen peroxide increased in the meristem zone, decreased in the elongation zone and increased in the mature zone. The different levels of enzyme activities in these growth zones are related to hydrogen peroxide. Furthermore, Zeng et al. (2024) highlight that genetic differences have a significant influence on these responses. In particular, the significant enzyme activities observed in meristem and elongation zones may assist in the identification of crucial biochemical pathways that could be targeted for the management of plant diseases.

This study aimed to examine the expression profiles of two ceRNA networks, ceR396 consisting of miR396-GRF15-lnc89 and ceR319 consisting of miR319-TCP38-lnc319, in three different growth zones (meristem, elongation, and mature) of the sixth leaf of maize seedlings under two conditions: control and *F. verticillioides*-infected. The role of these networks on leaf growth was also evaluated. The study examined two maize lines in two growth conditions representing four groups which are called ADK1188 susceptible control (SC), ADK1188 susceptible infected (SI), ADK310 tolerant control (RC) and ADK310 tolerant infected (RI).

The expression of miR319 was found to be elevated 11-fold and 7-fold in the elongation and mature zones of infected plants that exhibited susceptibility, in comparison to the control group, respectively. These increases were observed to be statistically significant ($p < 0.05$). It was up-regulated in both zones compared to the meristem. In plants exhibiting tolerance, miR319 was downregulated in the elongation and mature zones compared to the meristem. miR319 demonstrated elevated expression 174-fold and 130-fold in the meristem zone of infected group compared to the elongation and mature zones of infected zone, respectively. However, no notable differences were discerned between them ($p > 0.05$). It was up-regulated in the meristem zone and down-regulated in the elongation and mature zones compared to the meristem zone. Its target TCP38 was expressed 1.3-fold more in the elongation zone of the SI group than in the meristem ($p < 0.05$). lnc319 was expressed 2-fold and

1-fold more in the elongation zone of the SI group compared to the meristem and mature zones.

The elevated expression of miR319 in the elongation and mature zones suggests that leaf growth processes are regulated by miR319. Additionally, Fang et al. (2021) have indicated that miR319 exerts control over leaf growth by targeting TCP transcription factors. Moreover, Zhao et al. (2015) discovered that the tomato miR319-TCP4 pathway has a significant influence on the plant's ability to resist root-knot nematodes. This is achieved by modulating the expression of genes that are involved in the synthesis of jasmonic acid (JA), a key plant hormone associated with defense responses. Additionally, miR319-TCP4 regulates the levels of endogenous JA in the leaves, thereby contributing to the plant's enhanced resistance to nematode infection by adjusting both the biochemical pathways and the hormonal balance within the plant. TCP genes are more expressed in elongation and mature zones while miR319 is expected to be more expressed in the meristem [Aydinoglu and Lucas, 2019]. In the SI group, TCP38 was down-regulated in growth zones and miR319 was up-regulated in the elongation and mature zones. lnc319 was up-regulated in the elongation and mature zones. In the RI group, TCP38 was down-regulated in the elongation zone, while miR319 was up-regulated in the meristem and down-regulated in the elongation and mature zones. The fact that lnc319 was up-regulated in all growth zones in the RI group may explain the down-regulation of miR319 in the elongation and mature zones. lncRNAs can act as decoys for miRNAs. These expression differences between RI and SI may have caused the decrease in LER in the S group.

miR396 was expressed 24-fold and 17-fold more in the elongation and mature zones of SI group than in the meristem zone, respectively. There was a statistically significant difference in the elongation zone compared to the meristem zone ($p < 0.05$). Elongation and mature zones were up-regulated compared to the meristem zone. GRF15 and lnc89 demonstrated expression alterations across a range of growth zones, with notable upregulation observed in certain zones of tolerant plants following infection. The elevated expression of miR396 in the elongation and mature zones indicates that this microRNA plays a pivotal role in regulating leaf growth processes. As reported by Chandran et al. (2019), miR396 controls leaf growth by interacting with GRF transcription factors. During the process of leaf growth, miR396 plays a role in maintaining equilibrium between cell proliferation and elongation, thereby

influencing overall plant morphology. The elevated expression of miR396 in the elongation zone lends support to the hypothesis that this growth zone is regulated by miR396.

Growth Regulating Factor 15 (GRF15) was expressed 26-fold and 24-fold more in the meristem zone of the SI group than in elongation and mature zone respectively, and the difference between them was statistically significant ($p < 0.05$). The elongation and mature zones were downregulated compared to the meristem zone. GRF15 has been demonstrated to function in conjunction with miR396 in the meristem and elongation zones, thereby exerting a pivotal influence on leaf growth. GRF transcription factors occupy a pivotal role in cell division and growth in plants [Aydinoglu and Lucas, 2019].

lnc89 was expressed 3-fold and 2-fold more in the mature zone of the RI group than in the elongation and meristem zones, respectively, and there was no statistically significant difference between these zones. While elongation was downregulated compared to the meristem, the mature zone was upregulated ($p < 0.05$). lnc89 demonstrated differential expression in mature zone of the RI group. This study indicates that lnc89 plays a regulatory role in the mature zone, thereby supporting that it is part of the genetic network that supports leaf growth in plants. The down-regulation of GRF15 in the elongation and mature zones of both the R and S groups, alongside the up-regulation of miR396 in the elongation and mature zones of the S group, aligns with the findings of Aydinoglu and Lucas (2019). In the RI group, miR396 was down-regulated in the elongation and mature zones, while it exhibited up-regulation in the meristematic zone. This pattern may be attributed to the elevated expression of lnc89, another target of miR396, in the elongation and mature zones of the RI group compared to the RC group. These observed expression differences between the R and S groups could provide an explanation for the reductions in growth parameters, such as cell cycle duration, meristematic zone length, and the number of cells in the meristem, observed in the R group.

In this thesis, the ceRNA regulatory networks among miRNA-lncRNA-mRNA, antioxidant enzyme activities, and morphological effects in the growth zones of the sixth leaf of *Z. mays* under biotic stress caused by *F. verticillioides* were investigated. The findings revealed differences in the expression of miRNAs, lncRNAs, and mRNAs involved in the ceR319 and ceR396 networks, as well as variations in enzyme

activities and certain growth parameters in response to *F. verticillioides*. This study constituted the first observation of the effect of lncRNAs on maize leaf growth zones during biotic stress. Further studies could focus on generating mutants by overexpressing or silencing the identified mRNAs, miRNAs, or lncRNAs to better understand their roles in ceRNA networks. These efforts could contribute to the development of maize lines tolerant to this pathogen. Additionally, it was demonstrated that the activities of CAT, POX, APX, SOD and GR enzymes vary across the developmental stages of maize leaves during biotic stress. These enzymatic analyses were conducted using enzyme extracts obtained after total protein isolation. For future studies, protein-specific investigations could provide more detailed insights.



REFERENCES

- Alberts J. F., Van Zyl W. H., Gelderblom W. C., (2016). Biologically based methods for control of fumonisin-producing *Fusarium* species and reduction of the fumonisins. *Frontiers in microbiology*, 7, 548.
- Apel K., Hirt H., (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual review of plant biology*, 55(1), 373-399.
- Asada K., (1999). The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. *Annual review of plant biology*, 50(1), 601-639.
- Axtell M. J., Bowman J. L., (2008). Evolution of plant microRNAs and their targets. *Trends in plant science*, 13(7), 343-349.
- Aydinoglu F., (2020). Elucidating the regulatory roles of microRNAs in maize (*Zea mays* L.) leaf growth response to chilling stress. *Planta*, 251(2), 38.
- Aydinoglu F., Lucas S. J., (2019). Identification and expression profiles of putative leaf growth related microRNAs in maize (*Zea mays* L.) hybrid ADA313. *Gene*, 690, 57-67.
- Avramova V., Sprangers K., Beemster G. T., (2015). The maize leaf: another perspective on growth regulation. *Trends in Plant Science*, 20(12), 787-797.
- Bacon C. W., Glenn A. E., Yates I. E., (2008). *Fusarium verticillioides*: managing the endophytic association with maize for reduced fumonisins accumulation. *Toxin Reviews*, 27(3-4), 411-446.
- Battilani P., Pietri A., Barbano C., Scandolara A., Bertuzzi T., Marocco A., (2008). Logistic regression modeling of cropping systems to predict fumonisin contamination in maize. *Journal of Agricultural and Food Chemistry*, 56(21), 10433-10438.
- Boedijn K. B., (1956). Trypan blue as a stain for fungi. *Stain technology*, 31(3), 115-116.
- Bottalico A., (1998). *Fusarium* diseases of cereals: species complex and related mycotoxin profiles, in Europe. *Journal of plant Pathology*, 85-103.
- Cacique I. S., Pinto L. F., Aucique-Pérez C. E., Wordell Filho J. A., Rodrigues F. A., (2020). Physiological and biochemical insights into the basal level of resistance of two maize hybrids in response to *Fusarium verticillioides* infection. *Plant physiology and biochemistry*, 152, 194-210.
- Campos M. D., Patanita M., Varanda C., Materatski P., Felix M. D. R., (2021). Plant-pathogen interaction. *Biology*, 10(5), 444.

Chandran V., Wang H., Gao F., Cao X. L., Chen Y. P., Li G. B., Wang W. M., (2019). miR396-OsGRF s module balances growth and rice blast disease-resistance. *Frontiers in Plant Science*, 9, 1999.

Chen X., (2009). Small RNAs and their roles in plant development. *Annual Review of Cell and Developmental*, 25(1), 21-44.

Chulze S. N., (2010). Strategies to reduce mycotoxin levels in maize during storage: a review. *Food additives and contaminants*, 27(5), 651-657.

Correll J. C., Klittich C. J. R., Leslie J. F., (1987). Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. *Phytopathology*, 77(12), 1640-1646.

Dewitte W., Murray J. A., (2003). The plant cell cycle. *Annual review of plant biology*, 54(1), 235-264.

Dodds P. N., Rathjen J. P., (2010). Plant immunity: towards an integrated view of plant–pathogen interactions. *Nature Reviews Genetics*, 11(8), 539-548.

Dumanović J., Nepovimova E., Natić M., Kuča K., Jačević V., (2021). The significance of reactive oxygen species and antioxidant defense system in plants: A concise overview. *Frontiers in plant science*, 11, 552969.

Fang Y., Zheng Y., Lu W., Li J., Duan Y., Zhang S., Wang Y., (2021). Roles of miR319-regulated TCPs in plant development and response to abiotic stress. *The Crop Journal*, 9(1), 17-28.

Folcher L., Jarry M., Weissenberger A., Gerault F., Eychenne N., Delos M., Regnault-Roger C., (2009). Comparative activity of agrochemical treatments on mycotoxin levels with regard to corn borers and *Fusarium mycoflora* in maize (*Zea mays* L.) fields. *Crop Protection*, 28(4), 302-308.

Foley D. C., (1962). Systemic infection of corn by *Fusarium moniliforme*.

Foyer C. H., Noctor G., (2009). Redox regulation in photosynthetic organisms: signaling, acclimation, and practical implications. *Antioxidants and redox signaling*, 11(4), 861-905.

Foyer C. H., Noctor G., (2011). Ascorbate and glutathione: the heart of the redox hub. *Plant physiology*, 155(1), 2-18.

Foyer C. H., Shigeoka S. (2011). Understanding oxidative stress and antioxidant functions to enhance photosynthesis. *Plant physiology*, 155(1), 93-100.

Franco-Zorrilla J. M., Valli A., Todesco M., Mateos I., Puga M. I., Rubio-Somoza I., Paz-Ares J., (2007). Target mimicry provides a new mechanism for regulation of microRNA activity. *Nature genetics*, 39(8), 1033-1037.

Gao X., Hao K., Du Z., Zhang S., Guo J., Li J., Wu Y., (2023). Identification of ceRNA-vsiRNA-mRNA network for exploring the mechanism underlying pathogenesis of sugarcane mosaic virus in resistant and susceptible maize inbred lines. *Phytopathol Res* 5, 60.

Gassmann W., Bhattacharjee S., (2012). Effector-triggered immunity signaling: from gene-for-gene pathways to protein-protein interaction networks. *Molecular Plant-Microbe Interactions*, 25(7), 862-868.

Gelderblom W. C., Jaskiewicz K., Marasas W. F., Thiel P. G., Horak R. M., Vleggaar R., Kriek N., (1988). Fumonisin--novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. *Applied and environmental microbiology*, 54(7), 1806-1811.

Gerlach W., Nirenberg H., (1982). *The genus Fusarium: a pictorial atlas* (Vol. 209, pp. 1-406). Berlin: Kommissionsverlag P. Parey.

Giannitti F., Diab S. S., Pacin A. M., Barrandeguy M., Larrere C., Ortega J., Uzal F. A., (2011). Equine leukoencephalomalacia (ELEM) due to fumonisins B1 and B2 in Argentina. *Pesquisa Veterinária Brasileira*, 31, 407-412.

Gill S. S., Tuteja N., (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant physiology and biochemistry*, 48(12), 909-930.

Gupta S. D., (2010). Reactive oxygen species and antioxidants in higher plants. *CRC press*.

Headrick J. M., Pataky J. K., (1991). to Kernel Infection by *Fusarium moniliforme*. *Phytopathology*, 81, 268-274.

Inzé D., De Veylder L., (2006). Cell cycle regulation in plant development. *Annual Review Genetics.*, 40(1), 77-105.

Jha U. C., Nayyar H., Jha R., Khurshid M., Zhou M., Mantri N., Siddique K. H., (2020). Long non-coding RNAs: emerging players regulating plant abiotic stress response and adaptation. *BMC Plant Biology*, 20, 1-20.

Jones-Rhoades M. W., Bartel D. P., Bartel B., (2006). MicroRNAs and their regulatory roles in plants. *Annual Review Plant Biology*, 57(1), 19-53.

Kamle M., Mahato D. K., Devi S., Lee K. E., Kang S. G., Kumar P., (2019). Fumonisin: Impact on agriculture, food, and human health and their management strategies. *Toxins*, 11(6), 328.

Kedera C. J., Leslie J. F., Claflin L. E., (1992). Systemic infection of corn by *Fusarium moniliforme*. *Phytopathology*, 82, 1138.

Kurihara Y., Watanabe Y., (2004). Arabidopsis micro-RNA biogenesis through Dicer-like 1 protein functions. *Proceedings of the National Academy of Sciences*, 101(34), 12753-12758.

Kushanov F. N., Turaev O. S., Muhammadiyev O. A., Umarov R. F., Rakhimova N. M., Mamadaliyeva N. N., (2022). Maize (*Zea mays* L.) as a Model System for Plant Genetic, Genomic, and Applied Research. *Model Organisms in Plant Genetics*, 49.

Kutschera U., Niklas K. J., (2007). The epidermal-growth-control theory of stem elongation: an old and a new perspective. *Journal of plant physiology*, 164(11), 1395-1409.

Lainé C. M., AbdElgawad H., Beemster G. T., (2024). Cellular dynamics in the maize leaf growth zone during recovery from chilling depends on the leaf developmental stage. *Plant Cell Reports*, 43(2), 38.

Lanubile A., Bernardi J., Marocco A., Logrieco A., Paciolla C., (2012). Differential activation of defense genes and enzymes in maize genotypes with contrasting levels of resistance to *Fusarium verticillioides*. *Environmental and Experimental Botany*, 78, 39-46.

Lanubile A., Ferrarini A., Maschietto V., Delledonne M., Marocco A., Bellin D., (2014). Functional genomic analysis of constitutive and inducible defense responses to *Fusarium verticillioides* infection in maize genotypes with contrasting ear rot resistance. *BMC genomics*, 15, 1-16.

Lanubile A., Maschietto V., Borrelli V. M., Stagnati L., Logrieco A. F., Marocco A., (2017). Molecular basis of resistance to *Fusarium* ear rot in maize. *Frontiers in plant science*, 8, 1774.

Leslie J. F., Pearson C. A., Nelson P. E., Toussoun T., (1990). *Fusarium* spp. from corn, sorghum, and soybean fields in the central and eastern United States. *Ecology Study*, 44, 66.

Leslie J. F., Summerell B. A., (2008). The *Fusarium* laboratory manual. *John Wiley & Sons*.

Li Q., Shen H., Yuan S., Dai X., Yang C., (2023). miRNAs and lncRNAs in tomato: Roles in biotic and abiotic stress responses. *Frontiers in Plant Science*, 13, 1094459.

Li Z., An X., Zhu T., Yan T., Wu S., Tian Y., Wan X., (2019). Discovering and constructing ceRNA-miRNA-target gene regulatory networks during anther development in maize. *International journal of molecular sciences*, 20(14), 3480.

Logrieco A., Mule G., Moretti A., Bottalico A., (2002). Toxigenic *Fusarium* species and mycotoxins associated with maize ear rot in Europe. *Mycotoxins in Plant Disease: Under the aegis of COST Action 835 'Agriculturally Important Toxigenic Fungi 1998-2003', EU project (QLK 1-CT-1998-01380), and ISPP 'Fusarium Committee'*, 597-609.

Mansoor S., Ali Wani O., Lone J. K., Manhas S., Kour N., Alam P., Ahmad P., (2022). Reactive oxygen species in plants: from source to sink. *Antioxidants*, 11(2), 225.

Marasas W. F., (2001). Discovery and occurrence of the fumonisins: a historical perspective. *Environmental health perspectives*, 109(suppl 2), 239-243.

Mhamdi A., Queval G., Chaouch S., Vanderauwera S., Van Breusegem F., Noctor G., (2010). Catalase function in plants: a focus on Arabidopsis mutants as stress-mimic models. *Journal of experimental botany*, 61(15), 4197-4220.

Mi S., Cai T., Hu Y., Chen Y., Hodges E., Ni F., Qi Y., (2008). Sorting of small RNAs into Arabidopsis argonaute complexes is directed by the 5' terminal nucleotide. *Cell*, 133(1), 116-127.

Mittler R., (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in plant science*, 7(9), 405-410.

Mittler R., (2006). Abiotic stress, the field environment and stress combination. *Trends in Plant Science*, 11(1), 15-19.

Mittler R., Vanderauwera S., Suzuki N., Miller G. A. D., Tognetti V. B., Vandepoele K., Van Breusegem F., (2011). ROS signaling: the new wave?. *Trends in plant science*, 16(6), 300-309.

Munkvold G. P., (2003). Cultural and genetic approaches to managing mycotoxins in maize. *Annual review of phytopathology*, 41(1), 99-116.

Munkvold G. P., Desjardins A. E., (1997). Fumonisin in maize: can we reduce their occurrence?. *Plant disease*, 81(6), 556-565.

Munkvold G. P., Carlton W. M., (1997). Influence of inoculation method on systemic Fusarium moniliforme infection of maize plants grown from infected seeds. *Plant Disease*, 81(2), 211-216.

Munkvold G. P., McGee D. C., Carlton W. M., (1997). Importance of different pathways for maize kernel infection by Fusarium moniliforme. *Phytopathology*, 87(2), 209-217.

Munkvold G. P., Hellmich R. L., Showers W. B., (1997). Reduced Fusarium ear rot and symptomless infection in kernels of maize genetically engineered for European corn borer resistance. *Phytopathology*, 87(10), 1071-1077.

Nelissen H., Gonzalez N., Inzé D. (2016). Leaf growth in dicots and monocots: so different yet so alike. *Current opinion in plant biology*, 33, 72–76.

Nelissen H., Rymen B., Coppens F., Dhondt S., Fiorani F., Beemster G. T., (2013). Kinematic analysis of cell division in leaves of mono-and dicotyledonous species: a basis for understanding growth and developing refined molecular sampling strategies. *Plant organogenesis: Methods and protocols*, 247-264.

Noctor G., Foyer C. H., (1998). Ascorbate and glutathione: keeping active oxygen under control. *Annual review of plant biology*, 49(1), 249-279.

Omotayo O. P., Babalola O. O., (2023). Fusarium verticillioides of maize plant: Potentials of propitious phytomicrobiome as biocontrol agents. *Frontiers in Fungal Biology*, 4, 1095765.

Ori N., Cohen A. R., Etzioni A., Brand A., Yanai O., Shleizer S., Eshed Y., (2007). Regulation of LANCEOLATE by miR319 is required for compound-leaf development in tomato. *Nature genetics*, 39(6), 787-791.

Palatnik J. F., Allen E., Wu X., Schommer C., Schwab R., Carrington J. C., Weigel D. (2003). Control of leaf morphogenesis by microRNAs. *Nature*, 425(6955), 257-263.

Park M. Y., Wu G., Gonzalez-Sulser A., Vaucheret H., Poethig R. S., (2005). Nuclear processing and export of microRNAs in Arabidopsis. *Proceedings of the National Academy of Sciences*, 102(10), 3691-3696.

Park W., Li J., Song R., Messing J., Chen X., (2002). CARPEL FACTORY, a Dicer homolog, and HEN1, a novel protein, act in microRNA metabolism in Arabidopsis thaliana. *Current biology*, 12(17), 1484-1495.

Parvathaneni R. K., Bertolini E., Shamimuzzaman M., Vera D. L., Lung P. Y., Rice B. R., Eveland A. L., (2020). The regulatory landscape of early maize inflorescence development. *Genome biology*, 21, 1-33.

Peng Y., van Wersch R., Zhang Y., (2018). Convergent and divergent signaling in PAMP-triggered immunity and effector-triggered immunity. *Molecular plant-microbe interactions*, 31(4), 403-409.

Perianez-Rodriguez J., Manzano C., Moreno-Risueno M. A., (2014). Post-embryonic organogenesis and plant regeneration from tissues: two sides of the same coin?. *Frontiers in plant science*, 5, 219.

Pfaffl M. W., (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic acids research*, 29(9), e45-e45.

Picot A., Barreau C., Pinson-Gadais L., Piraux F., Caron D., Lannou C., Richard-Forget F., (2011). The dent stage of maize kernels is the most conducive for fumonisin biosynthesis under field conditions. *Applied and Environmental Microbiology*, 77(23), 8382-8390.

Reinhart B. J., Weinstein E. G., Rhoades M. W., Bartel B., Bartel D. P., (2002). MicroRNAs in plants. *Genes & Development*, 16(13), 1616-1626.

Rheeder J. P., Marasas W. F. O., (1998). Fusarium species from plant debris associated with soils from maize production areas in the Transkei region of South Africa. *Mycopathologia*, 143, 113-119.

Rodriguez R. E., Mecchia M. A., Debernardi J. M., Schommer C., Weigel D., Palatnik J. F., (2010). Control of cell proliferation in *Arabidopsis thaliana* by microRNA miR396. *Development*, 137(1), 103-112.

Rogers K., Chen X., (2013). Biogenesis, turnover, and mode of action of plant microRNAs. *The Plant Cell*, 25(7), 2383-2399.

Rubio-Somoza I., Weigel D., (2011). MicroRNA networks and developmental plasticity in plants. *Trends in Plant Science*, 16(5), 258-264.

Rymen B., Coppens F., Dhondt S., Fiorani F., Beemster G. T., (2010). Kinematic analysis of cell division and expansion. *Plant Developmental Biology: Methods and Protocols*, 203-227.

Salmena L., Poliseno L., Tay Y., Kats L., Pandolfi P. P., (2011). A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language?. *Cell*, 146(3), 353-358.

Shephard G., (2011). Fusarium mycotoxins and human health. *Plant Breeding and Seed Science*, 64, 113.

Li J., Wang L., Ackah M., Amoako F. K., Jiang Z., Shi Y., Zhao W., (2023). The Competing Endogenous RNAs Regulatory Genes Network Mediates Leaf Shape Variation and Main Effector Gene Function in Mulberry Plant (*Morus alba*). *International Journal of Molecular Sciences*, 24(23), 16860.

Sleewinski T. L., (2012). Non-structural carbohydrate partitioning in grass stems: a target to increase yield stability, stress tolerance, and biofuel production. *Journal of Experimental Botany*, 63(13), 4647-4670.

Sprangers K., Avramova V., Beemster G. T., (2016). Kinematic analysis of cell division and expansion: quantifying the cellular basis of growth and sampling developmental zones in *Zea mays* leaves. *Journal of Visualized Experiments: JoVE*, (118).

Torres M. A., (2010). ROS in biotic interactions. *Physiologia Plantarum*, 138(4), 414-429.

Van Breusegem F., Dat J. F., (2006). Reactive oxygen species in plant cell death. *Plant Physiology*, 141(2), 384-390.

Wang J. W., Wang L. J., Mao Y. B., Cai W. J., Xue H. W., Chen X. Y., (2005). Control of root cap formation by microRNA-targeted auxin response factors in *Arabidopsis*. *The Plant Cell*, 17(8), 2204-2216.

Wang Y., Zhou Z., Gao J., Wu Y., Xia Z., Zhang H., Wu J., (2016). The mechanisms of maize resistance to *Fusarium verticillioides* by comprehensive analysis of RNA-seq data. *Frontiers in Plant Science*, 7, 1654.

Yang H., Cui Y., Feng Y., Hu Y., Liu L., Duan L., (2023). Long non-coding RNAs of plants in response to abiotic stresses and their regulating roles in promoting environmental adaptation. *Cells*, 12(5), 729.

Zeng H., Cai H., Bai T., Ren X., Ci J., Yang W., (2024). Transcriptome analysis of maize resistance to *Fusarium verticillioides*. *Journal of Plant Interactions*, 19(1), 2393803.

Zhang G. Q., Xu Q., Bian C., Tsai W. C., Yeh C. M., Liu K. W., Liu Z. J., (2016). The *Dendrobium catenatum* Lindl. genome sequence provides insights into polysaccharide synthase, floral development, and adaptive evolution. *Scientific Reports*, 6(1), 19029.

Zhang J., Kirkham M. B., (1996). Antioxidant responses to drought in sunflower and sorghum seedlings. *New Phytologist*, 132(3), 361-373.

Zhang Y., Xu K., Pei D., Yu D., Zhang J., Li X., Li C., (2019). ShORR-1, a novel tomato gene, confers enhanced host resistance to *Oidium neolycopersici*. *Frontiers in Plant Science*, 10, 1400.

Zhao W., Li Z., Fan J., Hu C., Yang R., Qi X., Wang S., (2015). Identification of jasmonic acid-associated microRNAs and characterization of the regulatory roles of the miR319/TCP4 module under root-knot nematode stress in tomato. *Journal of Experimental Botany*, 66(15), 4653-4667.

Zhao Z., Zang S., Zou W., Pan Y. B., Yao W., You C., Que Y., (2022). Long non-coding RNAs: new players in plants. *International Journal of Molecular Sciences*, 23(16), 9301.

BIOGRAPHY

Erdem Emre Deligöz, Completed his undergraduate education at Gebze Technical University, Department of Molecular Biology and Genetics in 2022. In 2022, he completed his master's degree in 2025 with the title of ‘Investigation Of Competing Endogenous RNA (ceRNA) Network Role In Leaf Growth Regulation During Maize (*Zea Mays*) Plant And Pathogenic Fungus *Fusarium Verticillioides* Interactions’ under the supervision of Assoc. Prof. Fatma Aydınoglu.



PUBLICATIONS AND PRESENTATIONS FROM THE THESIS

Deligoz E., Aydınoglu F., (2023), “Pathogenicity Effect of *Fusarium verticillioides* On Growth of Maize Seedlings”, Gebze Technical University Graduate School of Natural and Applied Sciences 7th Graduate Research Symposium, Kocaeli, 31 May - 1 June.

Deligoz E., Aydınoglu F., (2024), “Observing Leaf Growth Response of Maize (*Zea mays*) Against Pathogenic Fungus *Fusarium verticillioides*”, Gebze Technical University Graduate School of Natural and Applied Sciences 8th Graduate Research Symposium, Kocaeli, 30 May - 31 May.

