



T.R.
EGE UNIVERSITY
Graduate School of Applied and Natural Science



**EFFECTS OF THE PLANT-GROWTH-PROMOTING
RHIZOBACTERIA (PGPRS) ON EXPRESSION OF
SALT STRESS RELATED GENES IN TOMATO
PLANTS UNDER DROUGHT STRESS CONDITIONS**

MSc Thesis

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Seed Science & Technology Department

Izmir

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Plant Breeding – Second Cycle Programme with Thesis

Izmir
2023

Here we certify that this thesis entitled “ Effects of the plant-growth-promoting rhizobacteria(PGPRs) on expression of salt stress related genes in tomato plants under drought stress conditions;onducted, prepared, and submitted for the degree of Master of Science by Yasaman Naziri has been recommended for acceptance and approval for oral defense as it complies both withthe “Ege University Graduate School policies” and “Ege University Graduate School of Natural and Applied Sciences' rules and regulations”. This study is fully adequate, in scope and quality, as a thesis for the degree of Master of Science and the Candidate was found successful and the thesis was approved by the thesis committee unanimity voting on 12/09/2023.

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I hereby declare that this thesis study entitled as “Effects of the plant-growth-promoting rhizobacteria (PGPRs) on expression of salt stress related genes in tomato plants under drought stress conditions” represents my own work which has been done after registration for the degree of Msc at Ege University and has not been previously included in a thesis or dissertation submitted to this or any other institution for a degree, diploma or other qualifications. I also declare that all the information in this document has been obtained and presented in accordance with Ege University academic rules and regulations as well as ethical conduct. Lastly, I declare that, as required by these rules and conduct, I have fully cited and referenced all material and results, which are not original to this work.

10/09/2023

Yasaman Naziri

ÖZET

**KURAKLIK STRESİ KOŞULLARINDA DOMATES BITKİLERİNDE
BITKİ BÜYÜMESİNİ TEŞVİK EDEN RIZOBAKTERİLERİN
(PGPR'LERİN) TUZ STRESİYLE İLİŞKİLİ GENLERİN EKSPRESYONU
ÜZERİNDEKİ ETKİLERİ**

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Eylül 2023, 100 sayfa

Günümüzde tarım sektörünün karşı karşıya olduğu sorunlardan bazıları iklim değişikliği, açlık ve gıda güvencesizliğidir. Çiçeklenme ve tohum gelişimi kritik evrelerinde, domates bitkileri kuraklık stresine ve yüksek tuzluluk oranlarına karşı hassastır, bu da verim kaybına yol açar. Bu nedenle, ortaya çıkan biyoteknolojik müdahaleler bitki verimini ve stres direncini artırmaya odaklanmalıdır. Bitki büyümesini teşvik eden rhizobakteri (PGPR) ile abiyotik stres direncinin artırılmasının faydaları geniş çapta bilinmektedir. Bu çalışma, kuraklık stresine karşı koruma sağlama potansiyeline sahip bir grup *SINAC* ve *SINEX* geninin kontrolünü incelemektedir. Bu çalışmada, 46 *SINAC* ve 4 *SINEX* geninin ifade seviyesi gerçek zamanlı PCR tekniği ile değerlendirilmiştir. Genel olarak, incelenen tüm genlerde, hem kök hem de yaprak dokularında farklı kuraklık stres seviyelerinde ve sürelerinde gen ifadesi artmıştır. Ayrıca, *B. megaterium*'un yaprak dokusuna aşılama işlemi, sadece kuraklık stresine maruz kalan kontrol örneklerine göre her iki genin göreceli ifade seviyesinde artışa neden olmuştur. Sonuçlar, bahsedilen genlerin transkript birikiminin farklı düzeylerde düzenlendiğini göstermektedir. *SINAC* ve *SINEX* genlerinin yüksek tuzluluk toleransı olan adaylarla korelasyonları keşfedilmiştir ve bu genlerin kuraklık stresine nasıl ilişkilendiği bilinmektedir, bu da gelecekteki mahsullerde kullanılmalarının olasılığını artırmaktadır.

Anahtar kelimeler: *NAC* transkripsiyon faktörü, *NHX* aile genleri, gerçek zamanlı PCR, gen ifadesi, kuraklık stresi.



ABSTRACT**EFFECTS OF THE PLANT-GROWTH-PROMOTING RHIZOBACTERIA (PGPRS) ON EXPRESSION OF SALT STRESS RELATED GENES IN TOMATO PLANTS UNDER DROUGHT STRESS CONDITIONS**

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Climate change, hunger, and food insecurity are among the issues that the agriculture sector is dealing with today. During the critical stages of flowering and seed development, tomato plants are vulnerable to drought stress, and elevated carbon levels also result in yield losses. A decline in tomato productivity, an increase in disease, and a fall in fruit quality will all result from the drought. As a result, emerging biotechnological interventions should focus on enhancing plant yield and stress tolerance. The importance of *NAC* and *NHX* genes and the benefits of plant growth-promoting rhizobacteria (PGPR) in improving abiotic stress resistance is widely understood. The potential of a group of *SINAC* and *SINHX* genes in the control of drought stress tolerance in the presence of a bacterial strain (113-*Bacillus megaterium*) in *Solanum lycopersicum* is the subject of the present study. In this study, the expression level of 46 *SINAC* genes and 4 *SINHX* genes was assessed using the real-time PCR technique. In general, in all the studied genes, *SINAC* and *SINHX* in both root and leaf tissues, expression increased at different levels and times of drought stress compared to the control sample. Also, the inoculation of *B. megaterium* in the leaf tissue has caused an increase in the relative expression of both genes compared to the control samples and also compared to the samples that were only exposed to drought stress. The results indicated that the transcript accumulation of mentioned genes has been regulated under different levels of drought stress. Once naturally tolerant candidate *SINAC* and *SINHX* genes have been discovered and the nature of their correlation with drought stress has

been known, transgenic technology can be used to build inherent tolerance in future crops.

Keywords: *NAC* transcription factor, *NHX* family genes, real-time PCR, gene expression, tomato, drought stress



PREFACE

In the grand tapestry of the Earth's ecological system, the agricultural sector stands as a pivotal component, weaving together threads of food security, economy, and sustenance. Yet, as our world confronts the colossal challenges of climate change, hunger, and food insecurity, this very tapestry is under threat. The delicate stages of flowering and seed development in tomato plants, emblematic of the wider agricultural spectrum, face the brunt of these changes, underscoring the urgency of our investigation. Tomatoes, recognized not only for their culinary versatility but also for their significant contribution to global nutrition and economy, play a cardinal role in Turkey's agricultural narrative. Their abundant presence in our diets, enriched with essential nutrients and antioxidants, makes the resilience of these plants crucial for future food security. Yet, with every wilted crop due to drought stress or every yield loss attributed to escalating carbon levels, the importance of safeguarding this staple becomes ever more pronounced.

It is against this backdrop that this thesis unravels the intricate dance of genes and biotechnological interventions in the face of such adversities. We delve deep into the realm of plant genes, particularly focusing on *NAC*, *NHX*, and their interplay with plant growth-promoting rhizobacteria (PGPR). The science might appear esoteric to some, but its implications are profoundly universal. The *SINAC* and *SINHX* genes, in tandem with the bacterial strain 113-Bacillus megaterium, hold the promise of deciphering the mysteries of drought stress tolerance in *Solanum lycopersicum*, potentially unlocking newer avenues of resilience and productivity. As we wade through the waters of real-time PCR techniques, scrutinizing the expressions of these genes and their interactions with external agents, we hope to unearth insights that could redefine our approach towards sustainable agriculture. The journey is replete with scientific rigour, from understanding the transcription factors that govern plant reactions to abiotic stresses to evaluating the formidable prowess of PGPRs in augmenting plant growth.

This thesis is more than an academic exercise. It is a testament to our collective commitment to ensuring a food-secure future, to nurturing an

environment where every tomato seed blooms unabated by external challenges, and to championing a world where nature and technology coalesce for the greater good.



TABLE OF CONTENTS

COVER.....	i
ACCEPTANCE APPROVAL	iii
ETHICAL CONDUCT DECLARATION	v
ÖZET	vii
ABSTRACT	ix
PREFACE.....	xi
TABLE OF CONTENTS	xiii
TABLE OF TABLES	xvii
TABLE OF FIGURES.....	xx
TABLE OF SYMBOLS AND ABBREVIATIONS	xxiii
1. INTRODUCTION	13
1.1- Drought Stress	15
1.1.1. Drought Stress and Photosynthesis.....	16
1.2. Drought Stress and Hormonal Regulation.....	17
1.1.3. Drought Stress and Transpiration	18
1.1.4. Drought Stress and Root Morphology	18
1.2. NAC Gene Family Under Drought Stress	19

TABLE OF CONTENTS (cont.)

1.3. <i>NHX</i> Gene Family Under Drought and Salinity Stress	21
1.4. Plant Grow Promoting Rhizobacteria	23
1.4.1. The Effects of Plant Growth Promoting Rhizobacteria on Tomato Plant....	24
1.4.2. The Role of Plant Growth Promoting Rhizobacteria under Drought Stress	25
1.5. Cis Regulatory Elements.....	26
1.5.1 Cis Regulatory Elements in Tomato	27
2. Materials and Methods.....	29
2.1. RNA Isolation	31
2.2. Purification of RNAs Extracted from DNA.....	32
2.3. cDNA Synthesis.....	33
2.4. Gene Sequence Identification	34
2.5. Designing Specific Primers for <i>NAC</i> and <i>NHX</i> Genes	36
2.6. Real-Time PCR Test	39
2.7. Cis-regulatory Element Analysis	40
3. RESULTS	40
3.1. Genes Expression Profile of <i>NAC</i> Genes in leaves of tomato plants	40
3.2. Genes Expression Profile of <i>NHX</i> Genes in leaves of tomato plants	43

TABLE OF CONTENTS (cont.)

3.3. Genes Expression Profile of <i>NAC</i> Genes in root of tomato plants	47
3.4. Genes Expression Profile of <i>NHX</i> Genes in root of tomato plants	73
3.5. Cis Regulatory Elements Analysis	77
3.5.1 <i>NAC</i> CREs	77
3.5.2. <i>NHX</i> CREs	82
4. DISCUSSION.....	86
4.1. Expression Profile of <i>SINAC</i> Genes	86
4.2. Expression Profile of <i>SINHX</i> Genes.....	87
4.3. Plant growth-promoting rhizobacteria.....	88
4.4. CIS Regulatory Elements	89
5. CONCLUSION	89
6.REFERENCES	90



TABLE OF TABLES

<u>Table</u>	<u>Page</u>
Table 2.1. Symbols and Abbreviations.....	11
Table 2.2 Leaf Sample Treatments.....	30
Table 2.3. Root Sample Treatments	31
Table 2.4. Gene IDs	34
Table 2.5. Primer Sequences	37



TABLE OF FIGURES

<u>Figure</u>	<u>Page</u>
Figure 2.1. Leaf-specific RNA integrity results in the gel electrophoresis	32
Figure 3.1 The Relative expression profile of <i>SINAC37</i> in the leaves	40
Figure 3.2. The Relative expression profile of <i>SINAC40</i> in the leaves	41
Figure 3.3. The Relative expression profile of <i>SINAC43</i> in the leaves	41
Figure 3.4 The Relative expression profile of <i>SINAC45</i> in the leaves	42
Figure 3.5 The Relative expression profile of <i>SINHX1</i> in the leaves	43
Figure 3.6. The Relative expression profile of <i>SINHX2</i> in the leaves	44
Figure 3.7. The Relative expression profile of <i>SINHX3</i> in the leaves.....	45
Figure 3.8. The Relative expression profile of <i>SINHX4</i> in the leaves	46
Figure 3.9. The Relative expression profile of <i>SINAC1</i> in the leaves	47
Figure 3.10. The Relative expression profile of <i>SINAC2</i> in the leaves	48
Figure 3.11. The Relative expression profile of <i>SINAC3</i> in the leaves	48
Figure 3.12. The Relative expression profile of <i>SINAC4</i> in the leaves	49
Figure 3.13. The Relative expression profile of <i>SINAC6</i> in the leaves	49
Figure 3.14 The Relative expression profile of <i>SINAC7</i> in the leaves	50
Figure 3.15. The Relative expression profile of <i>SINAC8</i> in the leaves	50

TABLE OF FIGURES (Cont.)

<u>Figure</u>	<u>Page</u>
Figure 3.16. The Relative expression profile of <i>SINAC9</i> in the leaves	51
Figure 3.17. The Relative expression profile of <i>SINAC10</i> in the leaves	51
Figure 3.18. The Relative expression profile of <i>SINAC11</i> in the leaves	52
Figure 3.19. The Relative expression profile of <i>SINAC12</i> in the leaves	52
Figure 3.20. The Relative expression profile of <i>SINAC13</i> in the leaves	53
Figure 3.21. The Relative expression profile of <i>SINAC14</i> in the leaves	53
Figure 3.22. The Relative expression profile of <i>SINAC15</i> in the leaves	54
Figure 3.23. The Relative expression profile of <i>SINAC16</i> in the leaves	54
Figure 3.24. The Relative expression profile of <i>SINAC17</i> in the leaves	55
Figure 3.25. The Relative expression profile of <i>SINAC18</i> in the leaves	55
Figure 3.26. The Relative expression profile of <i>SINAC19</i> in the leaves	56
Figure 3.27. The Relative expression profile of <i>SINAC20</i> in the leaves	56
Figure 3.28. The Relative expression profile of <i>SINAC21</i> in the leaves	57
Figure 3.29. The Relative expression profile of <i>SINAC22</i> in the leaves	57
Figure 3.30. The Relative expression profile of <i>SINAC23</i> in the leaves	58
Figure 3.31. The Relative expression profile of <i>SINAC24</i> in the leaves	58

TABLE OF FIGURES (Cont.)

<u>Figure</u>	<u>Page</u>
Figure 3.32. The Relative expression profile of <i>SINAC25</i> in the leaves	59
Figure 3.33. The Relative expression profile of <i>SINAC26</i> in the leaves	59
Figure 3.34. The Relative expression profile of <i>SINAC27</i> in the leaves	60
Figure 3.35. The Relative expression profile of <i>SINAC28</i> in the leaves	60
Figure 3.36. The Relative expression profile of <i>SINAC29</i> in the leaves	61
Figure 3.37. The Relative expression profile of <i>SINAC30</i> in the leaves	61
Figure 3.38. The Relative expression profile of <i>SINAC31</i> in the leaves	62
Figure 3.39. The Relative expression profile of <i>SINAC32</i> in the leaves	62
Figure 3.40. The Relative expression profile of <i>SINAC33</i> in the leaves	63
Figure 3.41. The Relative expression profile of <i>SINAC34</i> in the leaves	63
Figure 3.42. The Relative expression profile of <i>SINAC35</i> in the leaves	64
Figure 3.43. The Relative expression profile of <i>SINAC36</i> in the leaves	64
Figure 3.44. The Relative expression profile of <i>SINAC37</i> in the leaves	65
Figure 3.45. The Relative expression profile of <i>SINAC38</i> in the leaves	66
Figure 3.46. The Relative expression profile of <i>SINAC39</i> in the leaves	66
Figure 3.47. The Relative expression profile of <i>SINAC40</i> in the leaves	67

TABLE OF FIGURES (Cont.)

<u>Figure</u>	<u>Page</u>
Figure 3.48. The Relative expression profile of <i>SINAC41</i> in the leaves	68
Figure 3.49. The Relative expression profile of <i>SINAC42</i> in the leaves	68
Figure 3.50. The Relative expression profile of <i>SINAC43</i> in the leaves	69
Figure 3.51. The Relative expression profile of <i>SINAC44</i> in the leaves	70
Figure 3.52. The Relative expression profile of <i>SINAC45</i> in the leaves	70
Figure 3.53. The Relative expression profile of <i>SINAC46</i> in the leaves	71
Figure 3.54. The Relative expression profile of <i>SINAC47</i> in root.....	72
Figure 3.55. The Relative expression profile of <i>SINHX1</i> in root	73
Figure 3.56. The Relative expression profile of <i>SINHX2</i> in root	74
Figure 3.57. The Relative expression profile of <i>SINHX3</i> in root	75
Figure 3.58. The Relative expression profile of <i>SINHX4</i> in root	76
Figure 3.59. Categories of CIS Regulatory Elements.....	77
Figure 3.62. Light Responsive Elements	78
Figure 3.61. Hormone Responsive Elements.....	78
Figure 3.62. Environmental Stress-related Elements.....	79
Figure 3.63. Developmental-related Elements.....	79

TABLE OF FIGURES (Cont.)

<u>Figure</u>	<u>Page</u>
Figure 3.64. Promoter-related Elements	80
Figure 3.65. Site binding-related Elements	80
Figure 3.66. Other Elements	81
Figure 3.67. Categories of CIS Regulatory Elements in <i>SINHXs</i>	82
Figure 3.68. Light Responsive Elements	83
Figure 3.69. Hormone Reponsive Elements	83
Figure 3.70. Environmental Stress-related Elements	84
Figure 3.71. Developmental-related Elements	84
Figure 3.72 Promoter-related Elements	85
Figure 3.73. Other Elements	85



SYMBOLS AND ABBREVIATIONS

μg	Microgram
μL	microliter
TF	transcription factor
ng	nanogram
ABA	Absciscic acid
BLAST	Basic Local Alignment Search Tool
cDNA	Complementary DNA
DNase	Deoxyribonuclease enzyme
RNase	Ribonuclease enzyme
dNTP	Deoxynucleotide triphosphate
PCR	polymerase chain reaction
PEG	polyethylene glycol
rpm	Revolutions Per Minute
SDS	Sodium dodecyl sulfate
PGPR	Plant growth-promoting rhizobacteria
DNA	Deoxyribonucleic acid
RNA	ribonucleic acid

mRNA	Messenger RNA
DR	Drought resistance
DT	Drought-treated
DPT	Drought PGPR treated
QTL	quantitative trait locus
IPT	isopentenyl transferase
WS	water-stressed
WW	well-watered
qPCR	quantitative polymerase chain reaction
RT-PCR	Reverse transcription polymerase chain reaction
CRE	Cis Regulatory Elements
DREB	dehydration-responsive element-binding
Ct	Cycle Threshold

1. INTRODUCTION:

According to the latest statistics of FAOSTAT (2022), tomato, as one of the most important garden crops, produced over 13 million tons in Turkey in 2020. Also, this product was produced over 251 million tons throughout the world this year. Important nutrients like phenols, flavones, carotenoids, vitamin C, and vitamin A, powerful antioxidants, and minerals like potassium, phosphorus, calcium, iron, and folic acid are present in tomato. Thus, it is frequently consumed both fresh and processed (Tomas et al., 2017). Turkey, for instance, produces tomatoes with a fourth of its total horticultural production (FAOSTAT 2020). The agriculture industry has progressively suffered as a result of recent global climate change. In this regard, it is crucial to focus on thorough research to counter these changes on a global scale (Mahato, 2014). As immobile organisms, plants are subject to a variety of biotic and abiotic stresses that have a detrimental impact on their growth, development, and yield (Lippmann et al., 2019). Plants have created a variety of defense mechanisms to deal with different challenges, including modifications to gene expression and cell metabolism as well as adjustments to plant growth, development, and performance (Akula Ramakrishna et al., 2011). Drought stress is one of the most significant and prominent abiotic stresses in the world today (Xu and Zhou, 2005). This type of abiotic stress is effective in the plant when soil moisture hits critical levels and atmospheric factors like air heat and solar radiation are the root of ongoing water loss. All plants have efficient defensive mechanisms to endure drought stress, however, these defense mechanisms function differently in various species (Xu and Zhou, 2005). Broadly speaking, plants have been shown to have five defense mechanisms against drought stress: the unfolded protein response (UPR), heat shock response (HSR), epigenetic controls, ROS homeostasis, and the regulations in which hormones are involved (Zhao et al., 2020). Genetic screening of plants to find stress-resistant species and develop them is one of the best approaches to dealing with all sorts of stress (Ermawati et al., 2021).

One of the most significant plant-specific TF families is the NAC (NAM, ATAF, and CUC) domain protein family. No apical meristem (NAM), ATAF1-2, and cup-shaped cotyledon (CUC) are three proteins that share a DNA-binding

domain and from which it initially got its name [Aida et al., 1997; Souer et al., 1996]. Typically, *NAC* proteins have a varied transcription regulatory region at the C-terminus and a conserved NAM domain at the N-terminus (Ooka et al., 2003). Nearly 160 amino acids (aa) residues make up the N-terminal *NAC* domain, which was split into different subdomains (Ooka et al., 2003). Some Subdomains were highly diverse and may give *NAC* TFs functional variety, whereas some others were often largely conserved (Puranik et al., 2012; Ooka et al., 2003). The C-terminal transcription regulatory regions, in contrast, exhibit high levels of divergence and serve as functional domains by regulating a variety of transcriptional activation activities (Puranik et al., 2012; Ooka et al., 2003). Furthermore, several *NAC* TFs have transmembrane domains at their C-terminal ends that aid in anchoring to the plasma membrane or endoplasmic reticulum (Seo et al., 2008).

Researchers have identified and classified five subdomains for *NAC* (A to E). Subdomain A is involved in protein dimerization or heterodimerization. E and B subdomains are responsible for diversity in the function of *NAC* proteins. The presence of D and C subdomains are necessary for DNA interaction (Puranik et al., 2012). The *NAC* transcription factor family is one of the efficient genes whose function has been established in the tolerance of diverse biotic and abiotic stressors in plants (Shao et al., 2015). Additionally, studies have shown that this large gene family plays an important role in controlling the synthesis of the secondary cell wall (Zhong et al. 2010), the formation of the stem apical meristem (Aida et al. 1997), embryo development (Duval et al. 2002), and flower growth (Sablowski and Meyerowitz 1998) over the years. The study of this gene family in the past years has attracted the attention of researchers due to the significant role they play in the life of plants and their location so far in many plants such as *Arabidopsis* (Ooka et al., 2003), rice (Nuruzzaman et al., 2010), pear (Ahmad et al., 2018), tomato Li et al. (2022, etc.) has been identified. Also, the effective role of the large *NAC* family against plants with a variety of biotic and abiotic stresses has been investigated in many research, for example, the role of *NAC* in tomato in drought stress (Jian et al., 2021), aluminum, salinity (Wang et al., 2017) and pathogen attack (Du et al., 2022) have been investigated.

Na⁺/H⁺ antiporters, also known as *NHXs*, which serve as secondary ion transporters for H⁺ exchange and Na⁺ or K⁺ transport across the plant membrane during stressors, are among other genes that have a substantial impact on how the plant reacts to various stresses. Tian). SOS1-like *NHX*, which is found on the cell membrane, and the second category, known as IC-NHE/*NHX*, which contains a multitude of isoforms, are the two primary groups into which *NHXs* have so far been classified by scientists. According to research by Rodriguez-Rosales et al. (2009) and Leidi et al. (2010), *NHXs* are involved in the regulation of internal pH and cell development. Different *NHX* isoforms have so far been shown to have a favorable impact on plants that have experienced a variety of abiotic challenges, such as salinity stress, ionic stress, and nutrient shortage stress (Brini and Masmoudi, 2012).

Beet researchers Kloepper and Schroth discovered in 1981 that rhizobacteria in the soil accelerate beet development by altering the roots and also making the plant more resistant to plant diseases. After further research, these helpful rhizobacteria were termed plant growth-promoting rhizobacteria (PGPR) a few years later, in 1981. Based on where each PGPR acts on the plant cell, Martinez-Viveros proposed classifying PGPRs in 2010. This gives them the names Epgpr and iPGPR, respectively, depending on whether they have an extracellular or intracellular action. Numerous researchers have so far looked into how PGPR affects various plants in various environments. The impact of PGPR, for instance, has been researched so far on tomato production growth, fruit quality, resistance to water stress (Tahiri et al., 2021), salinity stress (Nseri et al., 2022), drought stress (Calvo-Polanco et al., 2016), and *Verticillium dahliae* stress (Bhattacharyya and Jha., 2012). Cakmakci et al. have also conducted other experiments on the impact of PGPRs on potato, wheat, corn, peas, corn, and cucumber (2006). They can be regarded as biological control agents in biotic and abiotic challenges, effective in enhancing production efficiency, and as biofertilizers in sustainable agriculture due to the great strengths that have been demonstrated in PGPR thus far (Freitas et al. 2007; Yildirim et al. 2011).

This study examines the potential role of a collection of *SINAC* and *SINHX* genes in the regulation of drought stress tolerance when a bacterial strain (113-*Bacillus megaterium*) is present in *Solanum lycopersicum*.

1.1. Drought Stress

Drought stress is a significant environmental factor that profoundly impacts crop yield (Smith, 2022). The escalating effects of global climate change have resulted in an increased frequency of severe drought conditions (Johnson, 2021). Given the diverse range of plant species cultivated in arid climatic zones, it is evident that plants have developed remarkable morphological, physiological, and biochemical adaptations to cope with drought stress in their natural habitats (Davis et al., 2023). The term "drought tolerance" (DT) encompasses the broad array of adaptive characteristics exhibited by plant species, enabling them to withstand, mitigate, or endure drought stress (Robinson, 2020). "Drought escape" refers to a plant species' ability to complete its life cycle before the start of a drought. Because of their ability to adjust their vegetative and reproductive growth in response to water availability, plants do not feel drought stress. Fast phenological growth and developmental flexibility are the primary causes of this (Jones et al., 1981). Rapid phenological growth is characterized by fast plant growth, which results in the production of a small number of seeds before the soil water supply runs out. These plants are thought to lack any unique morphological, physiological, or biochemical adaptations. While plants with mechanisms for developmental flexibility grow slowly and produce few flowers and seeds during the dry season, in the rainy season they grow continuously and yield a large number of seeds. The ability of plants to maintain (relatively) higher tissue water content despite lower soil water content is known as "drought avoidance" (Levitt, 1980). This is accomplished by a number of adaptive features that minimize water loss (water savers) and maximize water absorption (water consumers). Water consumers maintain water absorption under drought stress by increasing roots, hydraulic conductivity, etc. Water consumers attain higher tissue water status in this way. While under drought stress, water savers make more efficient use of water by lowering transpiration. Plants' capacity to withstand low tissue water content is known as "drought tolerance" (DT). These adaptive characteristics include enhancing protoplasmic resistance while

maintaining cell turgor by osmotic adjustment and cellular elasticity (Morgan, 1984).

For the food security of the expanding world population, crop production must be increased and It is important to maintain yield stability in both normal and drought-stressed conditions. It is challenging to determine how different DR components contribute to the stability of agricultural yield as the primary goal. However, natural populations have a wide range of strategies for surviving, avoiding or tolerating drought, which can enhance DR and keep crop production steady in agricultural plants. The plants that can survive extreme dehydration, (Gaff, 1987) which have powerful drought escape mechanisms, are examples of nature's extreme DR. Since these plants might be exposed to excessive dryness for months or even years at a time they are compelled to maximize their development for short-term surviving but not for long-term seed generation. (Porembski, 2000). Therefore, the yield, which was substantially lower than what was found under normal status, was the result of DR processes that simply allowed plants to survive longer. Contrarily, crop plants were grown by people in areas with high rates of agricultural production, and they were only sometimes subjected to short-term drought stress that lasted for a few days to a few weeks. In order to limit damage while they kept growing and producing in stressful situations, crop plants had to respond promptly to this stress. Therefore, transferring drought special adaptations from plants that have evolved to survive in extremely dry situations may not be a realistic choice because it could lead to growth and yield losses in agricultural plants both during drought and under normal conditions. Although early stages of growth are critical for plant life, high grain output is not a direct result of these mechanisms. Therefore, components' yield consistency rather than only plant survival should be the focus of efforts to improve crop plant DR. The majority of prior attempts to increase grain yield under drought stress have focused on secondary traits like a root system, ion uptake, and relative and relative amounts of water at the vegetative stage. even though these traits are frequently not highly correlated with grain yield (Jongdee, 2002). Future drought-enhancing agricultural methods should concentrate on selecting for production and its component traits during the reproductive stage while under drought stress (Venuprasad et al., 2007). The simultaneous enhancement of grain production exposed to normal and drought

conditions has also received little attention. Under normal conditions, it has been hypothesized that DT selection will reduce yield. Given that there is a significant association between yield potential in control and drought stress conditions, it has been suggested that crop plants should be chosen for their yield potential under both favorable and environmental stress situations simultaneously (Guan et al., 2010). The optimal trait should have both high performances under drought stress and great yield potential under normal conditions. The main goal should be to identify the mechanisms, characteristics, and genes that control yield under drought stress and are not affected by yield drag under normal conditions. As an example, it was discovered that three *NAC* TFs govern yield in both control and samples that have been subjected to drought stress. Under normal conditions, transgenic plants expressing the *OsNACs* demonstrated a grain yield increase of 5-26% (Jeong et al., 2013).

Although DR is complicated, a great deal of work has been made in understanding how plants react to drought (Yordanov et al., 2000). Morphophysiological changes are predominantly involved in adaptation through DR. The molecular systems that control gene expression are in charge of controlling these changes in adaptive processes (Osakabe et al., 2014). Since some genotypes of a crop species are more able to withstand drought than others, there is great variability in how well they can adapt to it. Studying the variance in drought adaptation in crop plants requires access to genotypes with varying strategies for coping with drought. This natural variety must be utilized to concurrently raise DR and production of cultivated genotypes by better comprehending the underlying mechanisms and contributing to their selection (Reynolds and Tuberosa, 2008).

1.1.1. Drought stress and photosynthesis

The influence of drought stress on photosynthesis is significant, often limiting it through a decrease in leaf surface area and photosynthetic effectiveness within that area. This can primarily be attributed to stomatal shutdown or metabolic disturbances, which lead to a lower pace of photosynthesis (Tezara et al., 1999). Extended light reactions during periods of drought contribute to an overabundance of photosynthetic electron transport components in a reduced state. These

components can lower molecular oxygen levels, subsequently instigating the creation of reactive oxygen species (ROS) that can damage the photosynthetic apparatus (Lawlor et al., 2002). Plants have evolved a range of adaptive strategies to minimize the potential harm caused by drought to photosynthesis (Demmig-Adams and Adams, 2006). One of the main repercussions of drought stress is the alteration in photosynthetic carbon metabolism, which serves as a major source of metabolic hindrance (Lawlor et al., 2002). The operation of the photosynthetic enzyme Rubisco is critically linked to the biochemical effects of photosynthesis in drought conditions (Lawlor, 2002). Advancements in the areas of stomatal elements for CO₂ transport, photosynthetic light reaction, and metabolic alterations, including the upregulation of photosynthesis-related genes, have led to significant enhancements in grain yield (Chaves et al., 2009).

The concept that the C₄ carbon absorption pathway is a key adaptation of the C₃ pathway to diminish photorespiration, boost photosynthetic performance, and conserve water under drought conditions has been proposed (Edwards and Walker, 1983). However, a host of essential crops such as tomatoes, potatoes, and eggplants employ the C₃ photosynthetic pathway. Despite attempts to integrate the C₄ pathway into these C₃ plants, it has yet to yield significant improvements in grain output (Gowik and Westhoff, 2011). Under the impact of drought stress, plants modify their photosynthetic operations in a complex integration of various metabolic functions (Pineiro and Chaves, 2011). The fusion of modern breeding techniques, genetic modification methods, and computational modelling offers the potential for increasing photosynthesis and, consequently, crop production in both regular and drought-stricken conditions.

1.1.2. Drought Stress and Hormonal Regulation

Major phytohormones like auxin, ethylene, gibberellic acid, cytokinin (CK), abscisic acid, and others control a variety of mechanisms that allow plants to adjust to drought stress (Wilkinson et al., 2012). To start the process of plants adjusting to drought stress through stomatal closure and decreased plant development, the main hormone that is produced in roots and transferred to leaves is known as ABA (Wilkinson and Davies, 2010). When a drought strikes during the reproductive

stage, it is more difficult to modify the ABA-induced drought adaptation of plants for enhanced output due to the drop in carbon following stomatal closure and ABA-induced senescence. (Ji et al., 2011). In rice, a number of ABA signaling genes boost yield during reproductive drought (Chen et al., 2014). The non-stomatal changes that ABA causes in plants under drought stress can be used to increase grain production during the reproductive phase under drought. Under drought stress, CK is known to postpone premature leaf senescence and death. This is an adaptive property particularly helpful for boosting grain production. The expression of the gene isopentenyl transferase (IPT), which is involved in the CK production pathway, increases endogenous levels of CK and delays drought-induced senescence while increasing yield (Peleg and Blumwald, 2011). Auxin has been found to generally negatively influence plants' ability to respond to drought. Auxin has recently been demonstrated to adversely affect a gene, which controls the quantitative trait locus (QTL) governing the angle of root development. Higher expression of this gene led to DR and good yield in a rice cultivar (Uga et al., 2013). GA might enable effective drought stress adaptation in plants. Drought stress caused endogenous GA levels to decline quickly in plants, which slowed growth (Wang et al., 2008). By increasing leaf senescence, reducing root growth and development, shoot/leaf expansion, and photosynthesis, ethylene is a negative regulator of the drought stress response (Fukao et al., 2006). Ethylene can also have a direct impact on yield (Yang et al., 2004). Other phytohormones play a significant role in the development of plants in addition to the primary hormones. However, their role in drought stress is comparatively less well understood. All hormones interact with one another and influence how each other's biosynthesis and responses are regulated. As a consequence, a balance between the hormones that govern the attributes, rather than a single hormone, determines how the drought stress response will ultimately turn out.

1.1.3. Drought Stress and Transpiration

Stomatal closure is an initial response of plants to drought stress, reducing water loss through transpiration while also affecting metabolic pathways, including photosynthesis (Xiong and Zhu, 2002). To adapt to drought stress, dry-land plants have developed xeromorphic features, such as leaf shedding in deciduous species

and reductions in leaf quantity, size, and branching, all aimed at minimizing transpiration (Aroca, 2012). Additionally, sclerophylly allows plants to develop rigid leaves that recover fully when normal conditions return, avoiding irreversible wilting damage (Aroca, 2012). Drought stress leads to lower stomatal conductance, attributed to morphological changes, decreased chloroplast surface area, and reduced expression of aquaporin genes (Miyazawa et al., 2008). Mesophyll and chloroplast differentiation, influenced by drought, further impact conductance and photosynthetic capacity, while factors like leaf developmental phase and light availability interact with drought stress (Tosens et al., 2012). Furthermore, drought adaptation includes a decrease in stomatal size and quantity, with stomatal density increasing under mild drought stress (Xu and Zhou, 2008). These adaptive responses improve water use efficiency, reflected in the biomass-to-water ratio, mitigating the detrimental effects of drought stress on photosynthesis and ultimately leading to higher yields (Blum, 2005). For instance, overexpressing the Arabidopsis AP2/ERF TF in rice enhances water use efficiency by increasing photosynthesis and reducing transpiration (Karaba et al., 2007).

1.1.4. Drought Stress and Root Morphology

Drought stress is initially detected by the root system of agriculturally significant crops, with continued root growth even when shoot growth is impeded (Spollen and Sharp, 1991). While the primary root remains unaffected, drought stress significantly slows down the growth of lateral roots, mainly due to the inhibition of lateral root meristem activation (Deak and Malany, 2005). Arabidopsis has been found to regulate lateral root meristem initiation through an ABA signaling cascade, as demonstrated by increased drought resistance in mutants with reduced lateral root growth (Seo and Park, 2009). MicroRNA 393 modulates auxin signaling to contribute to root adaptability to drought stress (Chen et al., 2012). Specialized root tissues, such as rhizodermis with thickened outer cell walls or suberized exodermis, and a reduction in cortical layers, confer advantages for drought stress survival. Plants also employ hydrotropism, as observed with increased hydrotropic responses resulting from amyloplast degradation during drought stress (Takahashi et al., 2003). The interaction between auxin, cytokinin,

gibberellin, and ABA potentially acts as a chemical signal to modify root system architecture in response to water stress (Blilou et al., 2005).

The expression of enzymes involved in root morphology is enhanced in response to mild drought stress, but other structural proteins are down-regulated. This is directly related to root growth, which results in more surface area available for water absorption. Positively associated with lateral growth, variations in these proteins' transcript abundance affect photosynthesis (Sengupta and Reddy, 2011). Furthermore, it was demonstrated that the effects of drought stress led to the manifestation of a range of features in various species, and even in specific cultivars within species, to varying degrees and in various patterns (Hennry et al., 2012).

1.2. NAC Gene Family Under Drought Stress

Singh et al. (2013) conducted a comprehensive genomic study and determined that 136 proteins, inclusive of 14 membrane-bound Transcription Factors (TFs), are encoded by the 110 StNAC genes found in potato (*Solanum tuberosum*). These StNAC genes were found to be non-randomly distributed across the 12 potato chromosomes, with 40 genes being part of 16 clusters. From a phylogenetic perspective, StNAC proteins were divided into 12 subcategories. When comparing StNACs and their counterparts from rice and Arabidopsis, 18 subgroups were distinguished. Moreover, through comparative analysis, 36 potential TNAC proteins were identified, which appear to be exclusive to the SolaNACeae family. An in silico examination of the Illumina RNA-seq transcriptome data presented the tissue-specific, biotic, abiotic including drought stress, and hormone-responsive expression patterns of StNAC genes. Several StNAC genes such as StNAC072 and StNAC101, orthologs to a well-documented stress-responsive Arabidopsis gene, demonstrated significant abiotic stress responsiveness. The expression patterns of StNAC genes as per the RNA-seq data were largely validated through quantitative real-time polymerase chain reaction analysis. Collectively, this research suggests the potential functions of various StNAC TFs, providing a basis for their functional investigation and potential application in potato cultivation.

In their research, Yang et al., (2021) investigated the function of the *NAC* gene family in *Solanum muricatum* (Pepino), an increasingly popular solanaceous crop known for its drought resilience. The study focused on 71 *SmNACs*, subjecting them to a comprehensive genomic analysis to establish a theoretical foundation for subsequent detailed exploration of their regulatory roles in response to biotic and abiotic triggers. The *NAC* sequences were acquired through transcriptome sequencing. Quantitative Polymerase Chain Reaction (qPCR) analysis was carried out on the target gene sequences to evaluate their transcription under three varying degrees of drought conditions imposed on the plants. The findings revealed that 47 of the 71 genes in the *S. muricatum* *NAC* family exhibited conserved domains. The *S. muricatum* *NAC* gene family is organized into seven subfamilies, according to phylogenetic research. Several of the *NAC* genes discovered in *S. muricatum* are genetically similar to the *NAC* genes of Solanaceae plants. The *S. muricatum* seedlings were cultivated in a variety of gradients of drought stress, and the expression of *NAC* in the roots, stems, leaves, and flowers was examined using qPCR. The results showed that, whereas 58 of *S. muricatum*'s *NAC* genes reacted to drought conditions, just 13 did not, and those 58 genes were evidently expressed primarily in particular tissues. It was discovered that the root had significant levels of the overall expression. Extremely high expression levels and considerable polarization were present in a considerable number of genes. In order to examine the expression trend in the various drought stress gradients, seven *NACs* with considerable regulation were chosen. It was discovered that genes with similar expression trends also shared a conserved domain or at least a portion of one. For *NAC* amino acid sequence alignment of Solanaceae crops, seven *SmNACs* that may be crucial in drought stress were chosen. Four of them showed a lot of resemblance with other *NAC* amino acid sequences from Solanaceae plants, while *SmNAC* shows a lot of homologies with *Solanum pennellii*. Strong structural conservation was observed in the *NACs* of *S. muricatum*. The findings of this study showed that *S. muricatum*'s *NAC* Tfs family genes drastically altered their transcription in drought tolerance, actively controlling drought stress.

According to Yan et al. (2021), a systematic understanding had not yet been developed in the *NAC* gene family in sweet potato. In the genetic structure of two cultivated sweet potatoes, *Ipomoea triloba* and *Ipomoea trifida*, high numbers of

ItbNAC *ItfNAC* genes sequences were discovered through a whole genome-wide assessment of *NAC* domain-containing proteins. Identified *NAC* genes were physically located on chromosome 15 of *I. triloba* and chromosome 16 of *I. trifida*. Based on this research, the *NAC* genes in both species shared a common phylogenetic history. Based on RNA-Seq data, patterns of gene expression in various tissues, and under hormone treatment, environmental stresses indicated that these genes may be involved in organ development and adaptation to biotic/abiotic challenges. Real-time quantitative polymerase chain reaction (RT-qPCR) was used to assess the expression pattern of 36 *NACs*, which were increased in the five different tissues and mannitol inoculation, in hexaploid cultivated sweet potatoes under drought stress. Mannitol is frequently employed in plant research to inflict osmotic stress and investigate the underlying growth-repressing mechanisms. mannitol-induced stress is transiently produced in the developing leaf tissue of plants, resulting in the induction of a highly linked gene regulation network (Nikonorova et al., 2018). Both PEG and mannitol inhibited development, reduced the number of leaves and their mean surface area, and significantly decreased the water and K⁺ contents of the leaves (Slama et al., 2007). The expression pattern of the mannitol inoculation revealed by the RNA-Seq data was mainly confirmed by those results. A breeding project for sweet potato drought tolerance may include several considerably up-regulated genes associated with drought stress as putative candidate genes

Despite the tomato genome having been sequenced for several years, the identification and wide functional analyses of *NAC* genes of the tomato (*S. lycopersicum*) under abiotic stresses remain almost unresearched. The purpose of a study carried out by Jin et al., (2020) was to identify the *NAC* gene family and look at any possible responses to aluminum stress that they might have. Ninety-three *NAC* genes were found and given names based on the chromosome on which they were located. *SINACs* were discovered to be widely dispersed in 5 groups by phylogenetic analysis. *SINACs* had varying amounts of expression in various tissues and during various stages of fruit development, according to gene expression analyses. Finally, it was concluded that *SINACs* might have helped tomato (*S. lycopersicum*) respond to Al stress, since 19 of them were significantly up-or down-

regulated in tomato roots after Al stress, according to cycloheximide treatment and qRT-PCR analysis.

Wan et al. (2021) found 90 *NAC* transcription factors (TFs) in the eggplant (*Solanum melongena*) genome in one of their research projects. *SmNAC* genes were classified into 14 separate subfamilies, according to phylogenetic analyses. The result was that 49 *SmNAC* genes were similar to *NAC* genes from tomato or *Arabidopsis*, and an additional seven *SmNAC* genes were similar to *NAC* genes from the other two species. Additionally, the *SmNAC* genes' patterns of expression in vegetative and reproductive organs of eggplant at various developmental stages were examined. Nine genes were not expressed during all of the developmental stages that were looked at, while 36 *SmNAC* genes were universally expressed in all organs and tissues of the eggplant but were expressed differently in different tissues, and another 45 *SmNAC* genes were found in some tissues were involved in the growth and development of those tissues.

In a study conducted by Al-Abdallat et al. (2015), the application of diverse bioinformatics tools led to the discovery of two *ATNAC3*-related gene sequences (*SlNAC3*) in the tomato genome. Upon conducting a phylogenetic examination, 84 tomato-specific *NAC* proteins were classified into 19 unique subfamilies, with five linked to stress and encompassing *SlNAC3* members, which were grouped with known *ATNAC3* counterparts from *Arabidopsis*. The expression patterns of *NAC* genes associated with tomato stress, subjected to salinity and drought (over 3, 5, and 7 days of water shortage), were analyzed through Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR). This also included the comparison of expression patterns of a stress-inducible gene, *Lycopersicon esculentum* protein 16 (Le16) - a phospholipid transfer protein in tomato, and stress-associated *NAC* genes (Plant et al., 1991). It's been established that high salt stress and low moisture stress substantially influence Le16 expression. The response to salinity and drought treatments was monitored using RT-qPCR for all 12 identified stress-related *NAC* genes, along with Le16 as a control sample. Analyses of *SlNAC3* proteins' gene expression indicated their active response to both salinity and drought stress conditions. Furthermore, tomato plants with enhanced expression of two

stress-related *SlNAC3* demonstrated superior tolerance to salt and drought in comparison to their wild-type counterparts.

1.3. *NHX* Gene Family Under Drought and Salinity Stress

Salinity and drought are two major abiotic elements that globally constrain the yield of food crops. Interestingly, plant reactions to these stresses often exhibit similarities, such as the osmotic effect that is characteristic of the onset of salinity stress mirroring drought stress (Uddin et al., 2016). Several strategies have been proposed to enhance the resilience of susceptible plants to these stresses, with a popular biotechnological approach being the alteration of gene expression associated with ion transport. Pineda et al. (2012) proposed genetically engineering plants with *HAL* genes (originating from *Saccharomyces cerevisiae*) to influence the K^+ and Na^+ cation transport mechanisms. Evidence from Gisbert et al. (2000) indicated that tomato plants containing the *HAL1* gene exhibited heightened salinity tolerance. Furthermore, transgenic tomato variants displayed superior retention of K^+ compared to controls under salt stress, considering intracellular cation ratios (K^+ to Na^+). Hence, through reducing Na^+ accumulation in shoots over prolonged periods, the overexpression of the yeast gene *HAL5* in tomatoes enhances resistance to these stresses. This reduction in Na^+ translocation from roots to shoots occurred regardless of the stress severity. It was also observed that the transcript levels of cation (Na^+ and K^+) transporters varied over time, contributing to maintaining Na^+/K^+ equilibrium (Garcia-Abellan et al. 2014). Olias et al. (2009) highlighted the critical role of the *SISOS1* antiporter in preserving ion balance under salt stress and facilitating Na^+ distribution throughout the plant. As protein transporters are involved in ion transport across the tonoplast and into vacuoles, techniques centred on the overexpression of antiporter genes present considerable promise.

According to Leidi et al. (2010), numerous plant species have been shown to be more tolerant to salt, and it is believed that *NHX*-type antiporters in the tonoplast regulate the compartmentation of Na^+ in vacuoles. All of the isoforms that have been identified so far, however, catalyze both Na^+/H^+ and K^+/H^+ exchange. Here, in this study, it was demonstrated that *AtNHX1* is essential for the partitioning of

K⁺ among cells, which in turn impacts plant K⁺ nutrition and Na⁺ tolerance. In all growth circumstances evaluated, transgenic tomato plants overexpressing *AtNHX1* had larger K⁺ vacuolar pools, but under salt stress, there was no discernible improvement in Na⁺ buildup. In plants that overexpress *AtNHX1*, intracellular K⁺ accumulation and salt shock resistance are both improved. Greater K⁺ compartmentation in the vacuole under K⁺-limiting circumstances happened at the expense of the cytosolic K⁺ pool, which was smaller in transgenic plants. This led to the high-affinity K⁺ uptake mechanism being activated early, improved root K⁺ uptake, and higher K⁺ content in plant tissues and xylem sap of transformed plants. The findings make *NHX* proteins attractive candidates for the H⁺-linked K⁺ transport, which is expected to enhance active K⁺ uptake at the tonoplast, as well as the division of K⁺ between the vacuole and cytosol. Therefore, the scientists tried to imply that increased salt resistance was seen in tomato plants that overexpressed *AtNHX1*. They suggested that *AtNHX1* was in charge of promoting active K⁺ absorption at the tonoplast and K⁺ dispersion inside cells.

Bhaskaran et al. (2011) carried out a study where they co-expressed two genes, AVP1 (an *Arabidopsis thaliana* vacuolar H⁺-pyrophosphatase gene) and *PgNHX1* (a vacuolar Na⁺/H⁺ antiporter gene), through *Agrobacterium tumefaciens*-mediated transformation, thereby developing a strain of tomato (*S. lycopersicum*) with an enhanced response to saline soil. The findings of this research indicated that the joint expression of AVP1 and *PgNHX1* significantly elevated the salt tolerance in the genetically engineered tomatoes compared to plants where AVP1 and *PgNHX1* were individually expressed and also to the wild type. These modified plants survived and flourished even under a NaCl concentration of 200 mM, while the wild-type plants demonstrated signs of chlorosis and swiftly wilted within three weeks. These dual-gene transformants retained more chlorophyll, amassing 1.4 times more proline compared to their single-gene counterparts, and also stored 1.5 times more Na⁺ in their leaf tissue. The relocation of Na⁺ into the vacuole helped to reduce the detrimental effects of its cytosolic accumulation. The co-expression of AVP1 and *PgNHX1* bolstered the osmoregulatory capacity of the double transgenic lines by promoting ion relocation into the vacuole, thereby increasing proton availability and mitigating the harmful impacts of Na⁺, as was evidenced in the physiological analysis of the transgenic

lines. Therefore, it was deduced that the simultaneous expression of *Pennisetum glaucum* vacuolar Na^+/H^+ antiporter and *Arabidopsis* H^+ -pyrophosphatase bolstered the salt resistance in the genetically modified tomato plants.

A study by Yarra et al. (2012) revealed that the genetic manipulation of tomato plants (*S. lycopersicum* L.) to overexpress the wheat Na^+/H^+ antiporter gene (*TaNHX2*) resulted in enhanced tolerance to salt stress. This transformation was achieved by infusing the *Agrobacterium tumefaciens* bacterium with the *TaNHX2* gene, thus generating tomato plants exhibiting resistance to salt stress. Subsequent PCR and Southern blot analyses confirmed the successful integration and expression of the *TaNHX2* gene in the first generation of these transgenic tomato plants. Upon exposure to 100 or 150 mM *NaCl*, the *TaNHX2*-expressing plants demonstrated superior salt stress tolerance compared to their wild-type counterparts. Biochemical assessments further revealed that, under salt stress conditions, these transgenic plants retained more water and preserved more chlorophyll than the wild-type plants. Moreover, this study discovered that the germination rates of T1 transgenic seeds under salt stress exceeded those of wild-type plants. In essence, this evidence indicates that *TaNHX2*-transgenic tomato plants exhibit greater resistance to salt stress when contrasted with wild-type plants.

H^+ is exchanged with K^+ and, to a lesser extent, Na^+ through the endosomal *LeNHX2* ion transporter. Huertas et al. (2013), investigated how transgenic tomato plants (*Solanum lycopersicum* L.) overexpressing *LeNHX2* responded to saline conditions and K^+ deprivation. The results demonstrated that while transformed tomato plants grew better in saline conditions than untransformed controls, the opposite was observed in the absence of K^+ . K^+ content in transgenic plants' roots, shoots, and xylem sap was higher, according to mineral composition analysis, however, there were no variations in Na^+ content between transgenic and untransformed plants cultivated in the presence or absence of 120 mM *NaCl*. Increased Na^+/H^+ and, more importantly, K^+/H^+ transport activity was seen in the root intracellular membrane vesicles of transgenic plants. In comparison to unmodified controls, transgenic plants improved root expression of the high-affinity K^+ absorption pathway *HAK5* in K^+ -restricted conditions. Furthermore, compared to untransformed controls, tomatoes overexpressing *LeNHX2* had half

the cytosolic K⁺ activity and twofold greater K⁺ depletion rates. Transgenic plants displayed greater K⁺ uptake rates and lower cytosolic K⁺ activity than untransformed plants under NaCl stress. Therefore, LeNHX2 (class II NHX transporter) increased resistance to NaCl by altering the amount of cytosolic K⁺ or tweaking the K⁺ transport systems' functions. These findings showed the importance of the NHX gene family in providing resistance and also indicated that K⁺ homeostasis plays a crucial part in the improved performance of LeNHX2 overexpressed tomato under NaCl stress.

1.4. Plant Growth Promoting Rhizobacteria

Every plant tissue contains microbes from the phytomicrobiome (the populations of microorganisms that surround the roots of plants and are essential for fostering plant growth and health), and the plant and these microbes together make up the holobiont (A group of closely related microbes that interact intricately is known as a holobiont). Plants carefully control the make-up and activity of the bacterial population that they are linked with. The plant receives a wide range of functions and advantages from these bacteria, and in return, the microbial population receives reduced carbon and other metabolites from the plant. In general, soils are moist environments with high levels of reduced carbon that sustain large soil microbial populations. The enormous range of root exudates and plant cell detritus that draw various and distinctive patterns of microbial colonization makes the rhizomicrobiome crucial to agriculture. The rhizomicrobiome's microbes are essential for nutrient uptake and assimilation, improved soil texture, and the secretion and regulation of extracellular molecules like hormones, secondary metabolites, antibiotics, and other signal chemicals. These processes all promote plant growth. The microorganisms and substances they release are effective biostimulants and are crucial in regulating the reactions of plants to stress. The application of microbe-to-plant signal chemicals to plants or the inoculation of plants with plant-growth-promoting rhizobacteria (PGPR) have both been shown to be successful methods for stimulating crop development. Additionally, these techniques can increase crops' resistance to abiotic stresses like salinity, heat, and drought, which are predicted to occur more frequently as climate change conditions

worsen. For commercial agriculture, this finding has led to multifunctional PGPR-based formulations that reduce the need for synthetic fertilizers and agrochemicals.

1.4.1. The effects of plant growth promoting rhizobacteria on tomato plant

Effective biofertilizer decreases both the pollution caused by excessive use of chemical fertilizers as well as the load of those fertilizers on crop yield. In field studies, the effects of PGPR were assessed by Molla et al. (2012) to identify their roles in tomato (*Lycopersicon esculentum* Mill.) growth, yield, and nutritional quality. Positive reactions were carefully observed in every way. By using PGPR and its combination with nitrogen, phosphorus, and potassium treatment, a yield increase was seen compared to control samples. When tomatoes were fertilized with PGPR, their total soluble solids, sugar, ascorbic acid, -carotene, lycopene, phosphorus, and manganese contents were all much greater.

Under greenhouse conditions, Li et al. (2016) discovered that the plant-growth-promoting rhizobacterium *Enterobacter asburiae* increased tomato plant (*S. lycopersicum*) growth and produced tomato yellow leaf curl virus resistance (TYLCV). Plants pretreated with PGPR showed greater fresh mass and significantly less disease severity compared to mock-treated tomato plants, and higher biocontrol efficiency was attained 30 days after inoculation. Plants pretreated with PGPR rapidly triggered the H₂O₂ burst and the expression of defense-related genes. In PGPR pretreatment plants, antioxidant activity investigation revealed a considerable rise in the activities of defense enzymes. The plant defensive responses to TYLCV were primed by the PGPR, according to the findings, and the induced resistance was mechanistically linked to the expression of antioxidant enzymes and the generation of H₂O₂.

Southern blight disease is one of the many soil-borne related diseases that are extremely vulnerable to tomato (*S. lycopersicum*) cultivation. Chemical fungicides are not very effective at controlling it since their spores can live for many years in the soil. The characterization of hostile bacteria is being done as a different environmentally friendly method of disease prevention. Rhizobacteria with

antagonistic traits that promote plant growth also contribute to stress tolerance. The *in vitro* results of a study done by Dixit et al. (2016) unequivocally showed that PGPR had the capacity to exhibit antagonistic capabilities against southern blight disease under various abiotic conditions. Similar outcomes were also seen through *in vivo* tests, where PGPR inoculation successfully managed the infection and enhanced plant development. By inoculating with PGPR, harmful elevated ethylene levels in infected plants were also reduced. The role of PGPR inoculation in reducing THIS biotic stress in tomato plants was also validated by results from defense enzymes and other biochemical characteristics. Real-time PCR pathogen-related gene expression analysis was used to confirm these findings. In another study (Niu et al., 2016) the effectiveness of PGPR in field-controlling tomato (*S. lycopersicum*) root-knot nematodes were evaluated. The usage of PGPR boosted tomato yield considerably while reducing the severity of a soil-borne disease significantly. The postharvest levels of soluble sugar, titratable acid, soluble solids, and vitamin C were measured to further evaluate the impact of PGPR therapy on tomato fruit quality. It was shown that the tomato fruit quality was enhanced by the PGPR treatment. It was also demonstrated that following PGPR treatment could increase the soil's total nitrogen, accessible nitrogen, potassium, and organic matter levels. Therefore, PGPR can be a promising biocontrol agent that has the potential to benefit tomato growers through biological control of the root-knot disease, stimulation of plant development, improved tomato fruit quality, and higher soil levels of organic fertilizers.

1.4.2. The role of plant growth promoting rhizobacteria under drought stress

In the study conducted by Wang et al. (2012), the mechanism of drought tolerance in tomato (*Lycopersicon esculentum*) induced by PGPR (Plant Growth-Promoting Rhizobacteria) was investigated. Various parameters including relative water content, malondialdehyde (MDA) content, relative electrical conductivity, chlorophyll content, root recovery intention, antioxidant enzyme activities, and the expression of cytosolic ascorbate peroxidase (cAPX) gene, monodehydroascorbate reductase (MDHAR) gene, and Rubisco gene were analyzed under drought stress conditions. The findings revealed that PGPR treatment significantly increased

relative leaf water content, doubled relative root recovery intention, enhanced relative chlorophyll content, and maintained a high level of relative Rubisco expression. Moreover, a notable decrease was observed in relative MDA content and relative electrical conductivity. Additionally, the application of PGPR led to elevated activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), along with up-regulated expression of cAPX and MDHAR under drought-stress conditions.

Regarding the role of PGPR under drought stress, extensive research was done on the possible contribution of host plants and PGPR interactions in the rhizosphere to the reduction of drought stress. In addition to facilitating significant nutrient and water uptake, these symbiotic partnerships may also be integrated as a long-term strategy to enhance crop growth and productivity in the face of water constraints. In order to create practical applications of these natural symbioses, a deeper understanding of the mechanisms underlying the interactions between PGPR and plants is becoming important. Tallapragada et al. (2016) reported that PGPR and its dual inoculation had positive impacts on the growth of tomato (*Lycopersicon esculatum*) and bell pepper (*Capsicum annuum*) and plants under drought stress. A complete randomized block design was used, with six replications of each treatment. The plants treated with these advantageous microbes showed a considerable increase in biomass, root length, shoot length, and chlorophyll content. Proline accumulation was shown to be reduced in PGPR-inoculated plants, pointing to its potential significance in reducing the effects of water stress. The findings of this study showed how crucial the PGPR is for DR.

In another investigation launched by Wang et al. (2019), greenhouse screening was used to assess the capacity of PGPR to induce drought tolerance in tomato plants (*S. lycopersicum*). According to the findings, PGPR considerably improved DR by raising root vigor, relative water content, and survival rates. Malondialdehyde concentrations were found to be lower and antioxidant enzyme activities to be more abundant. These modifications happened in the cellular defense responses. Moreover, plants exposed to PGPR showed higher expression levels of genes that respond to drought stress. Additionally, PGPR caused stomatal closure via a pathway controlled by abscisic acid. Additionally, biofilm formation

mutants were developed to examine how PGPR-induced drought tolerance relates to biofilm development. The findings demonstrated a favorable correlation between the capacity to generate biofilms and plant root colonization. Furthermore, while defective biofilm mutants were more susceptible to drought stress, plants inoculated with more PGPR were better able to resist drought stress. Together, these findings suggested that PGPR is beneficial for tomato plant root colonization and DR.

Tahiri et al. (2022) looked into the function that rewarding PGPR plays in enhancing tomato (*S. lycopersicum*) growth, yield, and DR. In this experiment, a completely random design was adopted, with the two treatments of control well-watered (WW) plants and water-stressed (WS) plants serving as the main factors and fertilization serving as a subfactor with eight treatments. Growth metrics, including fruit number and Weight, leaf number, and its area, shoot and root dry weight, productivity (sugar, protein, and polyphenols), mineral content (Ca^{2+} , Na^{+} , K^{+} , and P), and antioxidant enzyme activities (polyphenoloxidase, peroxidase, catalase, and superoxide dismutase), were evaluated to determine the effects of both factors. Following the experiment, the physicochemical and microbiological characteristics of the soil were analyzed to see how applied biofertilizers and water stress affected these variables. Plant growth features and yield were significantly impacted by water stress, and the antioxidant enzymes were out of balance. However, the deleterious effects of drought stress were lessened by the use of biofertilizers. For instance, PGPR treatments resulted in a substantial increase in shoot biomass when compared to the control. In fact, the treatment greatly enhanced the number of fruits per plant. The PGPR treatment was successful in increasing fruit yield. In terms of fruit quality, the results revealed a favorable impact on the amount of sugar and a significant reduction in the level of polyphenol content.

1.5. Cis Regulatory Elements

The Cis Regulatory Elements (CRE) are non-coding DNA segments that provide binding sites for transcription factors (TF) or other regulatory molecules. They ultimately direct how plants grow and develop, how cells differentiate, and how they react to different stresses (Lu et al., 2018). In general, CREs can be considered promoters and enhancers. It seems that enhancers are more variable than

promoters (Meng et al., 2021). Numerous distant enhancers with varied spatiotemporal activity can regulate a single gene because enhancers that control transcription are not location- or distance-dependent with respect to their homologous promoters (Meng et al., 2021).

To understand transcriptional control and utilize it for quality improvement, it is crucial to systematically identify CREs in plant genomes. By using some methods that investigated sequence conservation in the regions which are close to target genes, some significant results have been attained (Huang et al., 2020). Additionally, some databases, like PlantCARE (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>), can be used to find important CREs for each gene. However, there are a number of limitations, including the following: sequence conservation can be limited, detection of CREs far from their target genes seems impossible, and a lack of knowledge about their tissue specialization and their exact activities (Lu et al., 2018).

According to Espaa et al. (2017), CREs typically prefer to be placed in accessible chromatin areas. As a result, CREs are found and studied using genomes and epigenomics. RNA sequencing was used to identify CREs related to rice's ability to withstand salinity and drought stress (Mishra et al., 2018).

Modification of cis-regulatory elements within non-coding segments of DNA could lead to fine-tuning gene expression and produce a phenotypic variation (Swinnen et al., 2016). Crop improvement was seen in downregulating the genes of interest by modifying cis-regulatory areas in genes that control a desirable characteristic (Duan et al., 2016). Consequently, the development of modified alleles in these elements may result in the modification of transcript abundance and expression patterns, which would be extremely beneficial for crop improvement.

1.5.1 Cis Regulatory Elements in Tomato

In the promoter areas of genes, TFs bind to certain sequences. These areas, also known as cis-elements, are principally responsible for controlling the transcriptional regulation of biological processes. The variety of expression patterns

found in nature is a result of naturally occurring variance in promoter regions. As an example, the application of CRISPR/Cas9 technology to introduce mutations in the promoters of key regulators of meristematic activity resulted in enhanced inflorescence branching, increased locule number, and larger fruits in tomato (*S. lycopersicum*). These modifications within regulatory regions can give rise to novel alleles that have the potential to modify crop traits (Rodriguez-Leal et al., 2017).. It is believed that by combining these new deep learning frameworks and employing larger "omic" datasets, prediction ability may be improved even more. Additional layers of control, such as TF indirect binding through protein interactions and chromatin changes, could also be addressed. Designing unique alleles with particular expression patterns will be made possible by integrating these many CREs (Rodriguez-Leal et al., 2017).

Many controllers of organ weight and size in plants are members of the CYP78A family Dai et al., 2018). In a study carried out by Li et al. (2022), a single-nucleotide polymorphism was inserted into the tomato KLUH promoter to create allelic variation (SNP). Fruit weight was strongly correlated with this SNP. The SNP was found in a CRE. There were created a number of mutant alleles with different sizes. In all experimental evaluations, some of these mutant alleles consistently increased fruit weight while significantly reducing the percentage of undersized fruits. Several favorable alleles increased the weight of all fruits, according to further examination of fruit weight based on fruit location on the plant. Additionally, it was discovered that SIKLUH's allele types and transcriptional variations did not reliably show alterations in weight in fruit. This research not only offered a method for finding CRE but also demonstrated the great importance of modifying CREs to increase tomato yield.

The widely distributed and structurally diversified class of pigments known as carotenoids are produced in tomato plant (*S. lycopersicum*) fruits in a tissue-specific way. Although the genes involved in the carotenoid biosynthesis pathway have been identified and studied in tomato and other plants, their control is still unknown. Forty different tomato accessions were gathered and examined in a study designed by Koul et al. (2019). This research showed that the principal carotenoids had accumulated in the mature fruit tissue in distinct ways. The cis-acting

components of all the carotenoid pathway genes were sequenced in order to better understand the underlying regulatory mechanisms in carotenoid production and accumulation. It was suggested that the binding of the transcription factors at certain places in the CREs effectively controls the expression of carotenoid genes as well as the production of protein in ripe tomato fruits. Finally, the possibility that CREs were involved in the process of carotenoid production and accumulation was supported by the differential expression of these genes in various developmental organs. Significant knowledge of the regulatory agents involved in carotenoid biosynthesis in tomato was created by this work.

The critical role of sugar transporter proteins (SUTs), which are products of sucrose transporter genes, is evident in the transfer of sucrose between the production and consumption sites in tomato (*S. lycopersicum*). Arefin et al. (2021) asserts that the promoter regions of these genes provide insight into fruit development and the control of biological processes within the plants. Three of the SUT gene family members in tomato, SUT1, SUT2, and SUT4, had their promoter regions analyzed. In-silico tools, PlantCARE (Lescot et al., 2002) and PLACE (Higo et al., 1999), were employed to study the dispersion of cis-regulatory elements. The findings indicated a majority of cis-elements to be stress-responsive, with the least number of cis-elements related to hormonal regulation found in most tomato sucrose transporter genes. The tomato SUT gene, SUT4, housed the most cis-acting regulatory elements connected to hormonal control and stress response. This suggests that tomato SUT genes may exhibit a stronger response to stress than hormonal regulation. Moreover, the expression pattern of the tomato SUT genes was also investigated. The study ultimately pointed out the importance of examining cis-acting regulatory elements in the promoter regions of tomato SUTs in understanding the mechanisms behind productivity and fruit development.

Investigation of DRE/CRT cis-regulatory elements in response to stressors and dehydration-responsive element-binding (DREB) transcription factors showed that they were very important for creating tolerance in plants against abiotic stress. DREB genes and their promoter elements in *S. lycopersicum* were examined by Maqsood et al. (2022) using bioinformatics analysis. 58 SIDREB genes on 12 chromosomes during genome-wide identification were discovered in this research

project. In the 2500 base pairs upstream regulatory elements of 58 SIDREB genes, 103 motifs were detected. These 103 motifs were subdivided into seven categories by the cis-regulatory element analysis. These elements were linked to promoter binding sites, abiotic and biotic stress responses, development, hormonal changes, and light response. To investigate the promoter regions, Phytozome database (Goodstein et al., 2012) was used for the extraction of 2500 base pairs upstream promoter sequence of the SIDREB genes of *S. lycopersicum*. Plant Care (Lescot et al., 2002) and Solgenomics databases (<https://solgenomics.net>) were the main sources for the finding of these CREs.



2. MATERIALS AND METHODS

The experimental component of this research was carried out on the model plant, *Solanum lycopersicum*, specifically, the MSC-50 variety. For a selected set of these samples, an inoculation procedure was implemented using Plant Growth Promoting Rhizobacteria (PGPR), in this case, *Bacillus megaterium*. The objective of this inoculation was to assess the response of the MSC-50 variety to PGPR. Following the inoculation process, the samples were subjected to an induced drought stress condition. This condition was implemented by administering three distinct concentrations of Polyethylene Glycol (PEG), a common tool used to simulate drought stress in laboratory conditions. Two separate time points were chosen for this application, specifically, 2 hours and 12 hours following the PGPR treatment. The specifics of the PEG dosages and the related effects on the plant samples are elaborated in Tables 2.1 and 2.2. After the completion of the drought stress induction, a systematic collection of the plant samples was carried out. This sampling process involved both the leaves and roots of the plants. Post-collection, the samples were meticulously pulverized using liquid nitrogen. This process aids in maintaining the cellular structure of the samples, thereby allowing for more accurate analysis. The ground samples were then stored in Falcon tubes at a maintained temperature of -80 degrees Celsius. This stringent temperature control was implemented to preserve the biological and biochemical integrity of the samples, a critical aspect for subsequent analytical procedure

Table 2.1. Leaf Sample Treatments

Applied Dose of PEG	Samples
(0.25 mM PEG) 31 g of PEG per liter – 1116 g of PEG was used for 36 liters. (36 pots - 1 liter per pot)	MC: Control group of MSC-50 tomato variety with no application
	MP1-2h: PEG-treated samples (2 hours)
	MP1-12h: PEG-treated samples (12 hours)
	MBC: Untreated control sample of MSC-50 variety inoculated with 113- <i>B. megatrium</i>
	MP1B-2h: PEG-treated samples (2 hours) included with 113- <i>B. megatrium</i>
	MP1B-12h: PEG-treated samples (12 hours) included with 113- <i>B. megatrium</i>
(0.50mM PEG) 50 g of PEG per liter – 1500 g of PEG was used for 30 liters. (30 pots - 1 liter per pot)	MP2-2h: PEG-treated samples (2 hours)
	MP2-12h: PEG-treated samples (12 hours)
	MP2B-2h: PEG-treated samples (2 hours) included with 113- <i>B. megatrium</i>
	MP2B-12h: PEG-treated samples (12 hours) included with 113- <i>B. megatrium</i>
(0.75mM PEG) 65.5 g of PEG per liter – 1179 g of PEG was used for 18 liters. (24 pots - 750 ml per pot)	MP3-2h: PEG-treated samples (2 hours)
	MP3-12h: PEG-treated samples (12 hours)
	MP3B-2h: PEG-treated samples (2 hours) included with 113- <i>B. megatrium</i>
	MP3B-12h: PEG-treated samples (12 hours) included with 113- <i>B. megatrium</i>

Table 2.2. Root Sample Treatments

Applied Dose of PEG	Samples
(0.25 mM PEG) 31 g of PEG per liter – 1116 g of PEG was used for 36 liters. (36 pots - 1 liter per pot)	MC: Control group of MSC-50 tomato variety with no application
	MP1-2h: PEG-treated samples (2 hours)
	MP1-12h: PEG-treated samples (12 hours)
(0.50mM PEG) 50 g of PEG per liter – 1500 g of PEG was used for 30 liters. (30 pots - 1 liter per pot)	MP2-2h: PEG-treated samples (2 hours)
	MP2-12h: PEG-treated samples (12 hours)
(0.75 mM PEG) 65.5 g of PEG per liter – 1179 g of PEG was used for 18 liters. (24 pots - 750 ml per pot)	MP3-2h: PEG-treated samples (2 hours)
	MP3-12h: PEG-treated samples (12 hours)

2.1. RNA Isolation

RNA isolation was performed based on a method adopted by Bray (1988) with a slight modification. First of all, 300 mg of the sample was weighed in 1.5 ml Eppendorf tubes, then 500 µl of the extraction solution containing 50 mM Tris with pH 9, 150 mM LiCl, 5 mM EDTA, 5% SDS was added to the Eppendorf using a pipette. Then it was vortexed for 15 seconds and centrifuged for 10 minutes in a centrifuge at +4°C, at 10,000 rpm. The upper phase was carefully taken and added to the solution at a ratio of 1/1 phenol chloroform isoamyl alcohol and vortexed for 15 seconds, and centrifuged at +4°C at 10,600 rpm for 5 minutes. In the next step, the liquid lying above (supernatant) was separated again and 10M LiCl was added to half of it (125 microliters in case the supernatant is 250 microliters) and incubated for 16 hours at +4°C. Then, the samples were centrifuged at +4°C at 11,000 rpm for 40 minutes and the upper phase was carefully discarded without damaging the pellet. In the next phase, 1000 µl of 70% ethanol was added to the Eppendorf tubes and centrifuged at 10600rpm at +4°C for 5 minutes and the supernatant was discarded (this step was repeated), afterward, the supernatant was removed and the

pellet was dried in the Eppendorf tube, and dissolved with 30 microliters of DEPC-treated Water.

2.2.Purification of RNAs Extracted from DNA

DNase I RNase Free (Thermo) was used to remove genomic DNA from total RNA using the manufacturer's protocol and controlled for quantity and quality on agarose gel.

Application of DNase to Total RNA Step Procedure:

- RNA 1 μ g
- 10X reaction buffer with $MgCl_2$ 1 μ L
- DNase I, RNase-free (#EN0521) 1 μ L (1 u)
- Water, nuclease-free total 10 μ L

Incubation at 37°C for 30 minutes.

- EDTA 50 mM 1 μ L

Incubation at 65°C for 10 minutes

The quality of RNA extracted in 1% agarose gel was evaluated by electrophoresis (Fig. 2.1). The obtained RNAs were kept at -20 degrees Celsius until the next step.

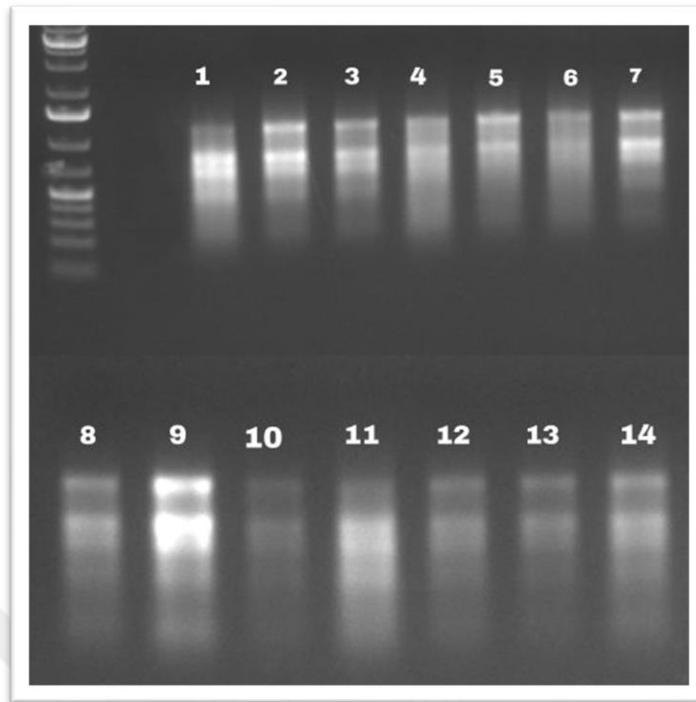


Figure 2.1. Leaf-specific RNA integrity results in the gel electrophoresis (1-MC, 2- MP1-2h, 3- MP1-12h, 4- MP2 -2h, 5- MP2 -12h, 6- MP3-2h, 7- MP3-12h, 8- MBC, 9- MP1B-2h, 10- MP1B-12h, 11- MP2B -2h, 12- MP2B -12h, 13- MP3B -2h, 14- MP3B -12h)

2.3. cDNA Synthesis

At this stage, the cDNA kit (Thermo Fisher, USA) was used to synthesize cDNA from total RNA, and the experiment was carried out according to the manufacturer instructions:

1- Each sample was analyzed using BiO1D software using 500 ng of RNA according to the ratio of mRNA bands determined by running on the gel.

2- A solution with a final volume of 12 microliters consisting of 500 nanograms of RNA, 1 microgram of oligo(dT)₁₈, and dH₂O was prepared and incubated in a heat block at a temperature of 65 degrees Celsius for 5 minutes. After incubation, the tubes were immediately placed on ice.

3- Then in a separate Eppendorf, 4 microliters of 5X reaction buffer, 1 microliter of RiboLock RNase Inhibitor, 2 microliters of dNTP and 1 microliter of

RevertAid Reverse Transcriptase RNA were mixed and 8 microliters of it were added to each sample.

4- The samples were incubated in a heat block at a temperature of 42°C for 1 hour and then placed at a temperature of 70°C for 5 minutes. After incubation, the tubes were immediately placed on ice.

5- Obtained cDNAs were divided into several Eppendorfs and stored at minus 20 degrees Celcius until the next step to prevent multiple melting.

2.4. Gene Sequence Identification

The nucleotide sequences of the genes whose expression will be analyzed were obtained via the Solgenomics database (<https://solgenomics.net/organism/Solanum%20lycopersicum/view>) and similar genes were searched using the NCBI and its Blast tool. The Gene ID of the genes studied in this research can be found in Table 2.3.

Table 2.3. Gene IDs

Gene Name	Ensembl Gene ID
SINAC1	Solyc01g009860
SINAC2	Solyc01g021730
SINAC3	Solyc01g094490
SINAC4	Solyc01g102740
SINAC5	Solyc01g104900
SINAC6	Solyc02g036430
SINAC7	Solyc02g061780
SINAC8	Solyc02g061870
SINAC9	Solyc02g061900
SINAC10	Solyc02g061910
SINAC11	Solyc02g062060
SINAC12	Solyc02g062070
SINAC13	Solyc02g069960

SINAC14	Solyc02g070140
SINAC15	Solyc02g077610
SINAC16	Solyc02g081270
SINAC17	Solyc02g084350
SINAC18	Solyc02g087920
SINAC19	Solyc02g088180
SINAC20	Solyc02g093420
SINAC21	Solyc03g059300
SINAC22	Solyc03g062670
SINAC23	Solyc03g062750
SINAC24	Solyc03g078120
SINAC25	Solyc03g080090
SINAC26	Solyc03g083880
SINAC27	Solyc03g097650
SINAC28	Solyc03g098190
SINAC29	Solyc03g114220
SINAC30	Solyc03g114260
SINAC31	Solyc03g115850
SINAC32	Solyc04g005610
SINAC33	Solyc04g009440
SINAC34	Solyc04g025760
SINAC35	Solyc04g072220
SINAC36	Solyc04g078670
SINAC37	Solyc04g079940
SINAC38	Solyc05g007550
SINAC39	Solyc05g007770
SINAC40	Solyc05g009840
SINAC41	Solyc05g021090
SINAC42	Solyc05g026180
SINAC43	Solyc05g055470
SINAC44	Solyc05g055480
SINAC45	Solyc06g008360
SINAC46	Solyc06g034340
SINAC47	Solyc06g060230

SINAC48	Solyc06g061080
SINAC49	Solyc06g063380
SINAC50	Solyc06g063430
SINAC51	Solyc06g065410
SINAC52	Solyc06g068580
SINAC53	Solyc06g069100
SINAC54	Solyc06g069710
SINAC55	Solyc06g073050
SINAC56	Solyc06g074170
SINAC57	Solyc07g006840
SINAC58	Solyc07g045030
SINAC59	Solyc07g053590
SINAC60	Solyc07g053680
SINAC61	Solyc07g062240
SINAC62	Solyc07g062840
SINAC63	Solyc07g063410
SINAC64	Solyc07g063420
SINAC65	Solyc07g066330
SINAC66	Solyc08g006020
SINAC67	Solyc08g007020
SINAC68	Solyc08g008660
SINAC69	Solyc08g028850
SINAC70	Solyc08g074300
SINAC71	Solyc08g077110
SINAC72	Solyc08g079120
SINAC73	Solyc09g010160
SINAC74	Solyc09g025310
SINAC75	Solyc10g005010
SINAC76	Solyc10g006880
SINAC77	Solyc10g047060
SINAC78	Solyc10g055760
SINAC79	Solyc10g079220
SINAC80	Solyc10g083450
SINAC81	Solyc11g005920

SINAC82	Solyc11g008000
SINAC83	Solyc11g008010
SINAC84	Solyc11g017470
SINAC85	Solyc11g018660
SINAC86	Solyc11g022480
SINAC87	Solyc11g065540
SINAC88	Solyc11g068620
SINAC89	Solyc11g068750
SINAC90	Solyc12g013620
SINAC91	Solyc12g017400
SINAC92	Solyc12g036480
SINAC93	Solyc12g056790
SINHX1	Solyc06g008820.2
SINHX2	Solyc04g056600.2
SINHX3	Solyc01g067710.2
SINHX4	Solyc01g098190.2

2.5. Designing Specific Primers for *NAC* and *NHX* Genes

The sequences of *SINAC* and *SINHX* genes of *Arabidopsis* was extracted from the TAIR database and in order to find the similar sequences of tomato, using the blast tool on the solgenomics database and the prepared sequences were re-checked for certainty in NCBI and the specific primers were designed in the Eurofins genomics database (<https://eurofinsgenomics.eu/en/ecom/tools/pcr-primer-design/>). Table 2.4 contains the primer sequences list used in this study. Each primer was evaluated for effectiveness with cDNA produced using standard PCR equipment, and the results were verified on a 1% agarose gel.

Table 2.4. Primer Sequences

Primer	Sequence
LeActinF	GCCGGGCGTGATCTTACTGA
LeActinR	AGCTACTCCTGGCGGTCTCC
<i>SINAC1F</i>	CTCGCCCGTTGTTTCAGTCA
<i>SINAC1R</i>	TTGAAGCTGCAACTCCATTCTG
<i>SINAC2F</i>	GCCTGCTCTAATGACGACGA
<i>SINAC2R</i>	TCCAATAACTGGTCCGCCTT
<i>SINAC3F</i>	GCGGAGAAGGAACCATGGGA
<i>SINAC3R</i>	CCAACACTTTCTTGGCTGGGT
<i>SINAC4F</i>	TTGGGAGCTTCCTGCTTTGG
<i>SINAC4R</i>	GACCGCGAATTCTCCGTTCT
<i>SINAC6F</i>	AAGCTGGGTACTGGAAGGCT
<i>SINAC6R</i>	CCGGTTCGAGTTCCATGAGG
<i>SINAC7F</i>	GGTCCTAAAGGGAGGCCCAA
<i>SINAC7R</i>	GCCTTTAGGTGCCCTTCCAC
<i>SINAC8F</i>	TGGCAACAAGAAGCCATCTCA
<i>SINAC8R</i>	AAGCGGAATCTTCTCTTGAAACCA

<i>SINAC9F</i>	GGAACGTTGGCCCAGTTACTT
<i>SINAC9R</i>	GTTGCGGGATGTTAGGAACCA
<i>SINAC10F</i>	TGTCGCATTACAAGGAGTGTCA
<i>SINAC10R</i>	ATGAACAGAGCCTTGACGCA
<i>SINAC11F</i>	GCGGCTCATGGAGAGCATAC
<i>SINAC11R</i>	TGACCACGTAGTCTCAAGTCCT
<i>SINAC12F</i>	ACTGTGAAATCCAGTGCTGCT
<i>SINAC12R</i>	TGCGCCGAACTAACAGAGG
<i>SINAC13F</i>	AGCCAACAAAGCTCAGGAACA
<i>SINAC13R</i>	AGTTCATCCCAGTTGCCATGT
<i>SINAC14F</i>	ACCAAACACAACCTAGACAATGTGG
<i>SINAC14R</i>	AGCCATCACCATGAACAACCA
<i>SINAC15F</i>	TCAACATACGTGGCCTCCCA
<i>SINAC15R</i>	TGAGCTTCATCCGTGTTGGC
<i>SINAC16F</i>	AGCTCCATGTCTCTCGTCTCC
<i>SINAC16R</i>	AGCAACATTCCCAAATCGGAACA
<i>SINAC17F</i>	TGGAAGGCCACTGGAAGTGA
<i>SINAC17R</i>	TCGTGCATCATCCAATCCGT

<i>SINAC18F</i>	CCAAAGGTTCTTGACAGTGGCT
<i>SINAC18R</i>	CCCTGCTTCTGTTGCTCTGT
<i>SINAC19F</i>	ATGAGGAAGCCAGTGTGGGA
<i>SINAC19R</i>	GCCCTGGTTCTTTGGCAACT
<i>SINAC20F</i>	ACTCCTTTGAAGAAGCGGAGGA
<i>SINAC20R</i>	ACCTGCACTTTCTCTTAAACCCT
<i>SINAC21F</i>	GGAAAGGGCAAACCGCAGAA
<i>SINAC21R</i>	CCTTAAAGAACTATCCGCAACACG
<i>SINAC22F</i>	TGGAGATCAACCACCATGGGA
<i>SINAC22R</i>	TTCTGCGGTTTGCCCTTTCC
<i>SINAC23F</i>	TTTGTTTGGGCTGCGTTGAA
<i>SINAC23R</i>	GTGGAAGCTTCGCCATTGGA
<i>SINAC24F</i>	GGCTGGTTGAGCCAGACTTC
<i>SINAC24R</i>	CGCCTTTAGCGCTGTAGCTT
<i>SINAC25F</i>	AGAGCCACAACAGCAGGATT
<i>SINAC25R</i>	AGACCATGAGGAGCCCTTCC
<i>SINAC26F</i>	TCCGAACGGATTCAGAACGA
<i>SINAC26R</i>	ACGTCTTCTTCATCCCAATAGCG

<i>SINAC27F</i>	ACGACTACTCCACGAGGGAA
<i>SINAC27R</i>	CAACTCCGTGGCCTTCTTGT
<i>SINAC28F</i>	TGGAGAAGAGAGAGACAGCGT
<i>SINAC28R</i>	TCTGCAACCACTCGTCAGGA
<i>SINAC29F</i>	TGAGGACAAACAGGGCAACT
<i>SINAC29R</i>	ACCTCTTGGAGCCCTTCCTC
<i>SINAC30F</i>	GCCAAATAGGGCAGCTGTGT
<i>SINAC30R</i>	TGGCCTAGTTGGTTGAGTCCT
<i>SINAC31F</i>	GCCCACACAATTAGCACCGA
<i>SINAC31R</i>	GCGCTCTGAACCTCCTTGTC
<i>SINAC32F</i>	CGGTGATAAAGAGCCAGCTGAA
<i>SINAC32R</i>	ACCCAATTCGTGATCTTTCCTTGT
<i>SINAC33F</i>	CTGTTTCCGGTACGGTCGTG
<i>SINAC33R</i>	ACGCGTACCCACCACAATTT
<i>SINAC34F</i>	TAGTTGTGCAGCCTGGACCT
<i>SINAC34R</i>	CTGTTGCTGCTGCTGGATGA
<i>SINAC35F</i>	CCATCGCGTTACCGGAGAAT
<i>SINAC35R</i>	TGCATCAGCAGCCATATTCAATG

<i>SINAC36F</i>	AGGCCATGCCCTGTCTCAAT
<i>SINAC36R</i>	TCAGTGCCAGTAGCCTTCCA
<i>SINAC37F</i>	AATGGTGGGACAGCGAGTCA
<i>SINAC37R</i>	CGGGTCCTAAACGCGCATAA
<i>SINAC38F</i>	AGCAACTGGTATTGACAAGCCT
<i>SINAC38R</i>	TGGAAGTTGGTGGGAGACGA
<i>SINAC39F</i>	TGGAGCAAGGTCTGCATCTG
<i>SINAC39R</i>	TCAGGGTTGTCAGGGTCAGG
<i>SINAC40F</i>	TGTTGGGCGGTATTCCTGCT
<i>SINAC40R</i>	AACCCGTCCATCCCATTGCT
<i>SINAC41F</i>	GGGACCACCAACTGGTGACA
<i>SINAC41R</i>	GCAATCTTGGGCGGAGCATC
<i>SINAC42F</i>	TGGACAATCATGAAGGAAGTATGCC
<i>SINAC42R</i>	GGCAAGGTCGATGGGCGAT
<i>SINAC43F</i>	TGTAGCTGCACCTCCTGGTT
<i>SINAC43R</i>	TGGAGCACTCGCCAATCAGT
<i>SINAC44F</i>	AACACGTGCTCTCACCCGAA
<i>SINAC44R</i>	TGCAGCGGCGACATCTGATA

<i>SINAC45F</i>	TGACCCATGGGACCTTCCAG
<i>SINAC45R</i>	TGTCTTTCCCTGTGGCTTTCCA
<i>SINAC46F</i>	GCCAGTACACCAGCAGCAAG
<i>SINAC46R</i>	ACATTGCAAAGTCGGGAATCA
<i>SINAC47F</i>	ACGCCGCAGAATGAGTGGTA
<i>SINAC47R</i>	TGTCTCTCCCTGTTGCCTTCC
<i>SINHX1F</i>	GCGTCGAGCACCATCTTAGG
<i>SINHX1R</i>	TCACGGTCAGTAGAGTGCCT
<i>SINHX2F</i>	CTCCTGCTCCTCGTTCTCCA
<i>SINHX2R</i>	AAGGACCTGGGTGAAGCTGT
<i>SINHX3F</i>	GCGAGGGCTGCTAATGTGTT
<i>SINHX3R</i>	TGACTGCAAAGCAAGGGCAA
<i>SINHX4F</i>	TGGTGGGCTGGTTTAATGCG
<i>SINHX4R</i>	TTGGGTGTGGCCAAATCTCG

2.6. Real-Time PCR Test

The Real-Time PCR reaction was carried out using a Real-Time PCR machine (LightCycler 480 II, Roche). RealQ Plus 2x Master Mix Green qPCR Master Kit (ref) was used in 96 plates and the actin gene was selected as the reference gene. The peak profile was determined for each analyzed gene in each

sample. Ct (Cycle Threshold) values were generated using peak profiles. Relative expression values were calculated based on the $2^{-\Delta\Delta CT}$ method using Ct values.

- 1µl of cDNA,
- 12.5µl RealQ Plus 2x Master Mix
- 2µL Forward primer
- 2µL reverse primer
- 7.5microliter dH2O

Propagation was done on tubes with a total volume of 25 µl.

2.7. Cis-regulatory Element Analysis

To assess the promoter regions, the Solgenomics database (<https://solgenomics.net/>) was adopted to identify the sequences. Phytozome database (<https://phytozome-next.jgi.doe.gov/>) was employed to obtain a 2000 base pairs in promoter sequence of the 93 *NAC* and 4 *NHX* genes of the *S. lycopersicum* v4.0 genome. Plant CARE database (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>) (Lescot et al., 2002) was used to investigate CREs.

3. RESULTS

3.1. Expression Profile of *SINAC* Genes in leaves of tomato plants

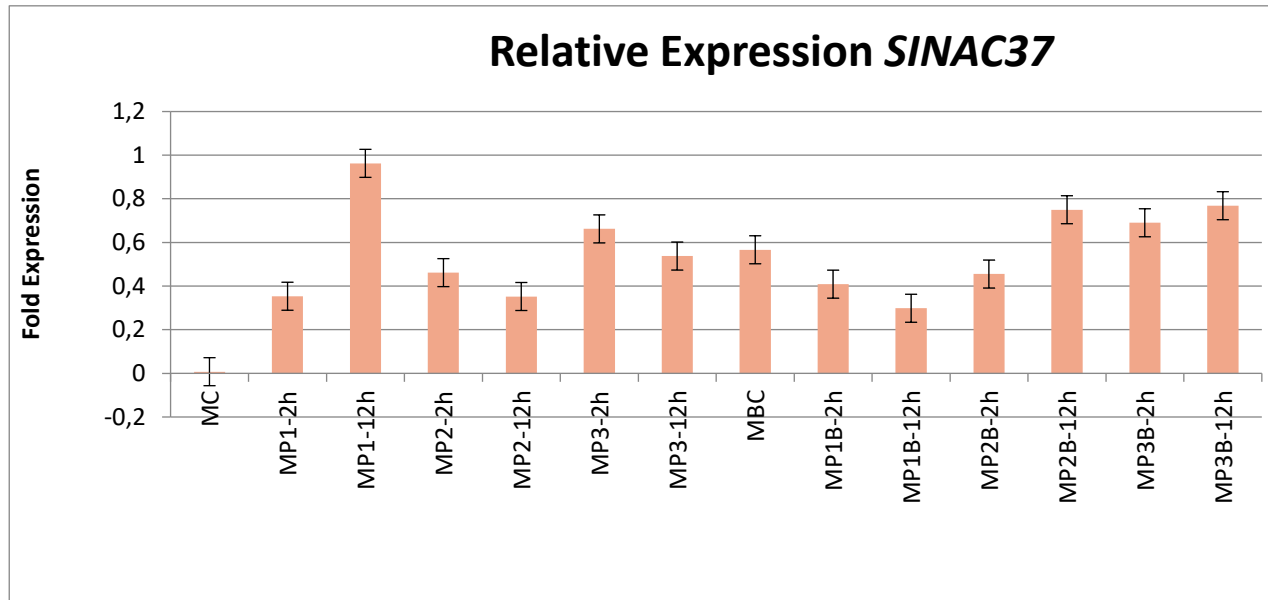


Figure 3.1. The Relative expression profile of *SINAC37* in leaves

SINAC37 gene expression in tomato plants was upregulated by polyethylene glycol (PEG) treatment at all three concentrations. The upregulation was significant after 12 hours of exposure in MP1, compared to the control sample. Additionally, the presence of PGPR strain 113-Bacillus megaterium positively regulated *SINAC37* expression. At MP2, *SINAC37* transcripts showed a slight downregulation at 2 hours and an upregulation at 12 hours. At MP3, *SINAC37* expression was slightly upregulated at both 2 hours and 12 hours. These findings highlight the influence of PEG-induced drought stress and PGPR treatment on *SINAC37* expression in tomato plants. (Fig.3.1).

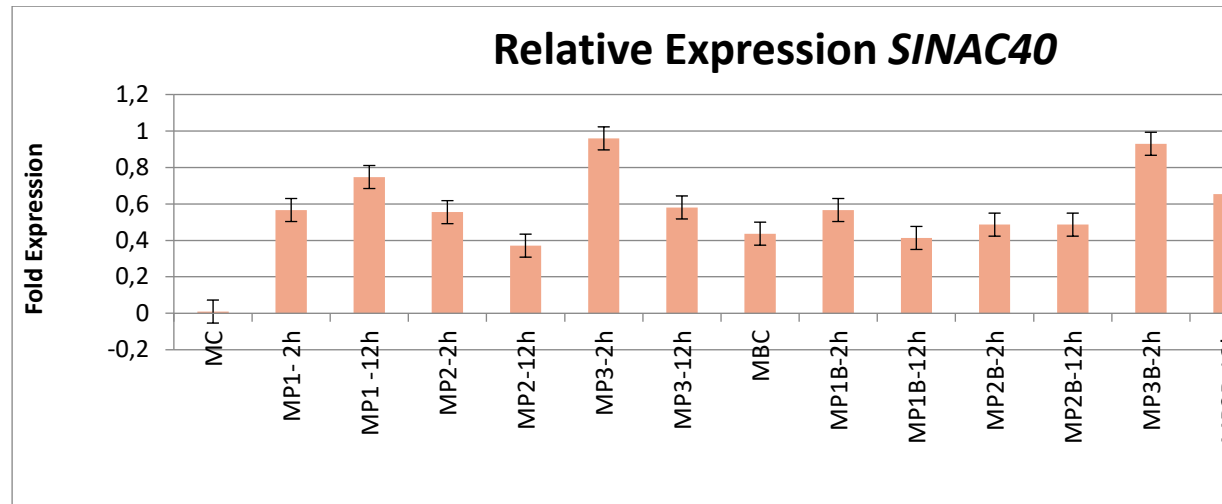


Figure 3.2. The Relative expression profile of *SINAC40* in leaves

The transcript abundance of the *SINAC40* gene was upregulated by polyethylene glycol (PEG) treatment at both 2 hours and 12 hours. PEG treatment led to increased expression levels of *SINAC40* compared to the control sample. Moreover, the presence of plant growth-promoting rhizobacteria (PGPR) strain 113-Bacillus megaterium positively regulated the expression of *SINAC40* in tomato leaves. These results indicate the influence of PEG-induced drought stress and PGPR treatment on the expression of *SINAC40* (Fig.3.2).

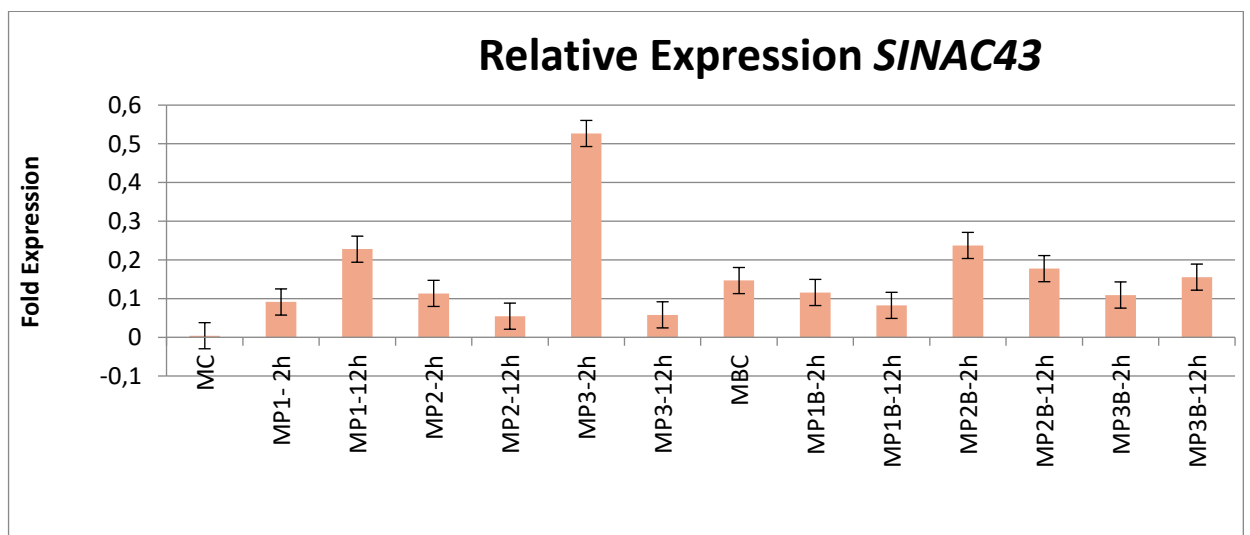


Figure 3.3. The Relative expression profile of *SINAC43* in leaves

The transcript abundance of *SINAC43* gene was upregulated by polyethylene glycol (PEG) treatment at both 2 hours and 12 hours. PEG treatment led to increased expression levels of *SINAC43* compared to the control sample. The presence of plant growth-promoting rhizobacteria (PGPR) strain 113-Bacillus megaterium further influenced the expression of *SINAC43*. These findings highlight the influence of PEG-induced drought stress and PGPR treatment on *SINAC43* expression in tomato plants (Fig.3.3).

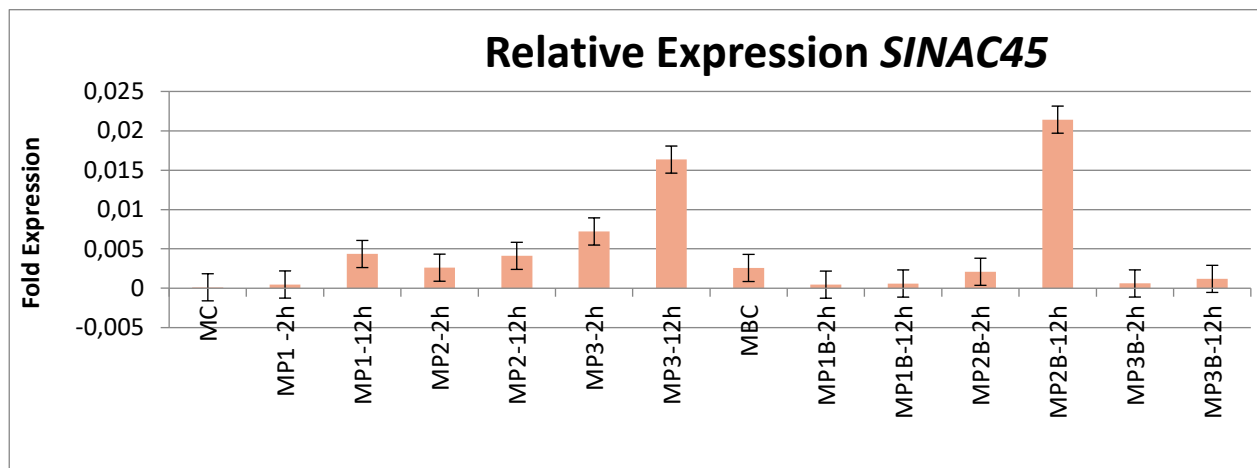


Figure 3.4. The Relative expression profile of *SINAC45* in leaves

The transcript abundance of *SINAC45* gene was upregulated by polyethylene glycol (PEG) treatment at both 2 hours and 12 hours. PEG treatment led to increased expression levels of *SINAC45* compared to the control sample. The presence of plant growth-promoting rhizobacteria (PGPR) strain 113-Bacillus megaterium further influenced the expression of *SINAC45*. These findings highlight the influence of PEG-induced drought stress and PGPR treatment on *SINAC45* expression in tomato plants (Fig.3.4).

3.2. Expression Profile of *SINHX* Genes in leaves of tomato plants

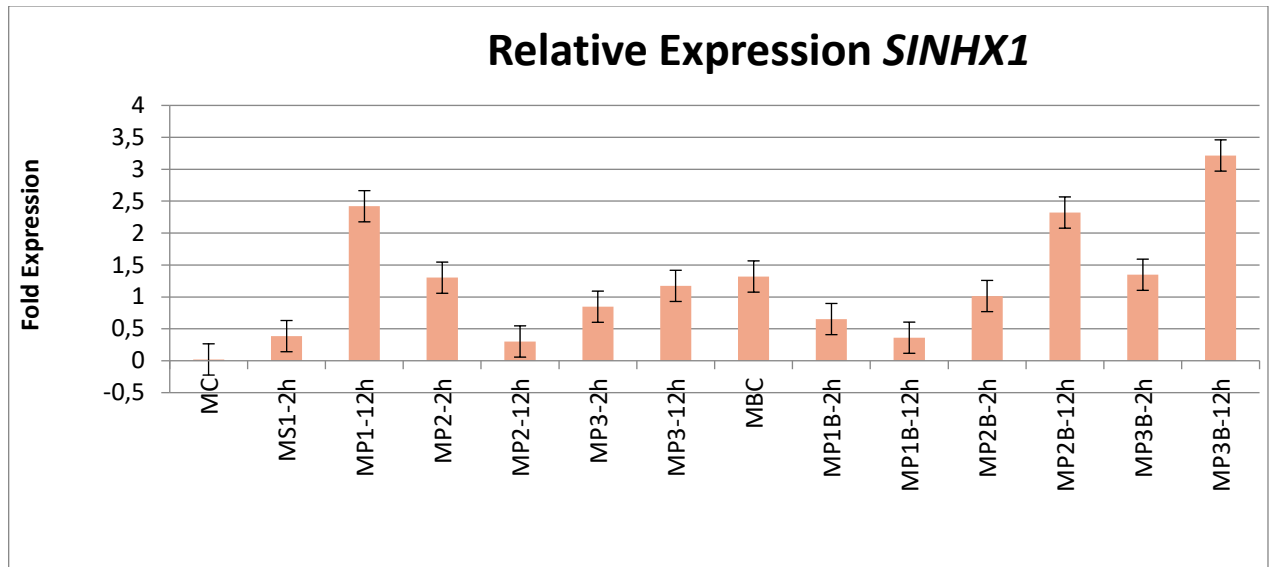


Figure 3.5. The Relative expression profile of *SINHX1* in leaves

The transcript abundance of *SINHX1* gene was upregulated by polyethylene glycol (PEG) treatment at both 2 hours and 12 hours. PEG treatment led to increased expression levels of *SINHX1* compared to the control sample. The presence of plant growth-promoting rhizobacteria (PGPR) strain 113-Bacillus megaterium further influenced the expression of *SINHX1*. These findings highlight the influence of PEG-induced drought stress and PGPR treatment on *SINHX1* expression in tomato plants. (Fig.3.5).

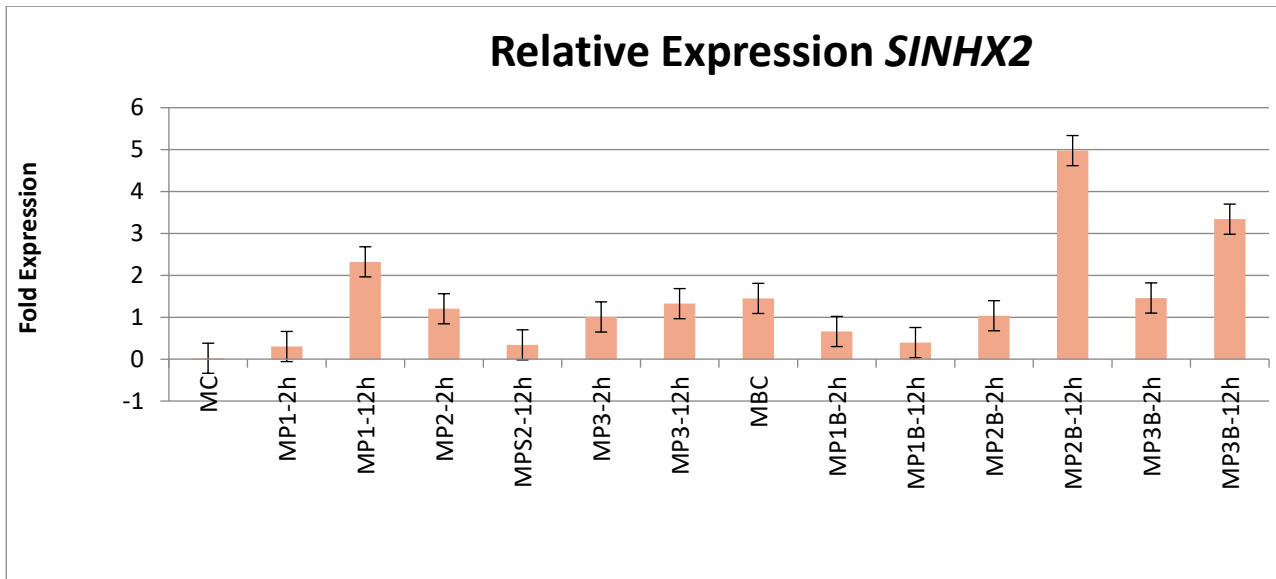


Figure 3.6. The Relative expression profile of *SINHX2* in leaves

The transcript abundance of *SINHX2* gene was upregulated by polyethylene glycol (PEG) treatment at both 2 hours and 12 hours. PEG treatment led to increased expression levels of *SINHX2* compared to the control sample. The presence of plant growth-promoting rhizobacteria (PGPR) strain 113-Bacillus megaterium further influenced the expression of *SINHX2*. These findings highlight the influence of PEG-induced drought stress and PGPR treatment on *SINHX2* expression in tomato plants (Fig.3.6).

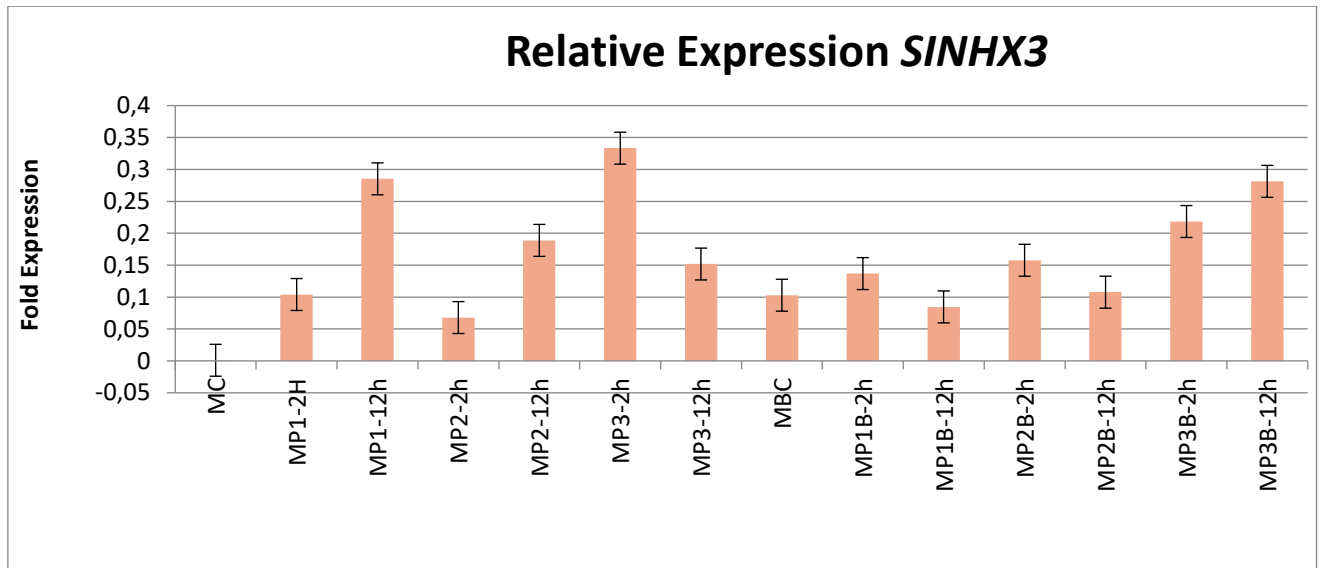


Figure 3.7. Relative expression profile of *SINHX3* in leaves

The transcript abundance of *SINHX3* gene was upregulated by polyethylene glycol (PEG) treatment at both 2 hours and 12 hours. PEG treatment led to increased expression levels of *SINHX3* compared to the control sample. The presence of plant growth-promoting rhizobacteria (PGPR) strain 113-Bacillus megaterium further influenced the expression of *SINHX3*. These findings highlight the influence of PEG-induced drought stress and PGPR treatment on *SINHX3* expression in tomato plants (Fig.3.7).

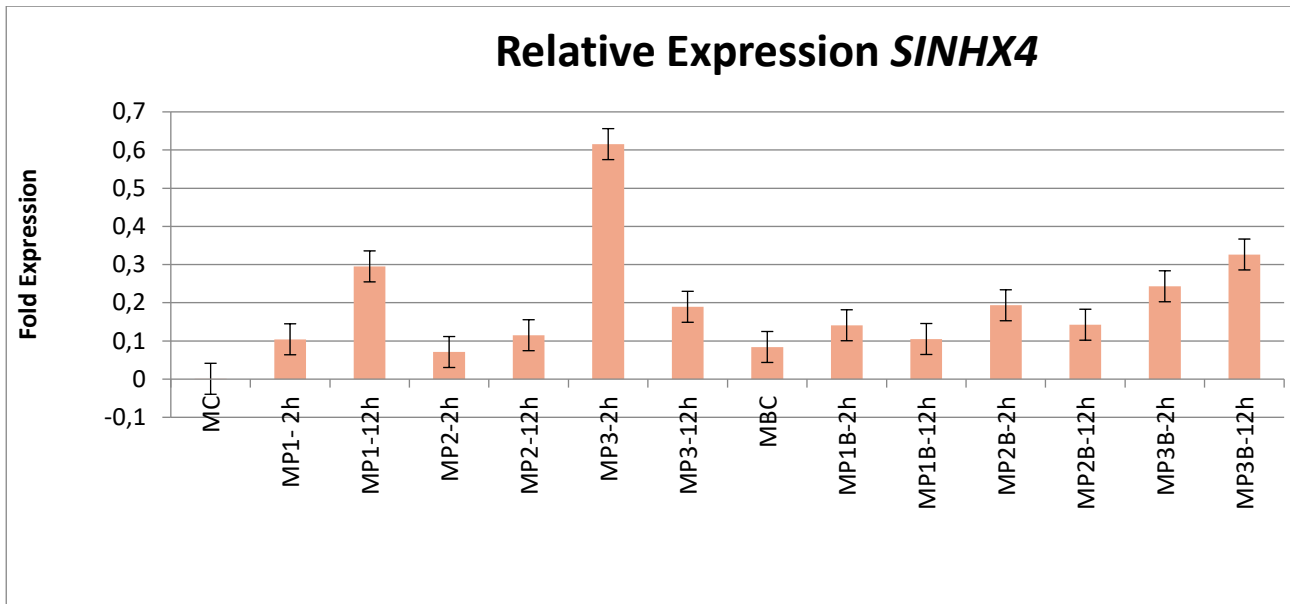


Figure 3.8. The Relative expression profile of *SINHX4* in leaves

The transcript abundance of *SINHX4* gene was upregulated by polyethylene glycol (PEG) treatment at both 2 hours and 12 hours. PEG treatment led to increased expression levels of *SINHX4* compared to the control sample. The presence of plant growth-promoting rhizobacteria (PGPR) strain 113-Bacillus megaterium further influenced the expression of *SINHX4*. These findings highlight the influence of PEG-induced drought stress and PGPR treatment on *SINHX4* expression in tomato plants (Fig.3.8).

3.3. Expression Profile of NAC Genes in roots of tomato plants

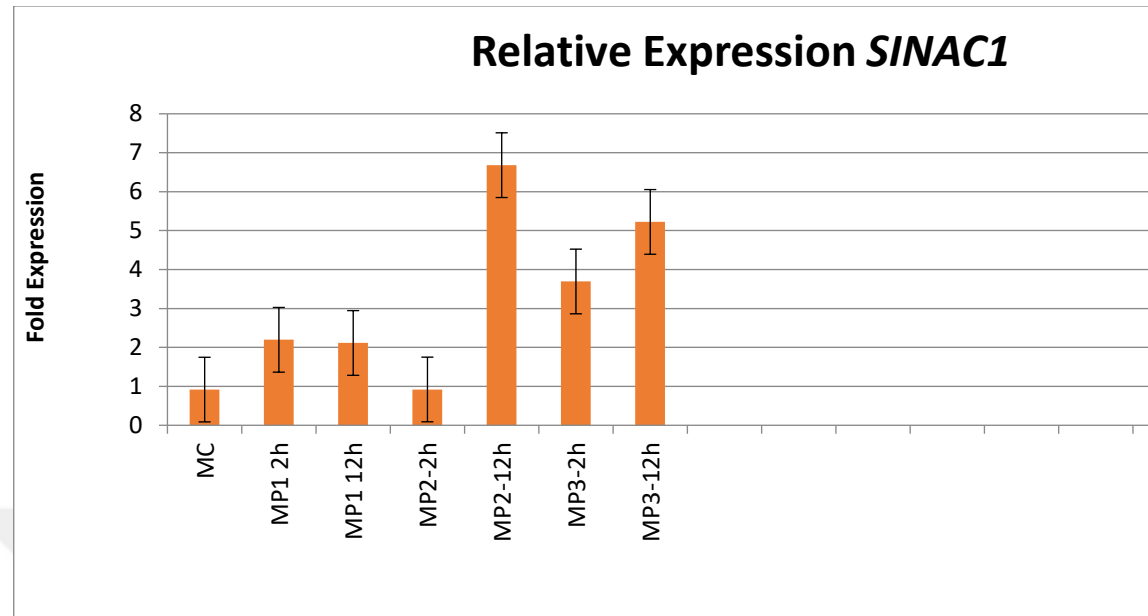


Figure 3.9. The Relative expression profile of *SINAC1* in roots

The transcript abundance of *SINAC1* gene was upregulated after 2 hours of treatment and remained elevated at 12 hours. These findings suggest a time-dependent response of *SINAC1* expression in tomato plants (Fig.3.9).

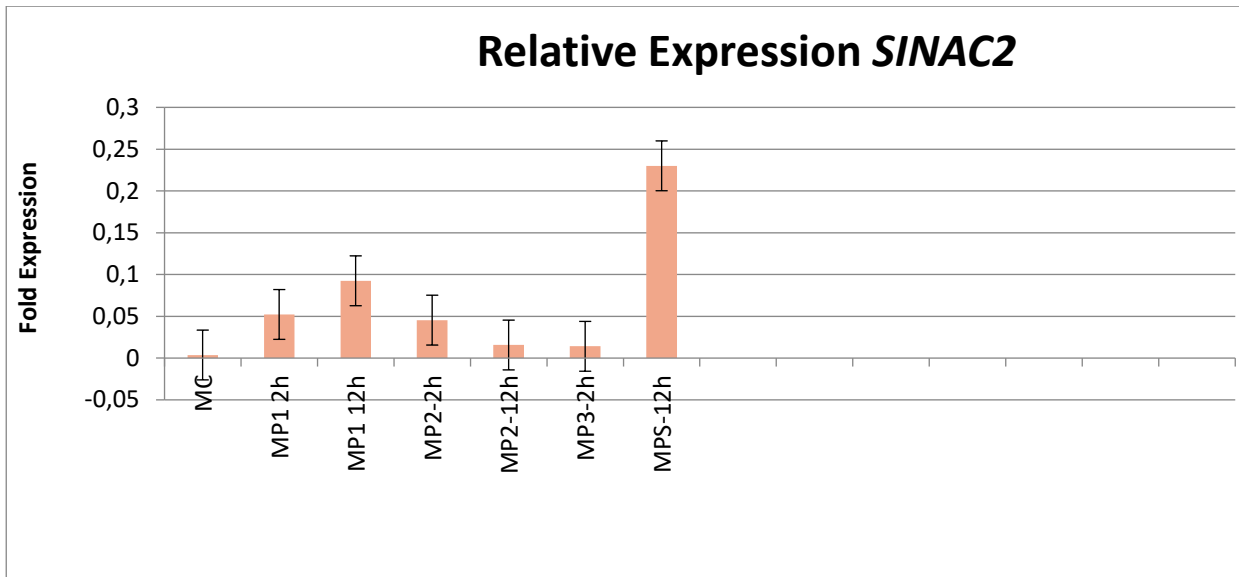


Figure 3.10. The Relative expression profile of *SINAC2* in roots

The transcript abundance of *SINAC2* gene was upregulated after 2 hours of treatment and remained elevated at 12 hours. These findings indicate a time-dependent response of *SINAC2* expression in tomato plants (Fig.3.10).

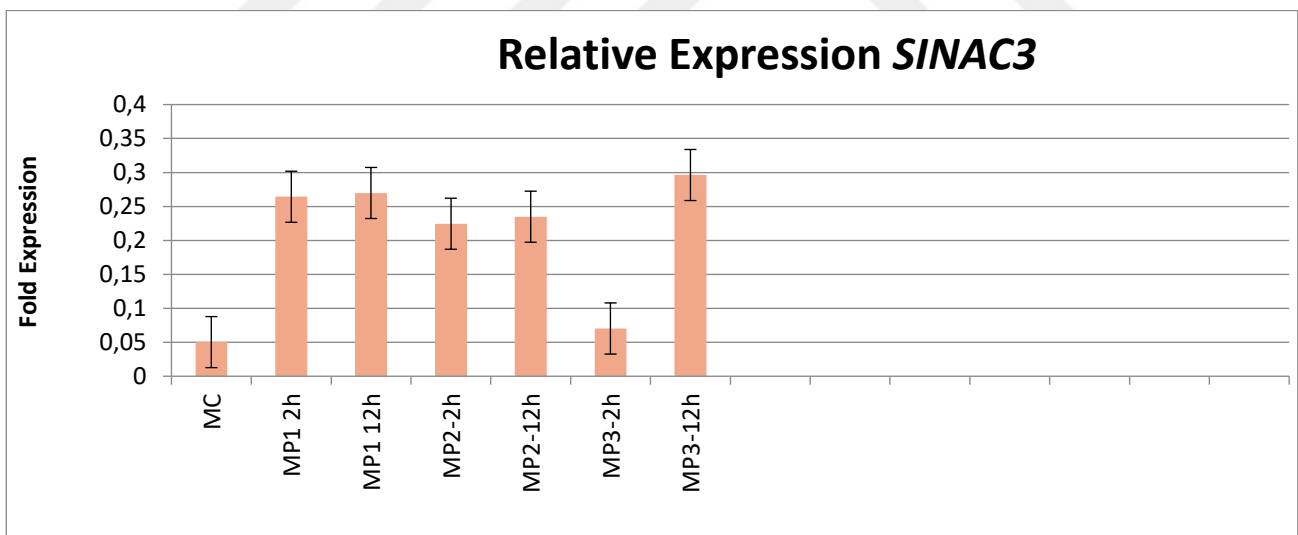


Figure 3.11. The Relative expression profile of *SINAC3* in root

The transcript abundance of *SINAC3* gene was slightly upregulated after 2 hours of treatment and remained relatively consistent at 12 hours. These findings suggest a time-dependent response of *SINAC3* expression in tomato plants (Fig.3.11).

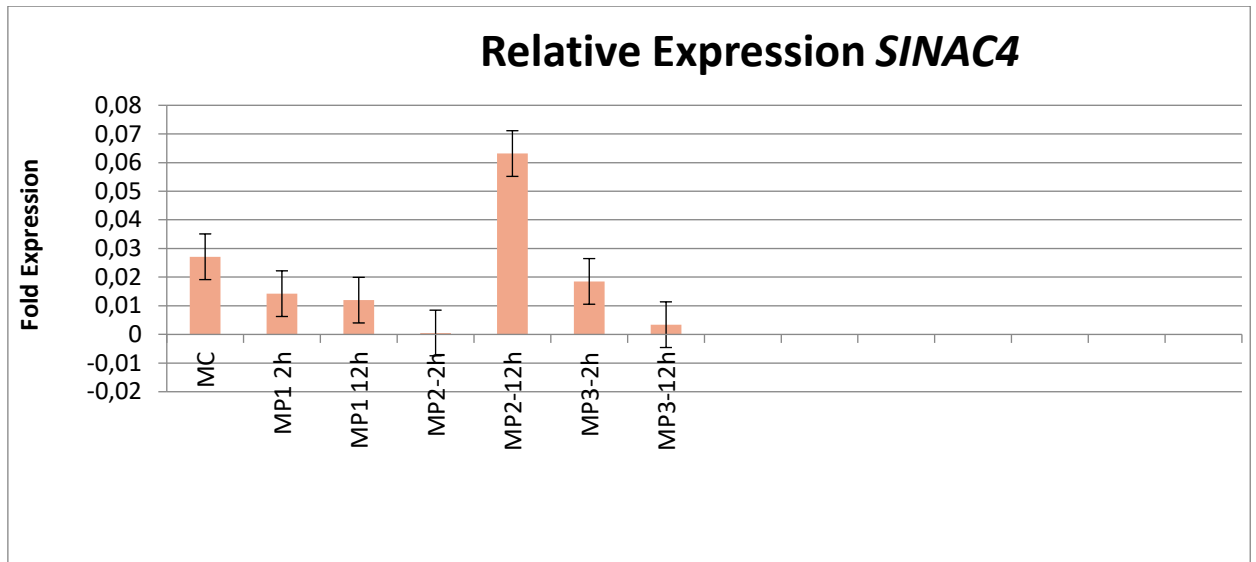


Figure 3.12. The Relative expression profile of *SINAC4* in root

The transcript abundance of *SINAC4* gene decreased after 2 hours of treatment and remained low at 12 hours. These findings suggest a time-dependent downregulation of *SINAC3* expression in tomato plants. (Fig.3.12).

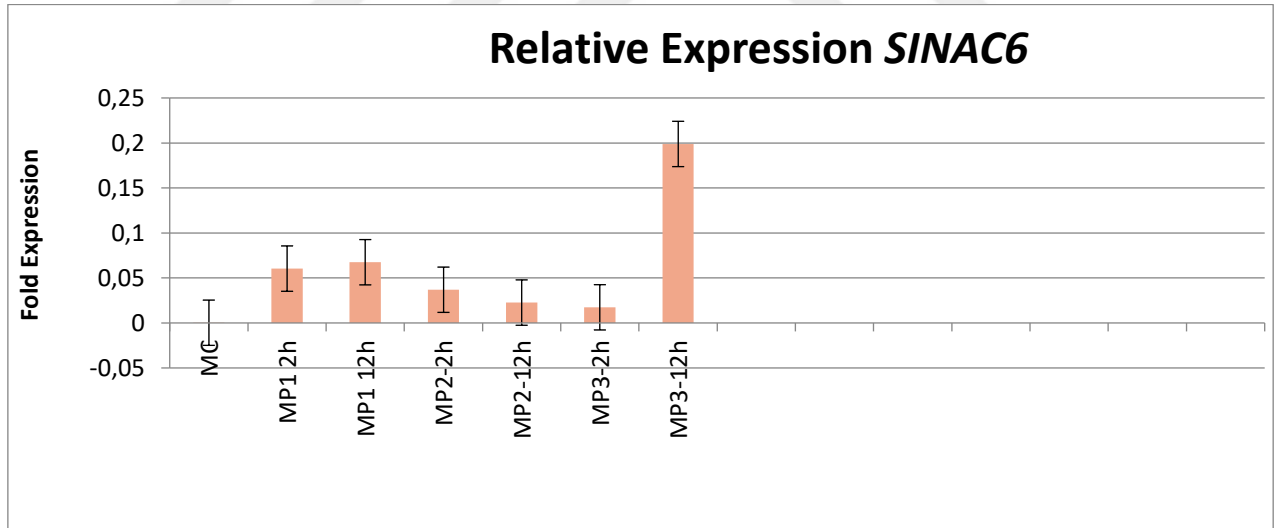


Figure 3.13. The Relative expression profile of *SINAC6* in root

The transcript abundance of the *SINAC6* gene exhibited a significant increase after 2 hours of treatment, with expression levels rising compared to the control sample. This upregulation was maintained at 12 hours, indicating a sustained response to the treatment. These findings suggest that the treatment applied to the

plants had a stimulating effect on the expression of the *SINAC6* gene, resulting in elevated expression levels. This time-dependent upregulation of *SINAC6* expression highlights the dynamic nature of gene regulation in response to the specific treatment applied to the tomato plants. (Fig.3.13).

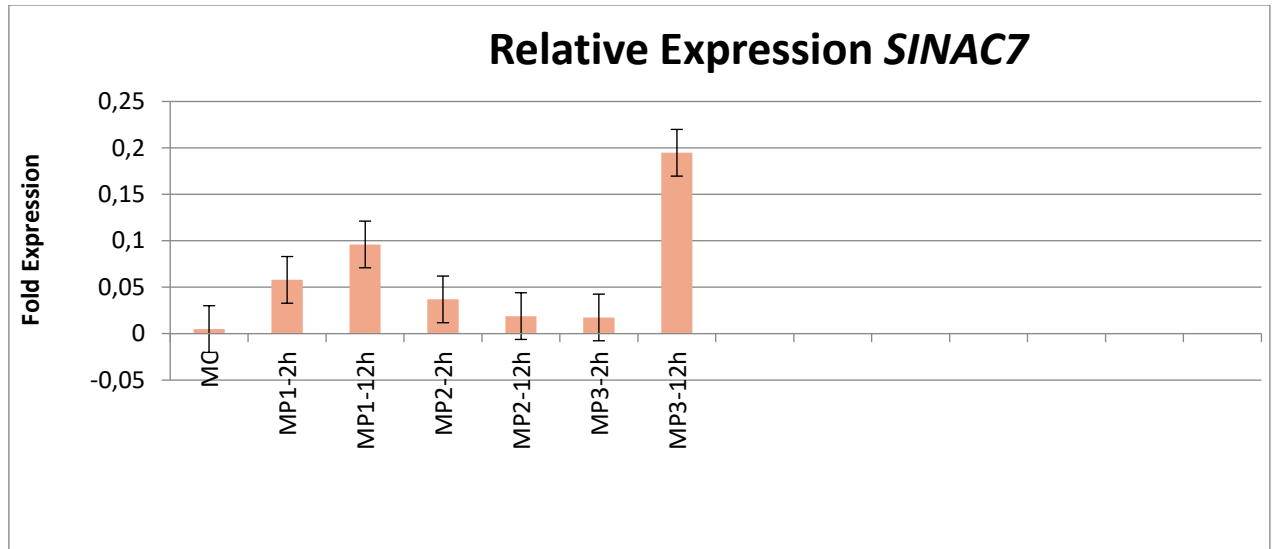


Figure 3.14. The Relative expression profile of *SINAC7* in root

The transcript abundance of *SINAC7* gene increased after 2 hours of treatment and remained elevated at 12 hours. These findings suggest a time-dependent upregulation of *SINAC7* expression in tomato plants (Fig.3.14).

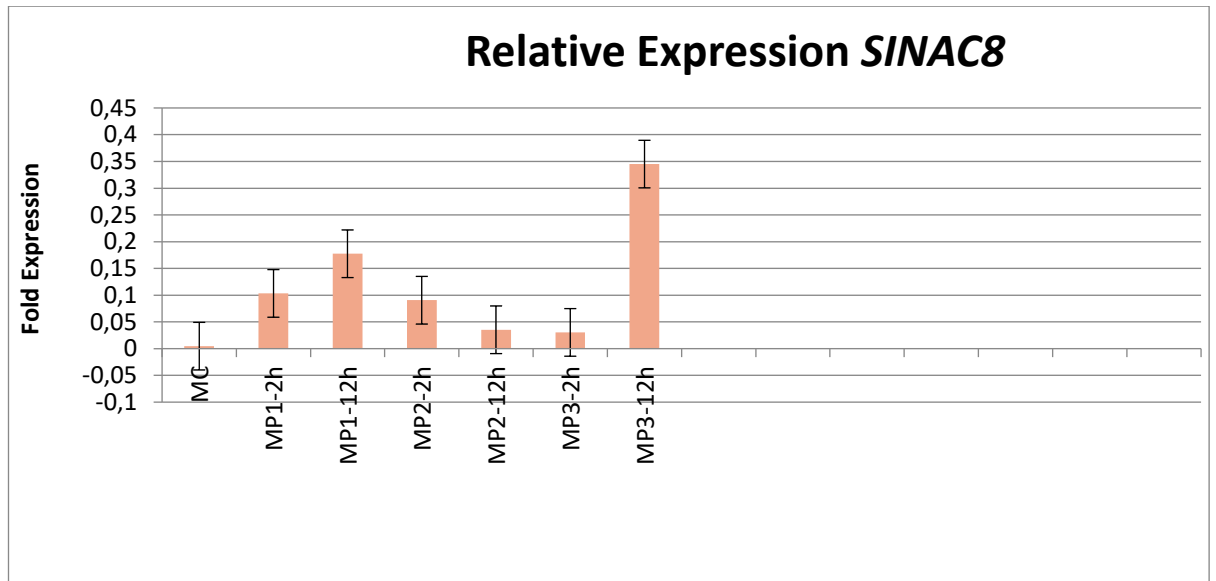


Figure 3.15. The Relative expression profile of *SINAC8* in root

The transcript abundance of the *SINAC8* gene exhibited a significant increase after 2 hours of treatment, with expression levels continuing to rise at 12 hours. These findings suggest a time-dependent upregulation of *SINAC8* expression in response to the treatment. (Fig.3.15).

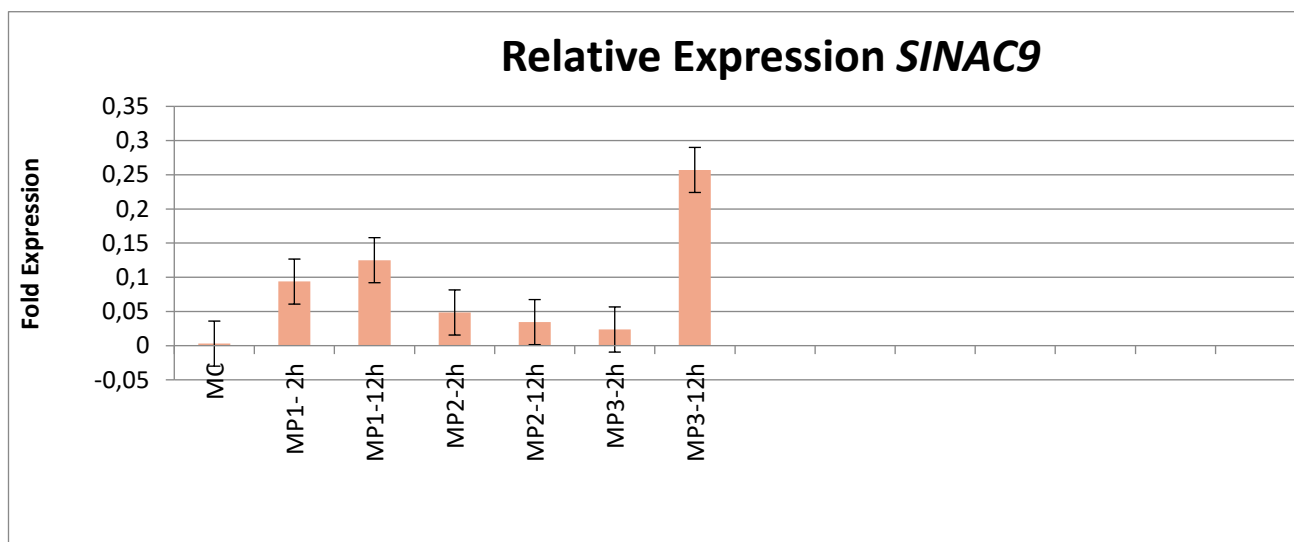


Figure 3.16. The Relative expression profile of *SINAC9* in root

The transcript abundance of the *SINAC9* gene exhibited a significant increase after 2 hours of treatment, with expression levels continuing to rise at 12 hours.

These findings suggest a time-dependent upregulation of *SINAC9* expression in response to the treatment. (Fig.3.16).

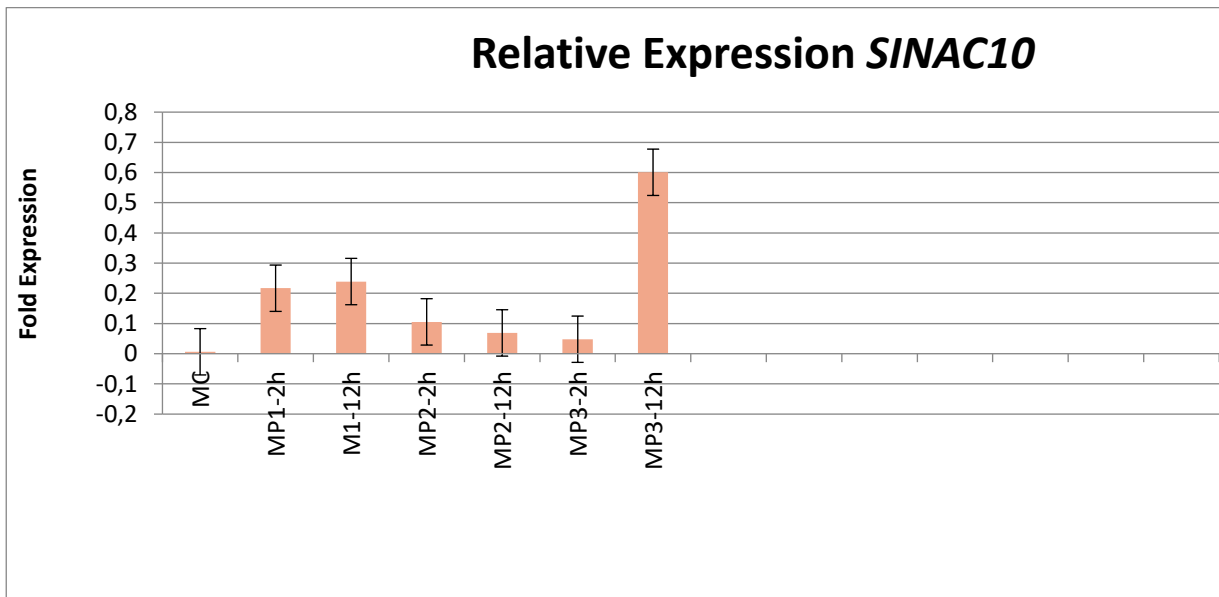


Figure 3.17. The Relative expression profile of *SINAC10* in root

The transcript abundance of the *SINAC10* gene increased significantly after 2 hours of treatment and remained elevated at 12 hours. These findings suggest a time-dependent upregulation of *SINAC10* expression in response to the treatment. The results indicate that the duration of treatment plays a role in influencing the expression levels of *SINAC10* in tomato plants. (Fig.3.17).

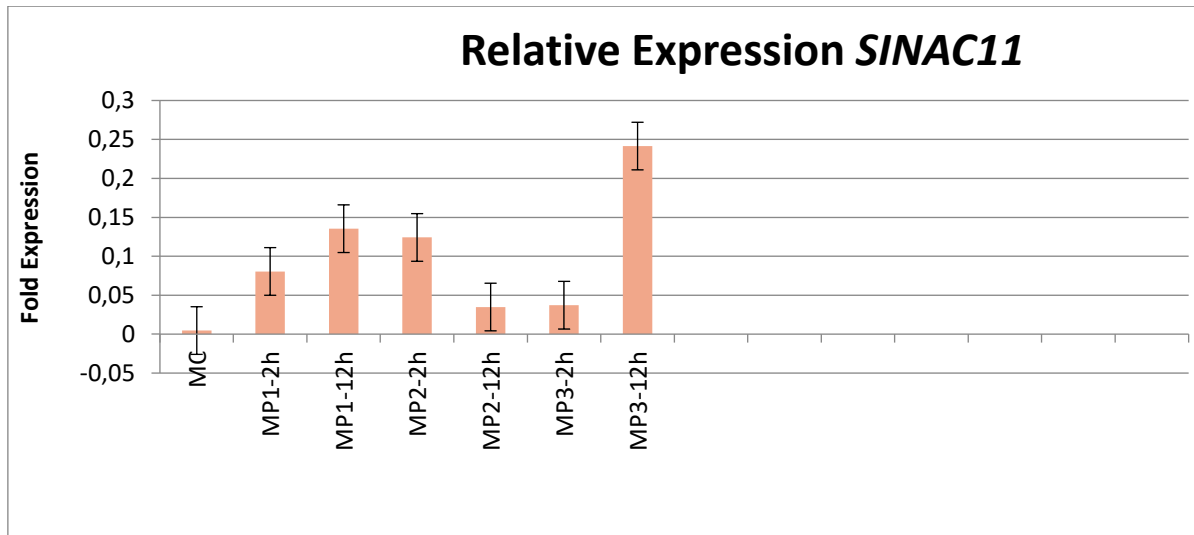


Figure 3.18. The Relative expression profile of *SINAC11* in root

The transcript abundance of the *SINAC11* gene increased significantly after 2 hours of treatment and remained elevated at 12 hours. These findings indicate a time-dependent upregulation of *SINAC11* expression in response to the treatment(Fig.3.18).

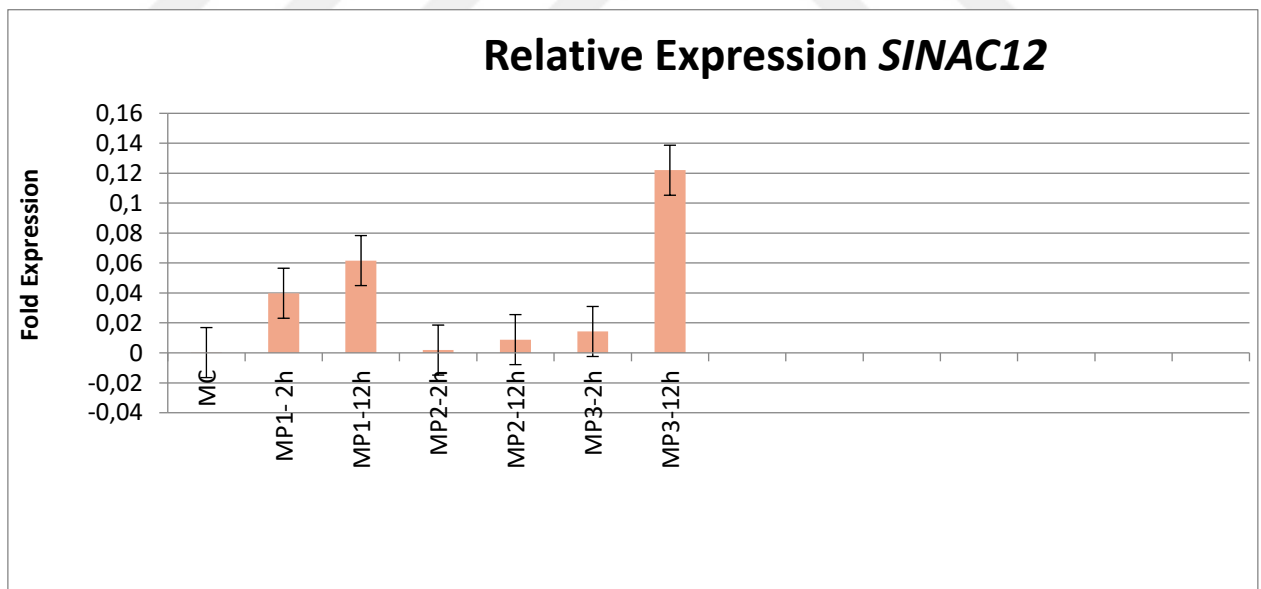


Figure 3.19. The Relative expression profile of *SINAC12* in root

The transcript abundance of the *SINAC12* gene increased significantly after 2 hours of treatment and remained elevated at 12 hours. (Fig.3.19).

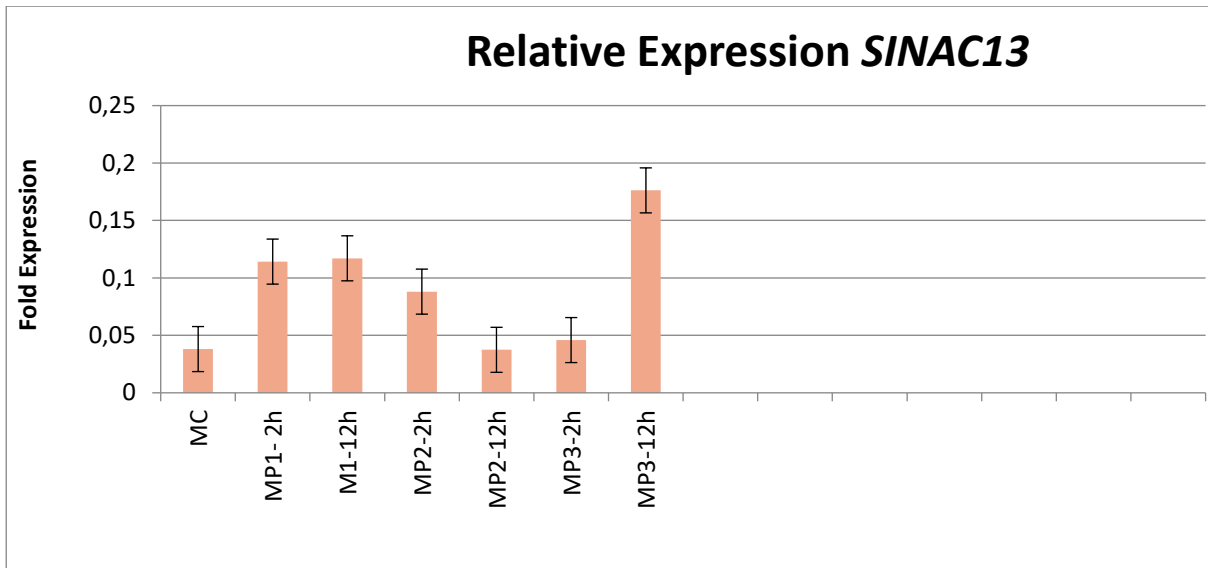


Figure 3.20. The Relative expression profile of *SINAC13* in root

The transcript abundance of the *SINAC13* gene exhibited a significant increase after 2 hours of treatment and remained elevated at 12 hours. the expression levels of *SINAC13* varied in the different treatment groups, suggesting potential regulatory mechanisms that are specific to the duration of treatment (Fig.3.20).

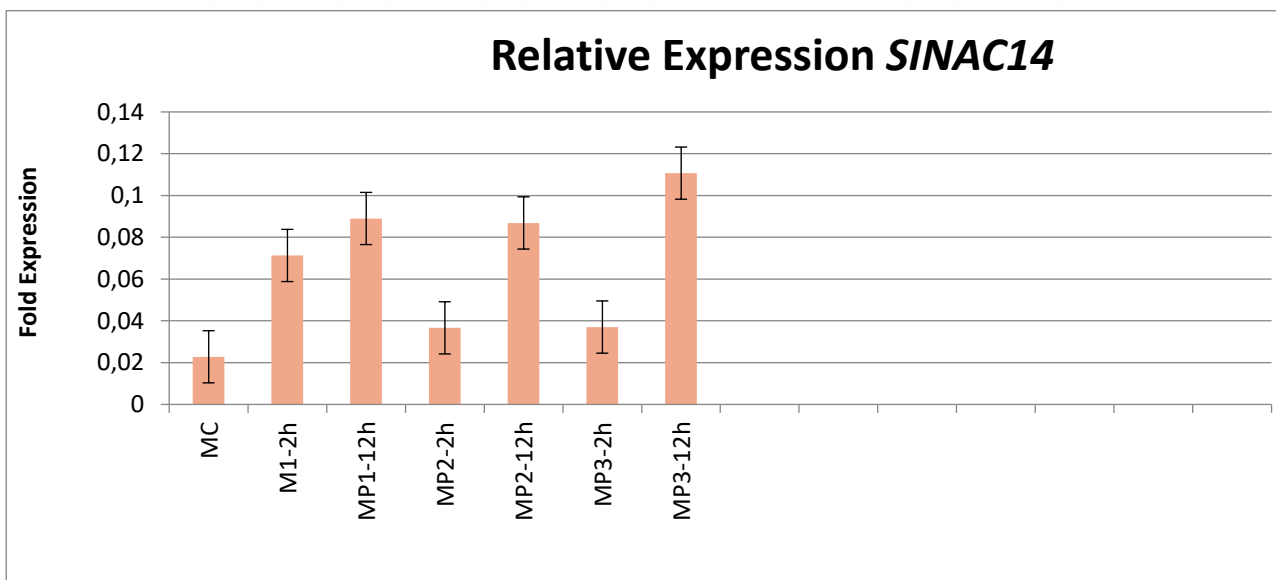


Figure 3.21. The Relative expression profile of *SINAC14* in root

The transcript abundance of the *SINAC14* gene significantly increased after 2 hours of treatment and remained elevated at 12 hours. The expression levels of

SINAC14 varied among the different treatment groups, indicating that the duration of treatment influences its expression in tomato plants (Fig.3.21).

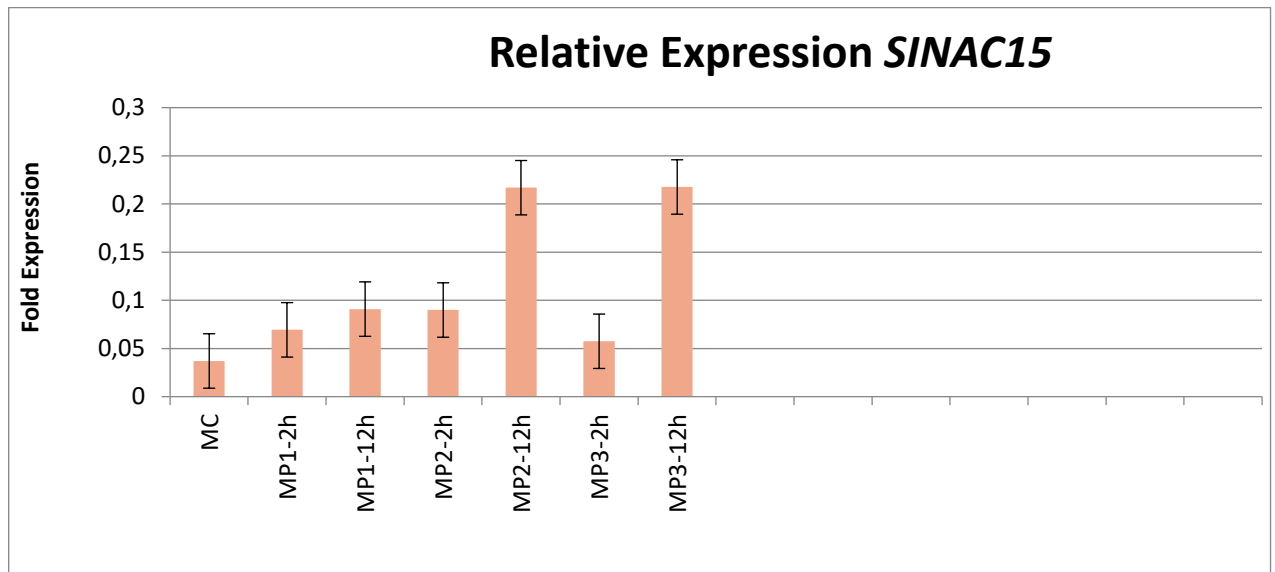


Figure 3.22. The Relative expression profile of *SINAC15* in root

The transcript abundance of the *SINAC15* gene significantly increased after 2 hours of treatment and remained elevated at 12 hours. These findings suggest a time-dependent upregulation of *SINAC15* expression in response to the treatment. (Fig.3.22).

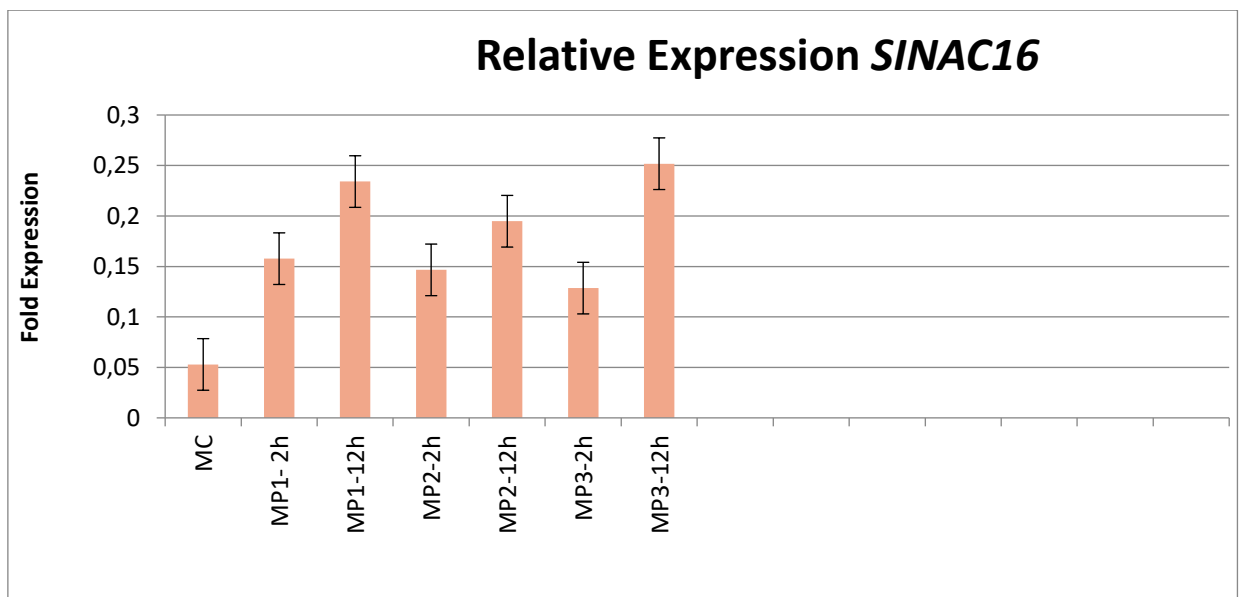


Figure 3.23. The Relative expression profile of *SINAC16* in root

The transcript abundance of the *SINAC16* gene exhibited a significant increase after 2 hours of treatment, which was further enhanced at 12 hours. These findings suggest a temporal regulation of *SINAC16* expression in response to the treatment(Fig.3.23).

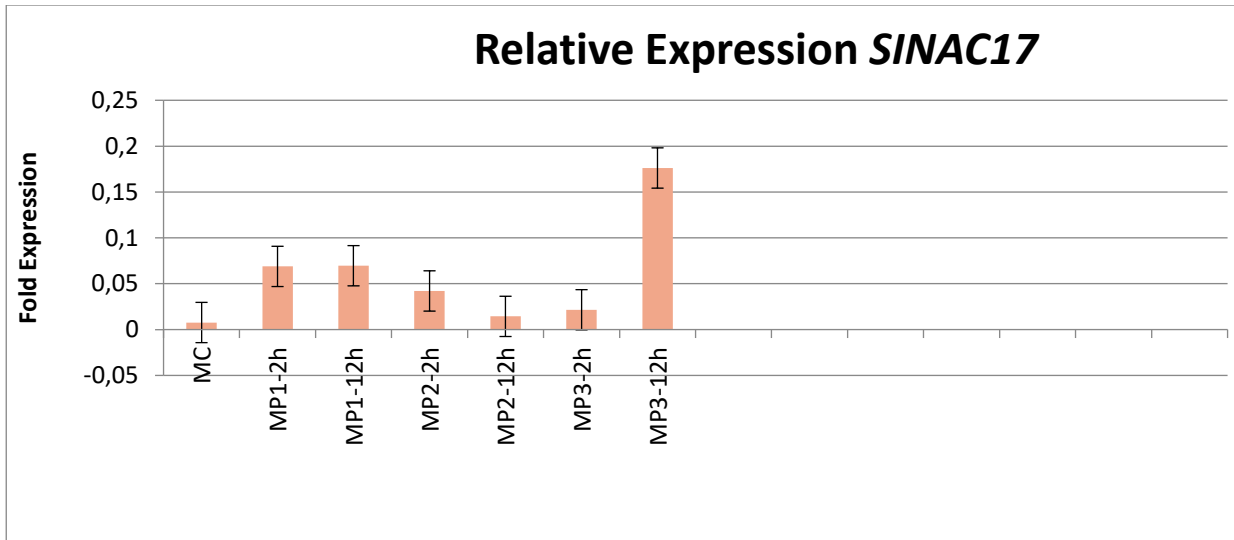


Figure 3.24. The Relative expression profile of *SINAC17* in root

The transcript abundance of the *SINAC17* gene increased after 2 hours of treatment, and this upregulation was sustained at 12 hours. These findings indicate a time-dependent modulation of *SINAC17* expression in response to the treatment.(Fig.3.24)

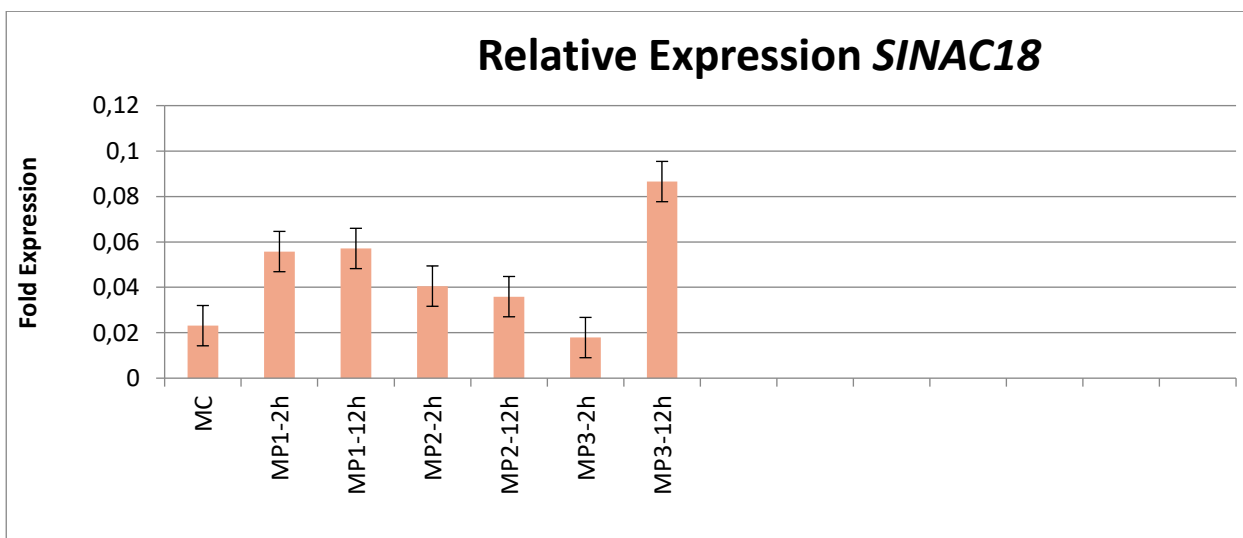


Figure 3.25. The Relative expression profile of *SINAC18* in root

The transcript abundance of the *SINAC18* gene increased after 2 hours of treatment, with sustained levels observed at 12 hours. However, no significant changes in expression were observed beyond this time point. (Fig.3.25).

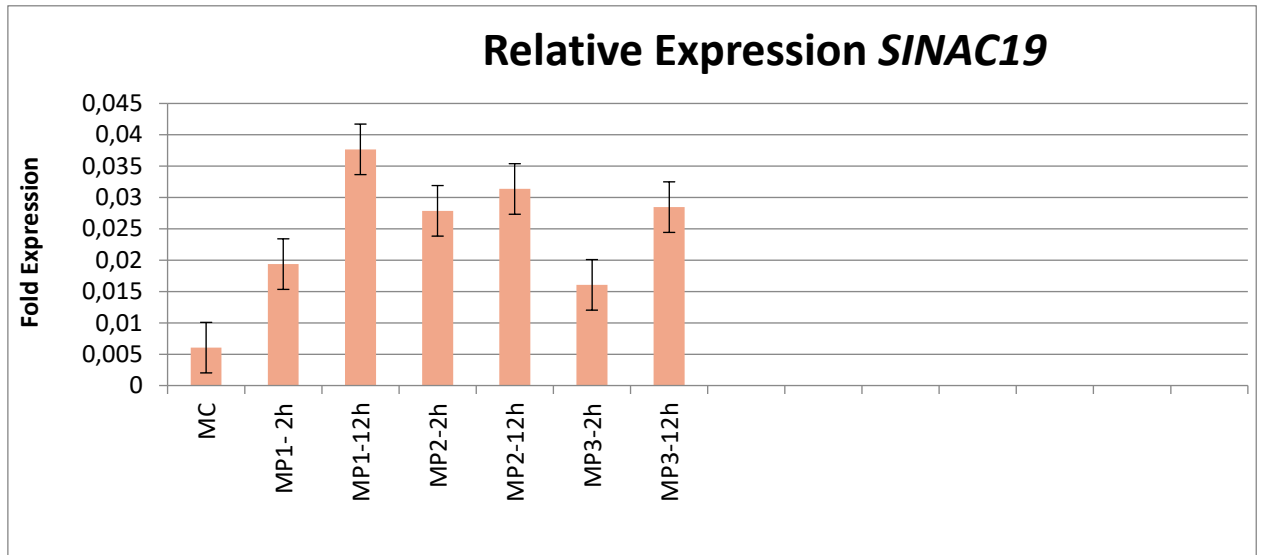


Figure 3.26. The Relative expression profile of *SINAC19* in root

The transcript abundance of the *SINAC19* gene exhibited a significant increase after 2 hours of treatment, which was further enhanced at 12 hours. These results indicate a time-dependent upregulation of *SINAC19* expression in response to the treatment (Fig.3.26).

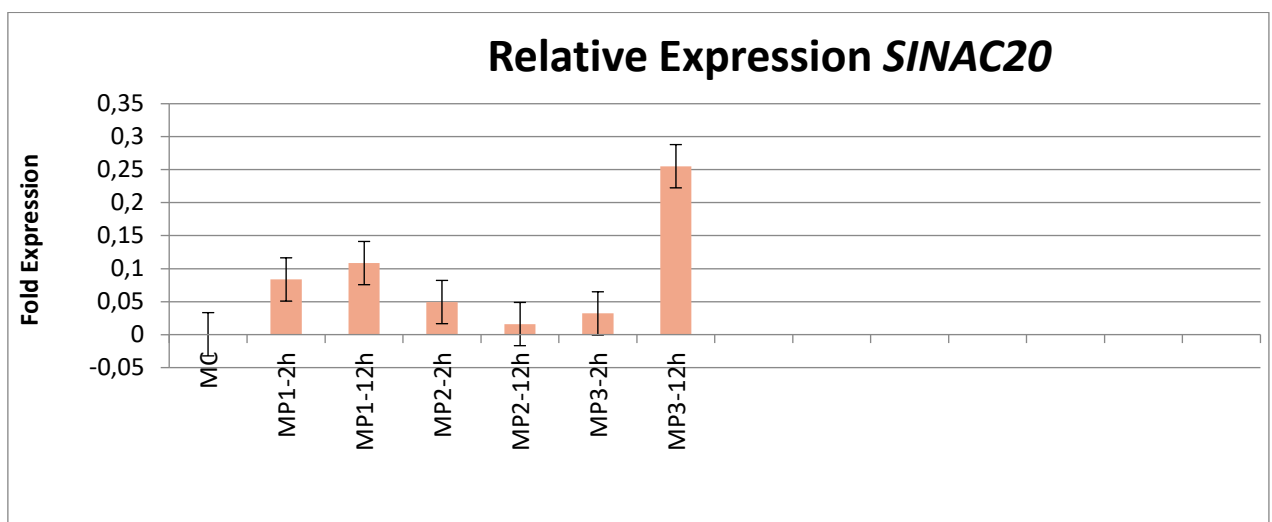


Figure 3.27. The Relative expression profile of *SINAC20* in leaves

The transcript abundance of the *SINAC20* gene increased after 2 hours of treatment and further elevated at 12 hours. These findings suggest a time-dependent upregulation of *SINAC20* expression in response to the treatment. (Fig.3.27).

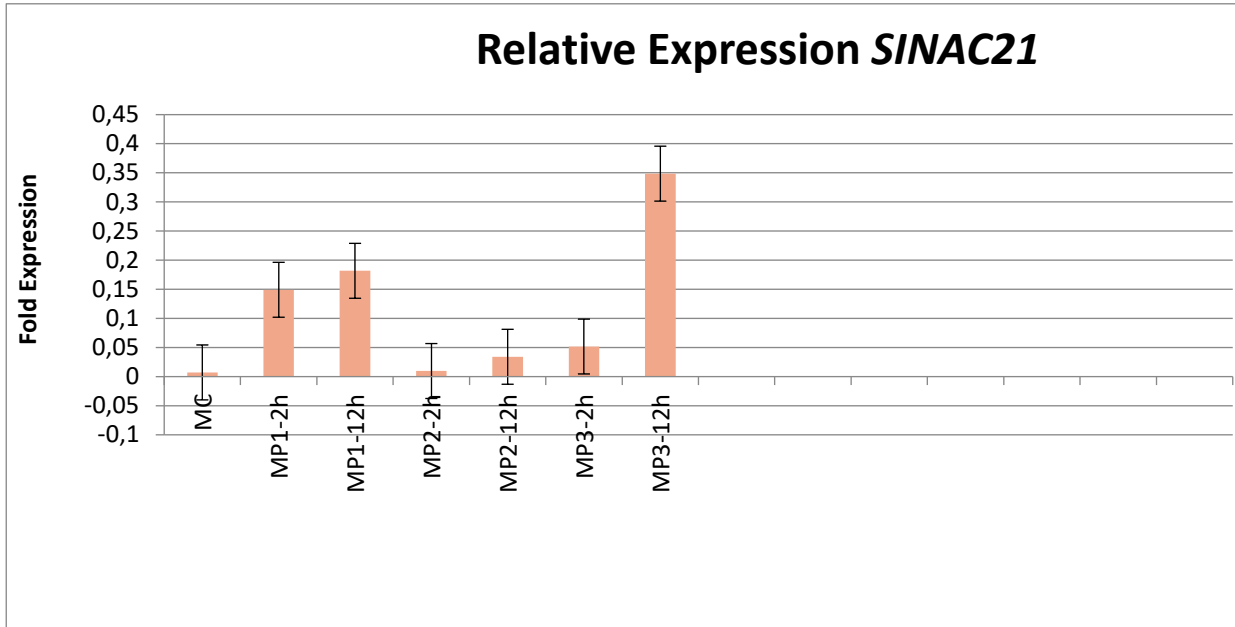


Figure 3.28. The Relative expression profile of *SINAC21* in root

The transcript abundance of the *SINAC21* gene increased after 2 hours of treatment and further elevated at 12 hours. These findings suggest a time-dependent upregulation of *SINAC21* expression in response to the treatment (Fig.3.28).

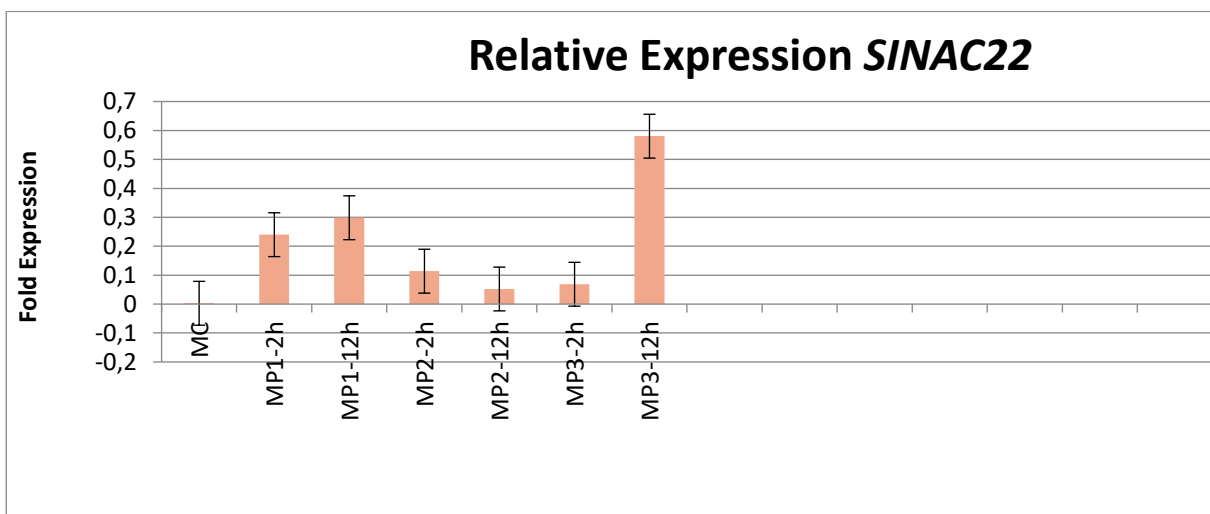


Figure 3.29. The Relative expression profile of *SINAC22* in root

The transcript abundance of the *SINAC22* gene showed significant upregulation after 2 hours of treatment, with further enhancement observed at 12 hours. These results suggest a time-dependent modulation of *SINAC22* expression in response to the treatment. (Fig.3.29).

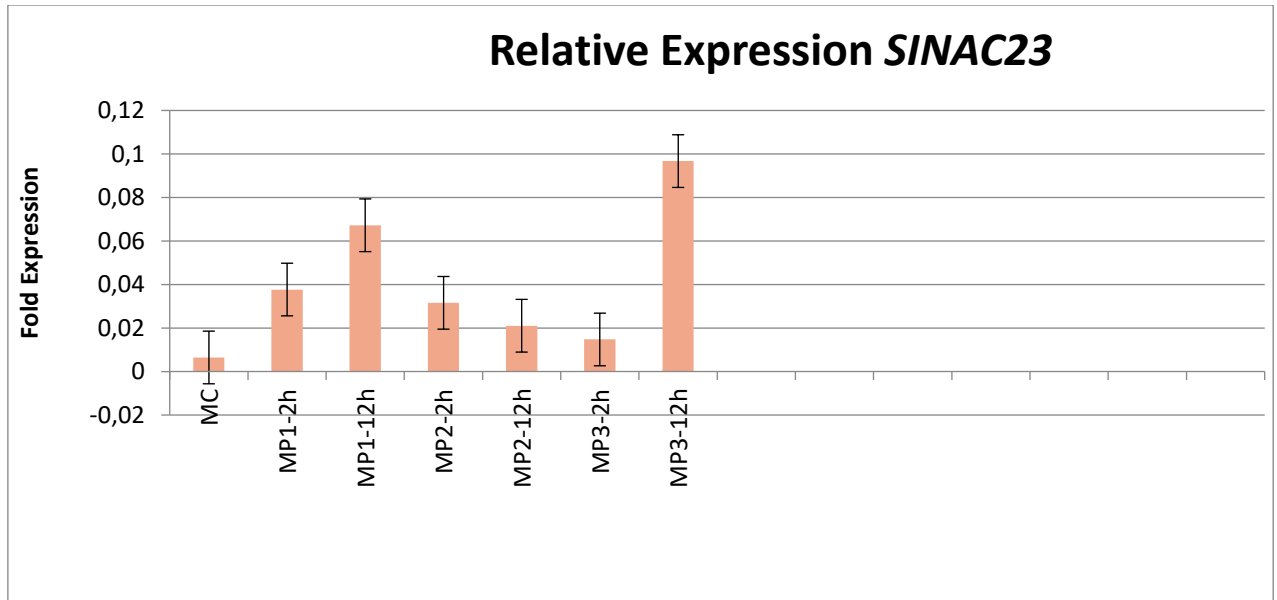


Figure 3.30. The Relative expression profile of *SINAC23* in root

The transcript abundance of the *SINAC23* gene increased after 2 hours of treatment, with further enhancement observed at 12 hours. These results indicate a time-dependent modulation of *SINAC23* expression in response to the treatment. (Fig.3.30).

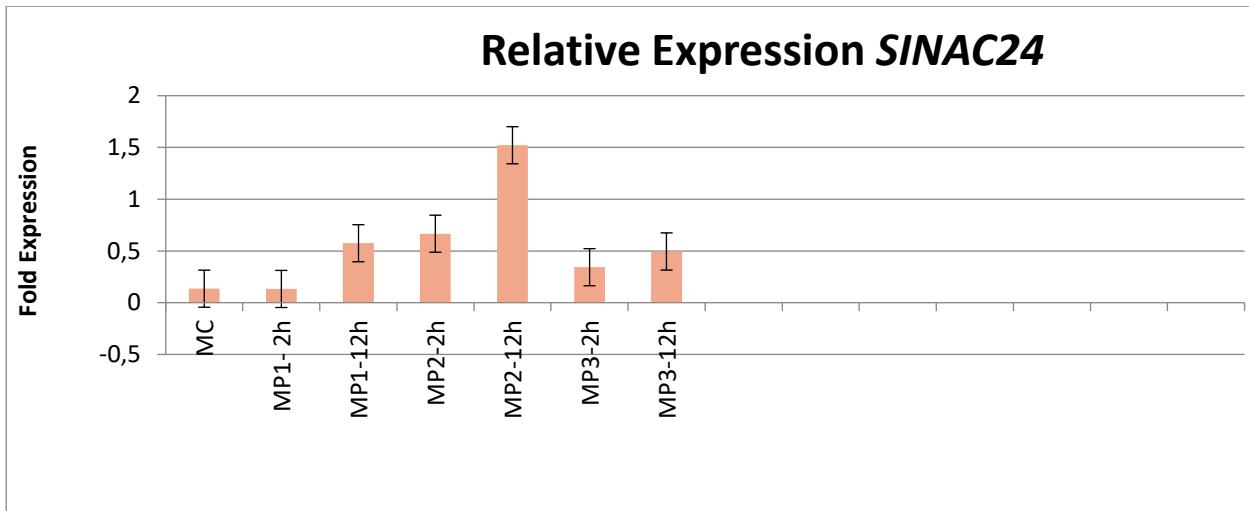


Figure 3.31. The Relative expression profile of *SINAC24* in root

The transcript abundance of the *SINAC24* gene varied under different treatment conditions and time points. While the expression remained stable after 2 hours, a significant increase was observed at 12 hours. Additionally, different treatment groups showed varying expression levels, with the highest expression at 2 hours in one group. These results indicate a time-dependent regulation of *SINAC24* expression (Fig.3.31).

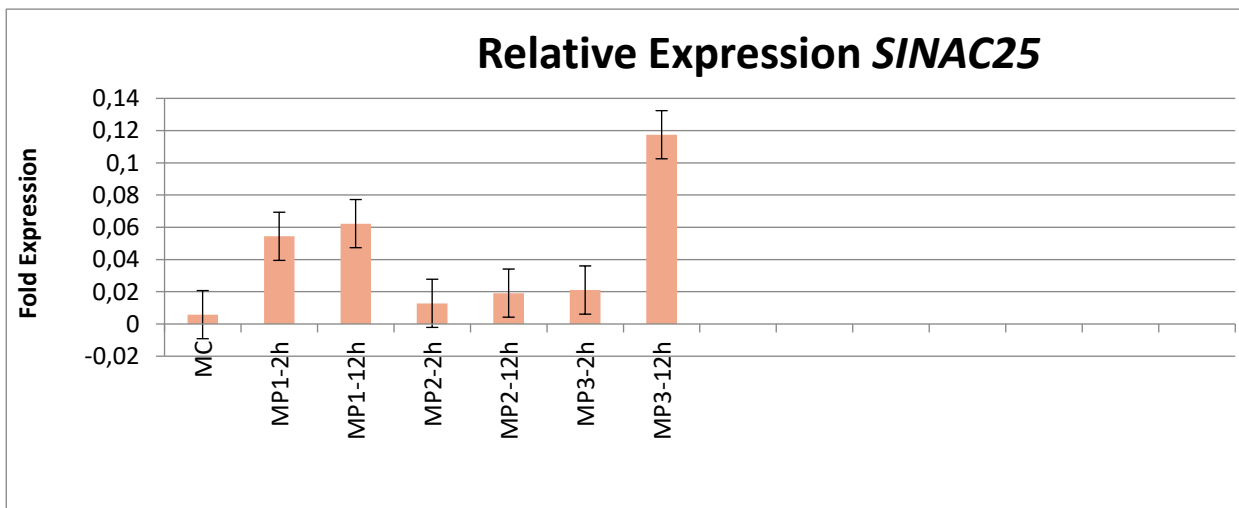


Figure 3.32. The Relative expression profile of *SINAC25* in root

The transcript abundance of the *SINAC25* gene was examined under different treatment conditions and time points. Treatment for 2 hours resulted in an increase in gene expression, which further slightly increased at 12 hours. Expression levels

varied across different treatment groups, indicating a time-dependent regulation. (Fig.3.32).

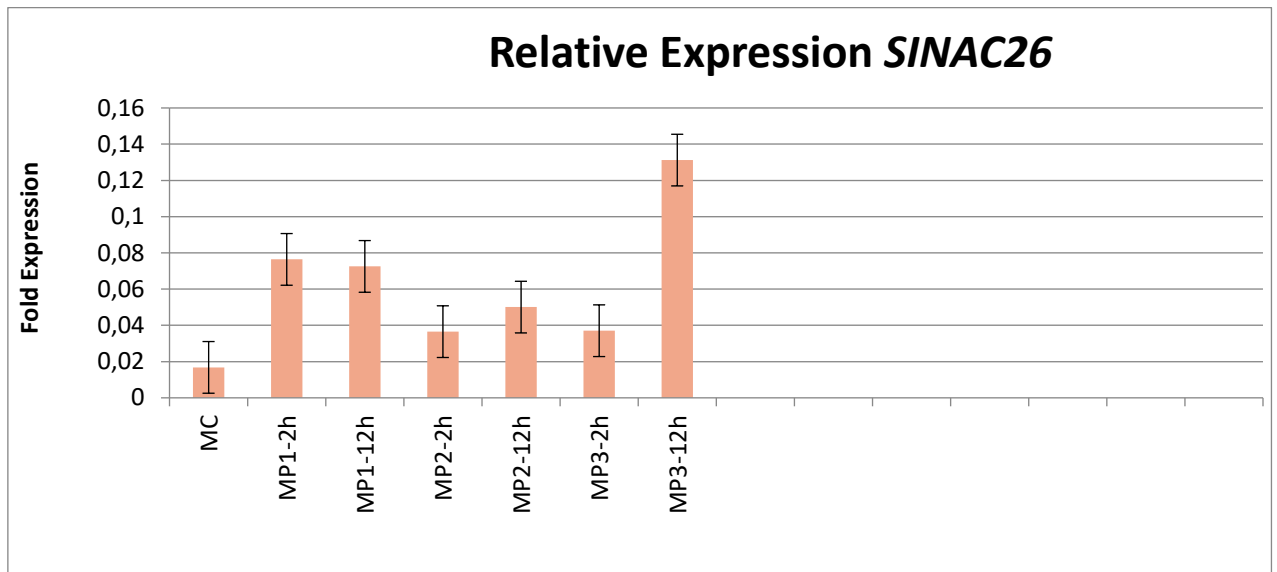


Figure 3.33. The Relative expression profile of *SINAC26* in root

The transcript abundance of *SINAC26* gene increased after 2 hours of treatment, with a slight decrease at 12 hours. These results indicate a time-dependent modulation of *SINAC26* expression in response to the treatment. (Fig.3.33).

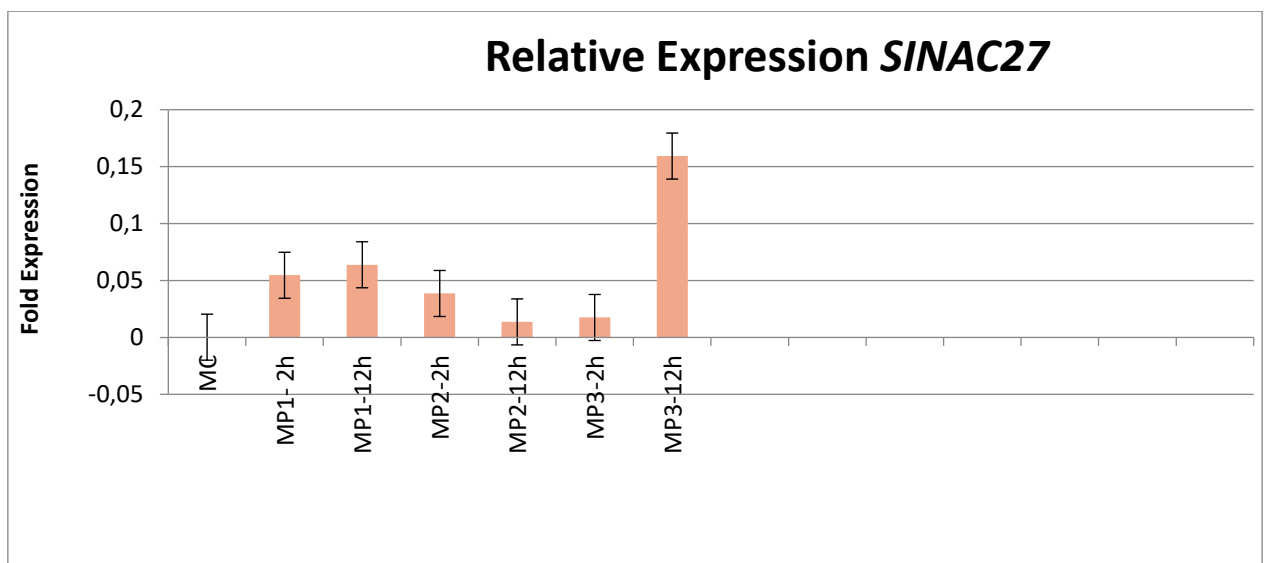


Figure 3.34. The Relative expression profile of *SINAC27* in root

The transcript abundance of the *SINAC27* gene was analyzed under different treatment conditions and time points. Treatment for 2 hours resulted in an increase in gene expression, which slightly increased further at 12 hours. Expression levels varied across different treatment groups, indicating a time-dependent regulation (Fig.3.34).

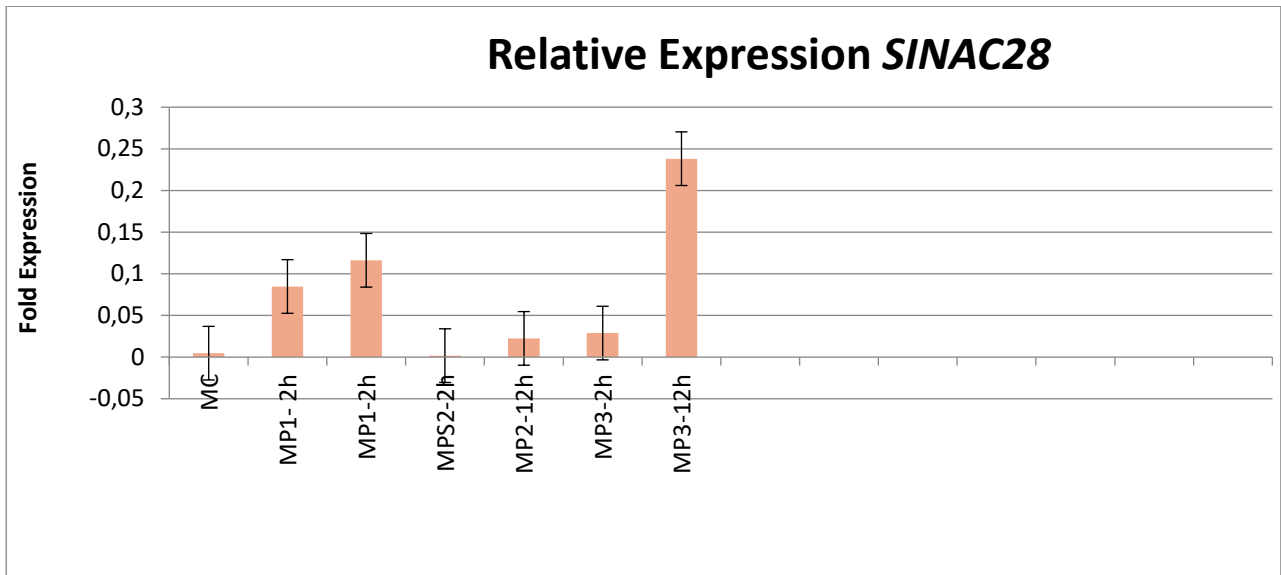


Figure 3.35. The Relative expression profile of *SINAC28* in root

The transcript abundance of the *SINAC28* gene increased after 2 hours of treatment and further increased at 12 hours. These results indicate a time-dependent modulation of *SINAC28* expression in response to the treatment (Fig.3.35).

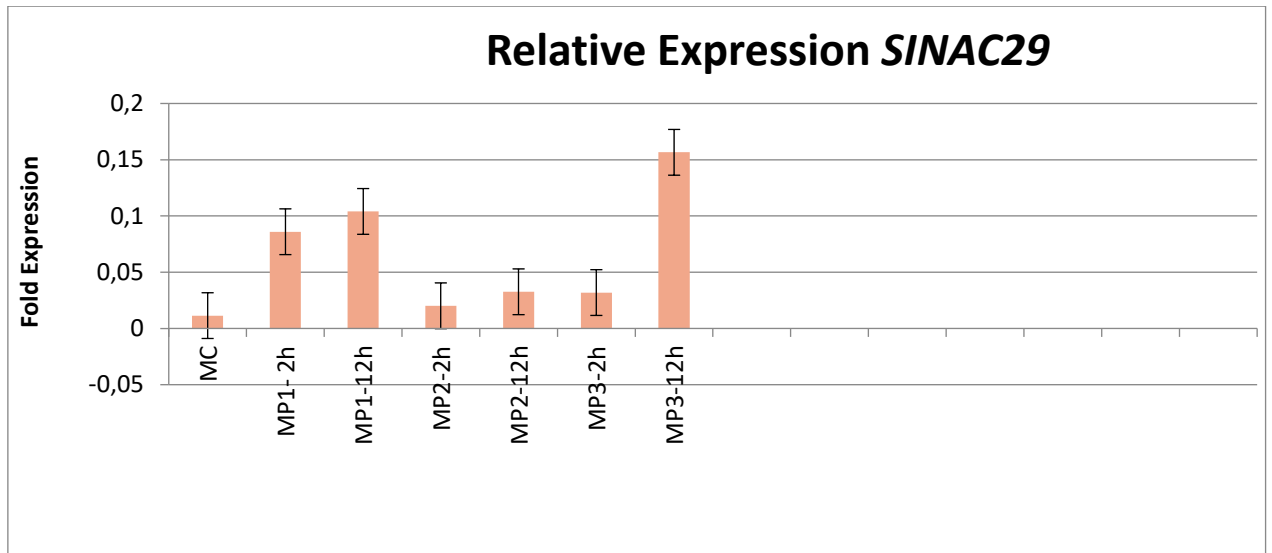


Figure 3.36. The Relative expression profile of *SINAC29* in root

The transcript abundance of the *SINAC29* gene was analyzed under different treatment conditions and time points. Treatment for 2 hours resulted in an increase in gene expression, which further increased at 12 hours. Expression levels varied across different treatment groups, indicating a time-dependent regulation (Fig.3.36).

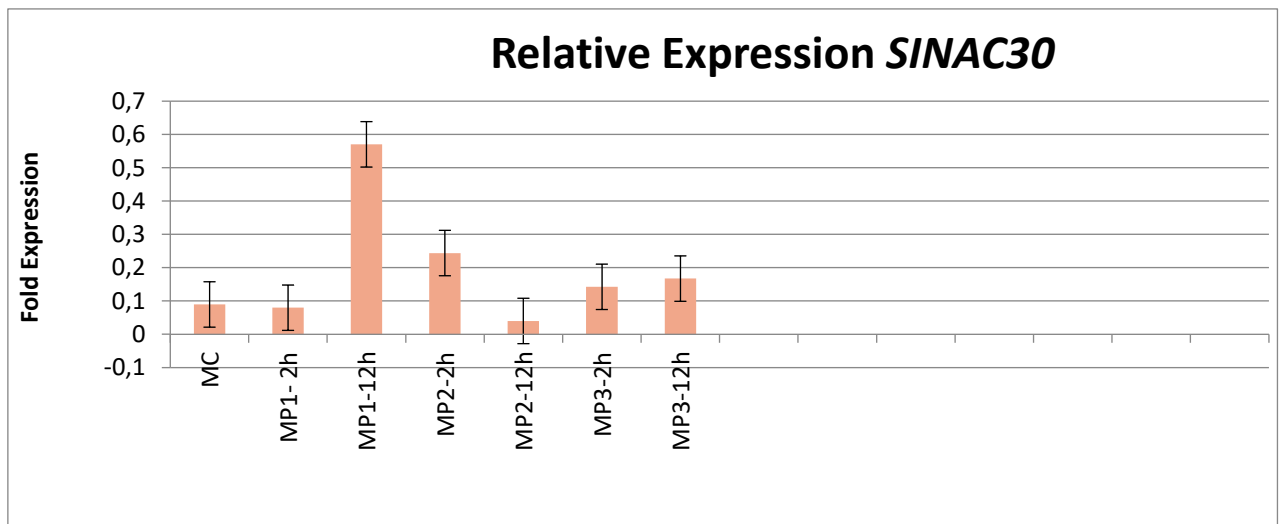


Figure 3.37. The Relative expression profile of *SINAC30* in root

The transcript abundance of the *SINAC30* gene slightly decreased after 2 hours of treatment but significantly increased at 12 hours. These results suggest a

time-dependent modulation of *SINAC30* expression in response to the treatment (Fig.3.37).

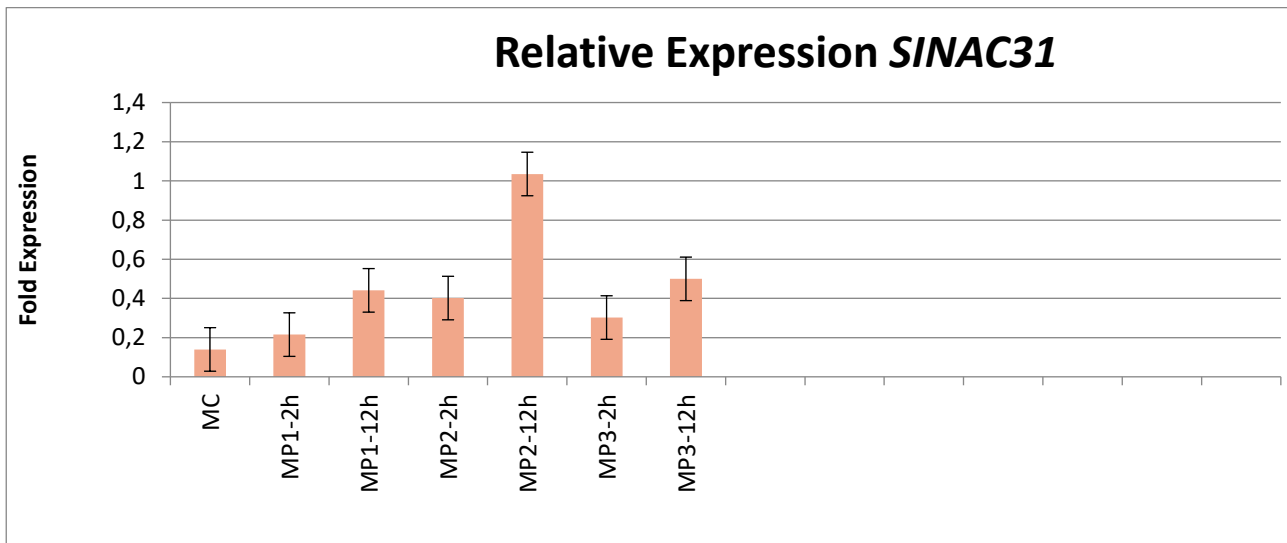


Figure 3.38. The Relative expression profile of *SINAC31* in root

The transcript abundance of the *SINAC31* gene increased after 2 hours of treatment and further increased at 12 hours. These results indicate a time-dependent modulation of *SINAC31* expression in response to the treatment (Fig.3.38).

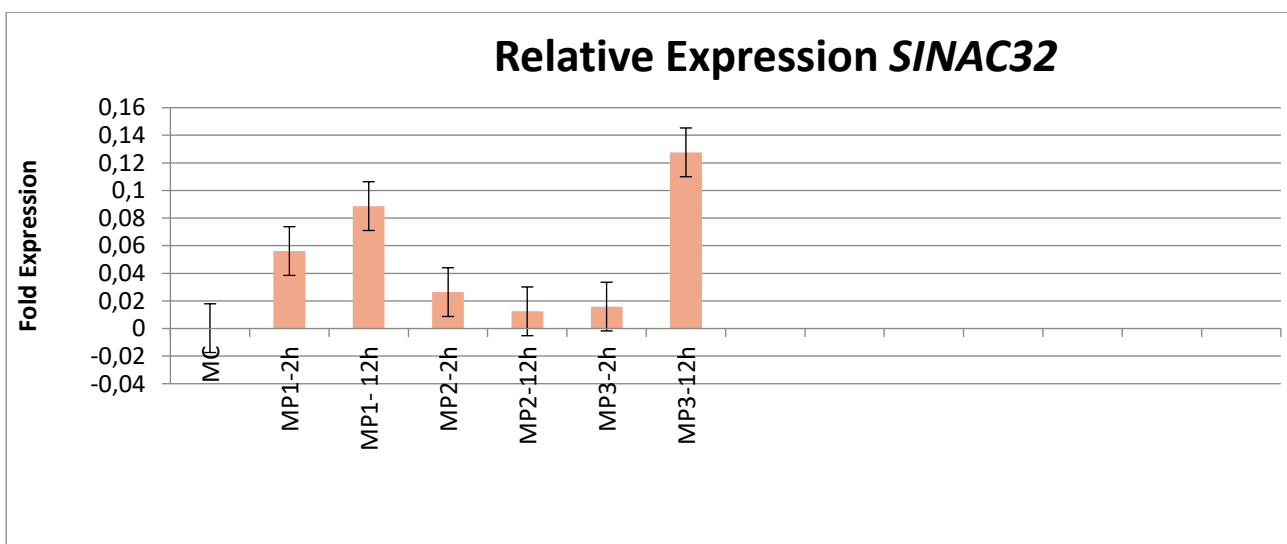


Figure 3.39. The Relative expression profile of *SINAC32* in root

The transcript abundance of the *SINAC32* gene were examined under different treatment conditions and time points. Treatment with the tested conditions resulted in varied expression patterns. After 2 hours of treatment, the expression of *SINAC32* increased, and a further increase was observed at 12 hours. These findings suggest a time-dependent regulation of *SINAC32* expression in response to the treatment compared to doz of PEG (Fig.3.39).

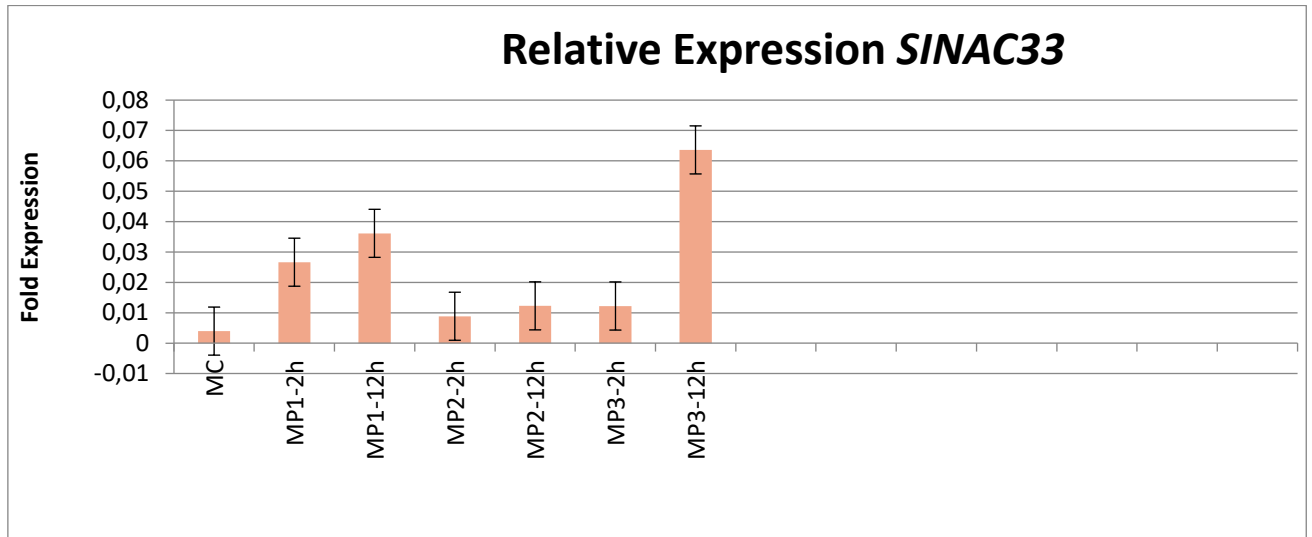


Figure 3.40. The Relative expression profile of *SINAC33* in root

The transcript abundance of the *SINAC33* gene were analyzed under different treatment conditions and time points. Treatment resulted in varied expression patterns. After 2 hours, the expression of *SINAC33* increased, and a further increase was observed at 12 hours. These findings suggest a time-dependent regulation of *SINAC33* expression in response to the treatment (Fig.3.40).

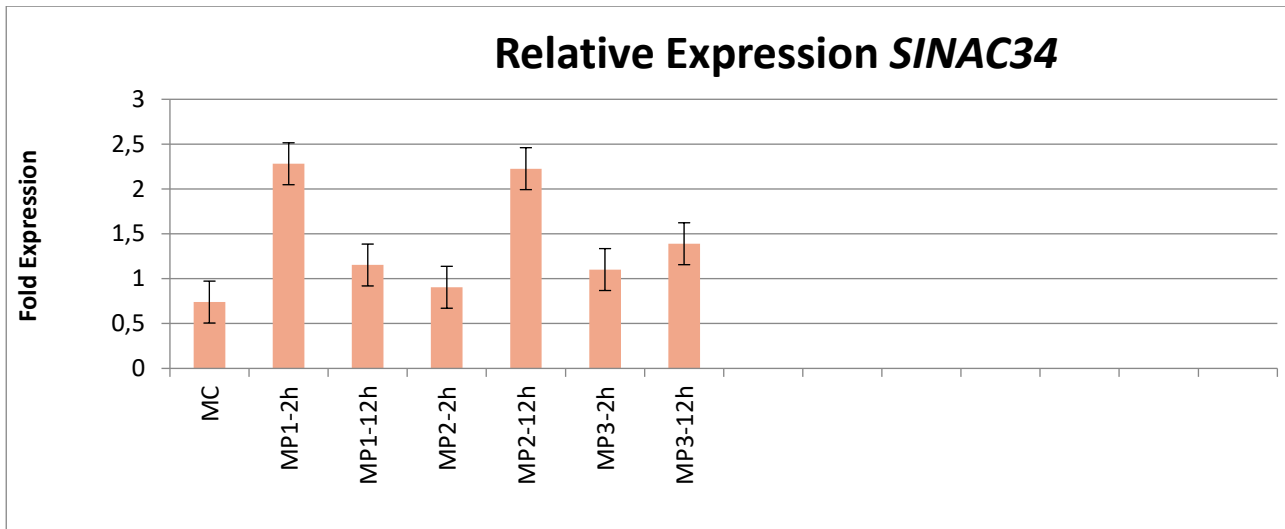


Figure 3.41. The Relative expression profile of *SINAC34* in root

The transcript abundance of the *SINAC34* gene were analyzed under different treatment conditions and time points. Treatment led to varied expression patterns. After 2 hours, the expression of *SINAC34* increased but decreased at 12 hours. Similar fluctuations were observed in other treatment groups. These findings indicate a time-dependent regulation of *SINAC34* expression (Fig.3.41).

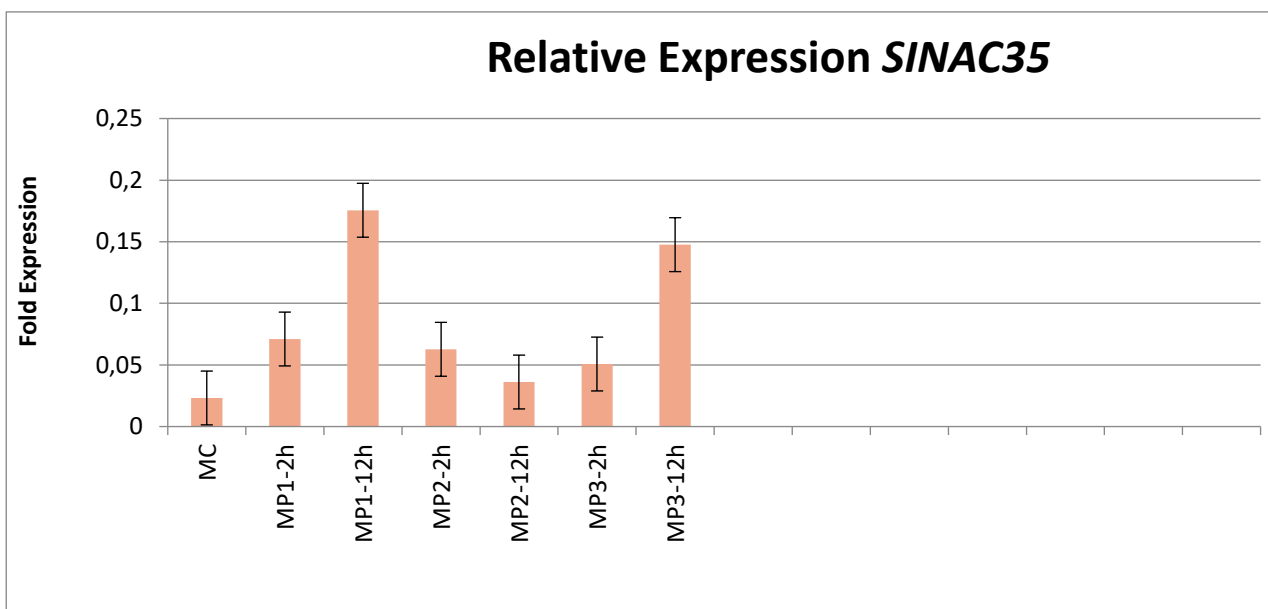


Figure 3.42. The Relative expression profile of *SINAC35* in root

The transcript abundance of the *SINAC35* gene was examined under different treatment conditions and time points. Treatment with MPS1 led to an increase in *SINAC35* expression at both 2 hours and 12 hours. Similar trends were observed in the MP2 and MP3 treatment groups, with varying expression levels. These findings highlight the dynamic regulation of *SINAC35* in response to the treatments (Fig.3.42).

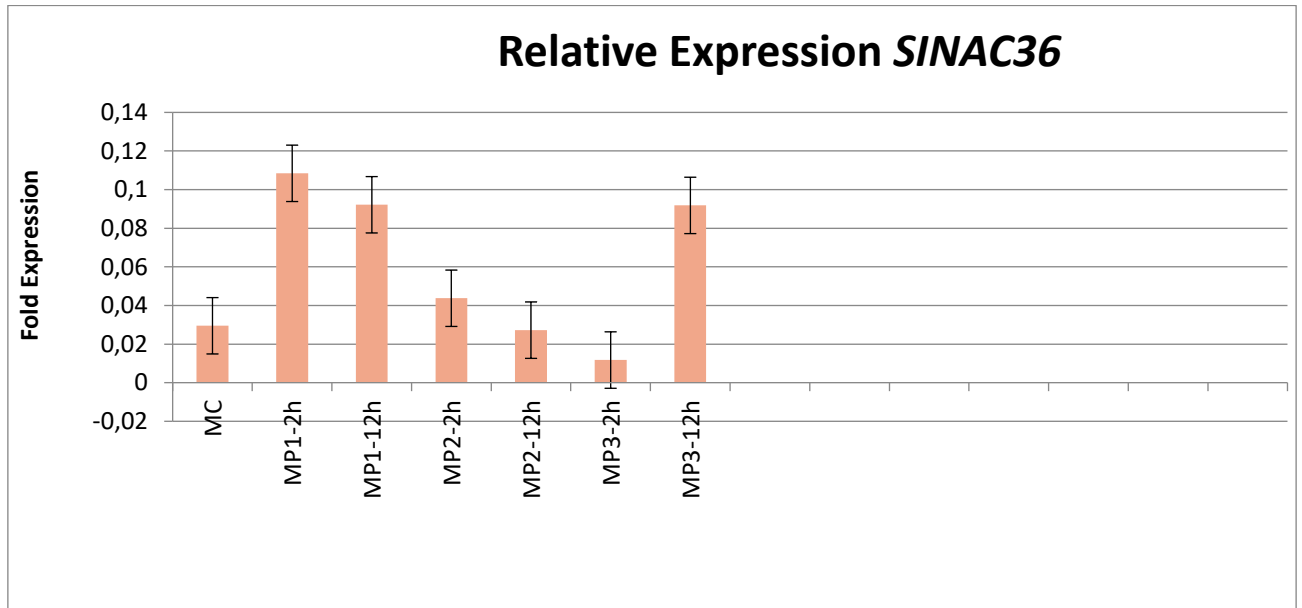


Figure 3.43. The Relative expression profile of *SINAC36* in root

The transcript abundance of the *SINAC36* gene was examined under different treatment conditions and time points. Treatment with MP1 led to an initial increase in expression at 2 hours, followed by a slight decrease at 12 hours. Similar patterns were observed in the MP2 treatment groups. However, the MP3 treatment groups showed consistently low expression levels (Fig.3.43).

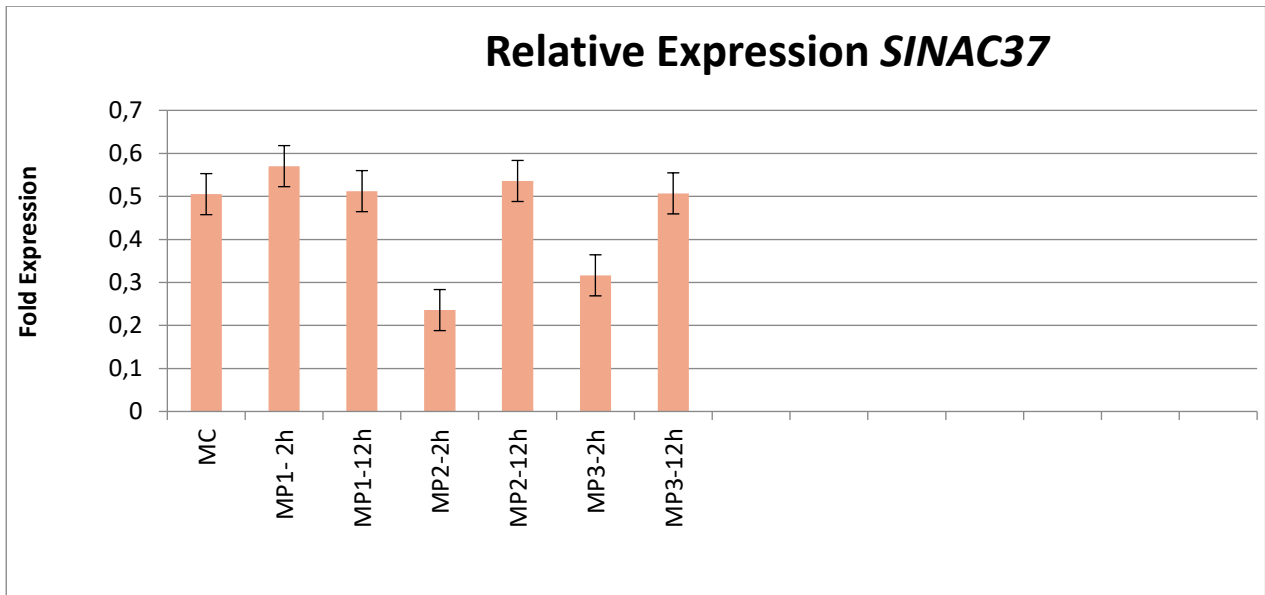


Figure 3.44. The Relative expression profile of *SINAC37* in root

The transcript abundance of the *SINAC37* gene were analyzed under different treatment conditions and time points. The results showed relatively stable expression in the control group (MP1 Control). Among the treatments, MP1 at 2 hours and 12 hours exhibited slightly higher expression levels compared to the control. MP2-2h and MP2-12h treatments also showed increased expression levels. MPS3-2h and MP3-12h treatments demonstrated moderate expression levels. These findings indicate that the expression of the *SINAC37* gene in tomato plants is influenced by the different treatments and time durations, suggesting a regulatory role in response to these conditions. (Fig.3.44).

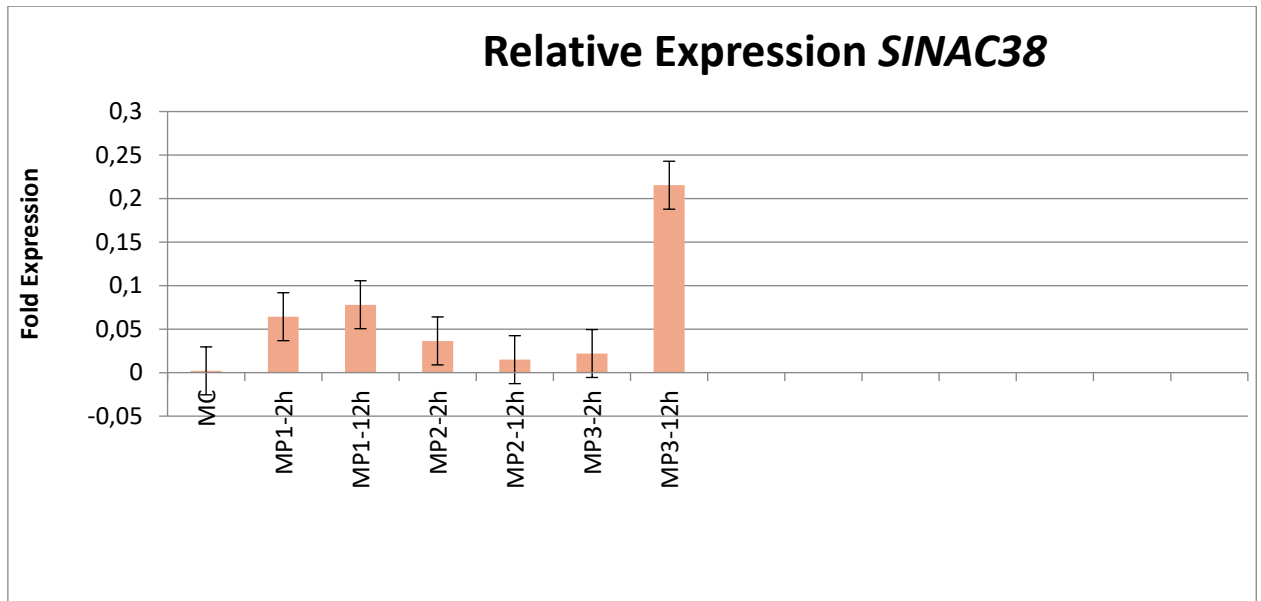


Figure 3.45. The Relative expression profile of *SINAC38* in root

The transcript abundance of the *SINAC38* gene was analyzed under different treatment conditions and time points. The control group (MC) exhibited relatively low expression levels. However, the MP1 treatment at 2 hours and 12 hours showed increased expression compared to the control. MP2-2h and MP2-12h treatments also demonstrated slightly higher expression levels. MP3-2h and MP3-12h treatments exhibited moderate expression levels. These findings suggest that the expression of the *SINAC38* gene is influenced by the different treatments and time durations, indicating its potential role in response to these conditions (Fig.3.45).

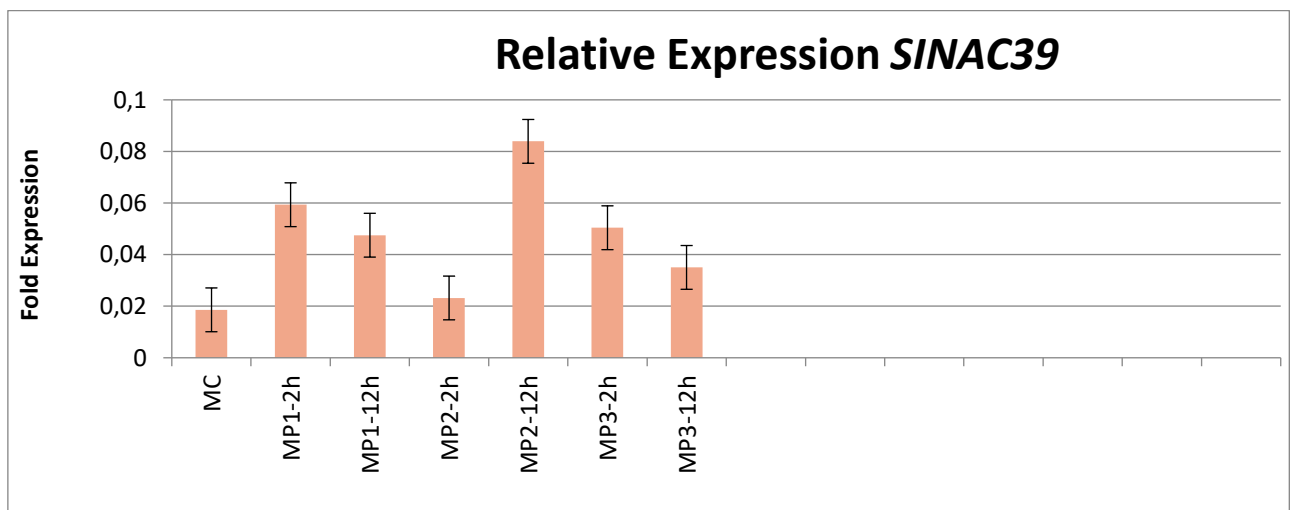


Figure 3.46. The Relative expression profile of *SINAC39* in root

The transcript abundance of the *SINAC39* gene was examined under various treatment conditions and time points. The control group (MC) showed a moderate expression level. The MP1 treatment at 2 hours exhibited increased gene expression compared to the control, while the expression level decreased at 12 hours. MP2-2h treatment also demonstrated a slightly higher expression, whereas MP2-12h treatment showed a substantial increase in gene expression. MP3-2h and MP3-12h treatments exhibited moderate to low expression levels. These results suggest that the expression of the *SINAC39* gene is influenced by the different treatments and time durations, indicating its potential involvement in the plant's response to these conditions (Fig.3.46).

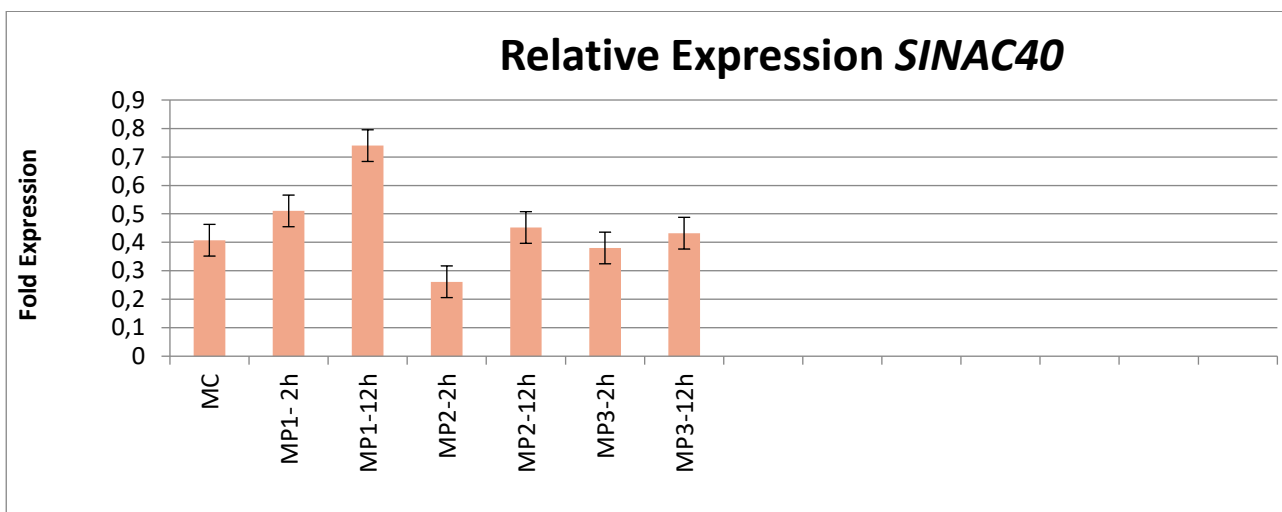


Figure 3.47. The Relative expression profile of *SINAC40* in root

The transcript abundance of the *SINAC40* gene was investigated under different treatment conditions and time points. The control group (MC) exhibited a moderate level of gene expression. The MP1 treatment at 2 hours showed a slight increase in gene expression compared to the control, while the expression level further increased at 12 hours. MP2-2h treatment resulted in a lower expression level, whereas MP2-12h treatment showed a significant increase in gene expression. MP3-2h and MP3-12h treatments displayed similar expression levels, which were moderately higher than the control. These findings suggest that the expression of the *SINAC40* gene is influenced by the applied treatments and time durations, indicating its potential role in the plant's response to these conditions. (Fig.3.47).

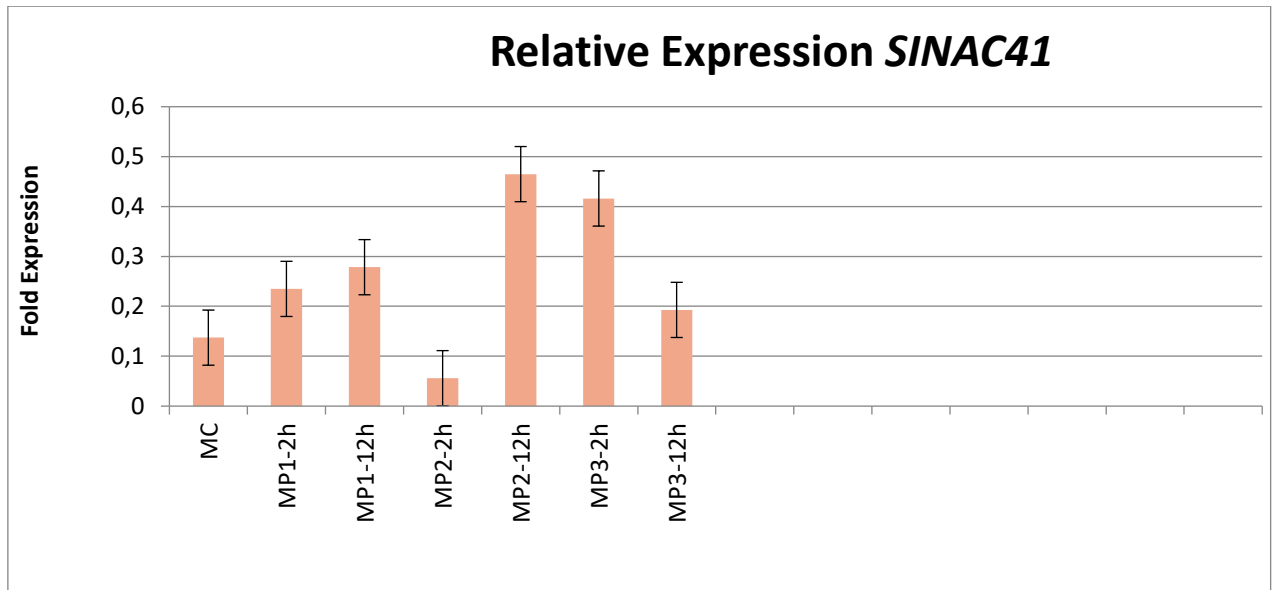


Figure 3.48. The Relative expression profile of *SINAC41* in root

The transcript abundance of *SINAC41* was examined under different treatment conditions and time points. In the control group (MC), the gene exhibited a moderate level of expression. Following MP1 treatment, there was an increase in gene expression at both 2 hours and 12 hours compared to the control. MP2-2h treatment resulted in a relatively lower expression level, whereas MP2-12h treatment showed a significant increase in gene expression. MP3-2h and MP3-12h treatments displayed relatively higher expression levels, with MP3-2h showing a slightly higher expression compared to MP3-12h. These findings suggest that the expression of the *SINAC41* gene is regulated by the applied treatments and time durations, indicating its potential involvement in the plant's response to these conditions (Fig.3.48).

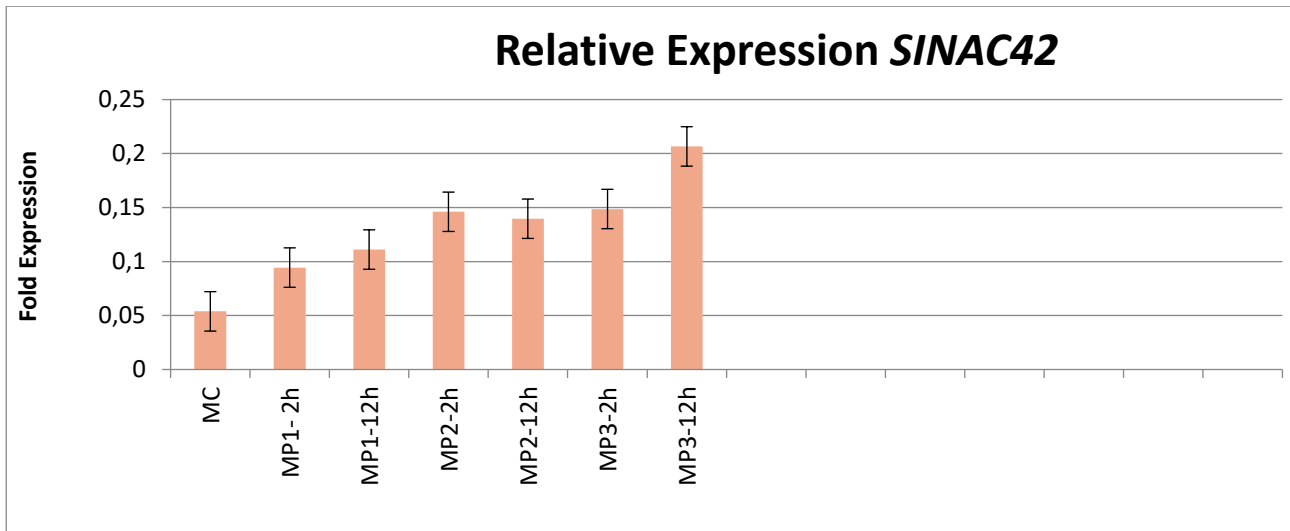


Figure 3.49. The Relative expression profile of *SINAC42* in root

The gene *SINAC42* was analyzed for its expression levels under various treatment conditions and time points. In the control group (MC), the gene displayed a moderate level of expression. Following MP1 treatment, there was a slight increase in gene expression at both 2 hours and 12 hours compared to the control. MP2-2h treatment showed a higher expression level, while MP2-12h treatment exhibited a slightly lower expression. MPS3-2h and MP3-12h treatments resulted in relatively higher expression levels, with MP3-12h showing a slightly higher expression compared to MP3-2h. These findings suggest that the expression of the *SINAC42* gene is influenced by the applied treatments and time durations, indicating its potential role in plant responses to these conditions (Fig.3.49).

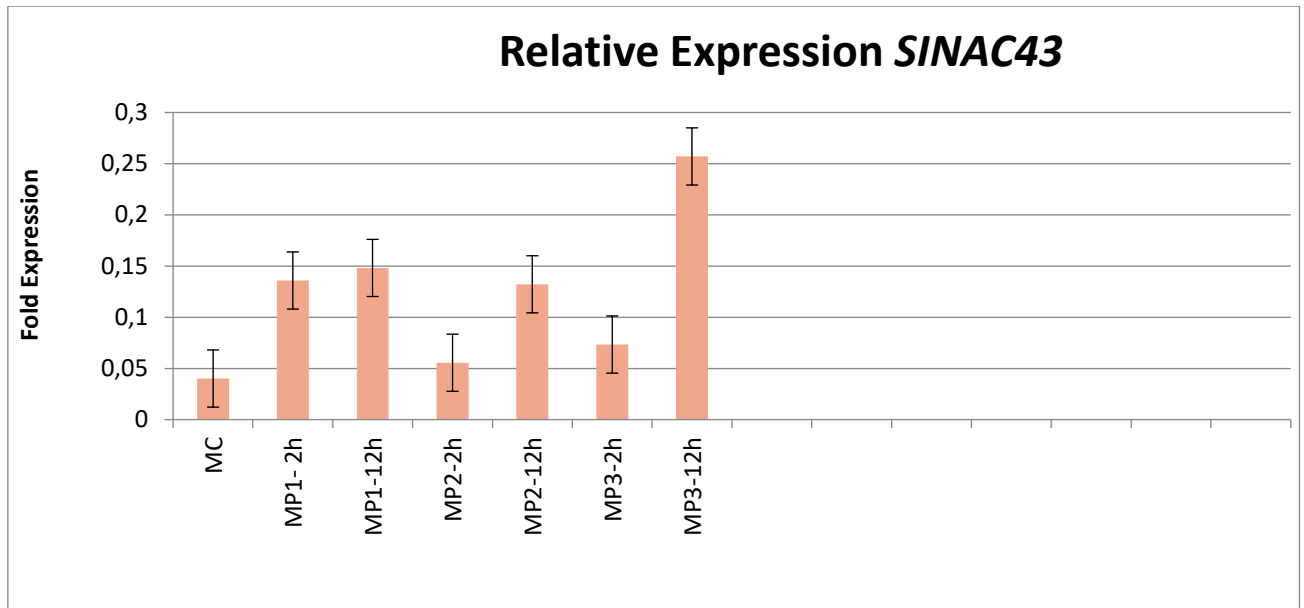


Figure 3.50. The Relative expression profile of *SINAC43* in root

In the control group, the gene displayed a moderate level of expression. Following MP1 treatment, there was an increase in gene expression at both 2 hours and 12 hours compared to the control. MP2-2h treatment resulted in a relatively lower expression level, while MP2-12h treatment showed a higher expression. MP3-2h treatment exhibited a moderate increase in gene expression, whereas MP3-12h treatment showed a significantly higher expression level. These results suggest that the expression of the *SINAC43* gene is responsive to the applied treatments and time durations, indicating its potential involvement in plant responses to these conditions (Fig.3.50).

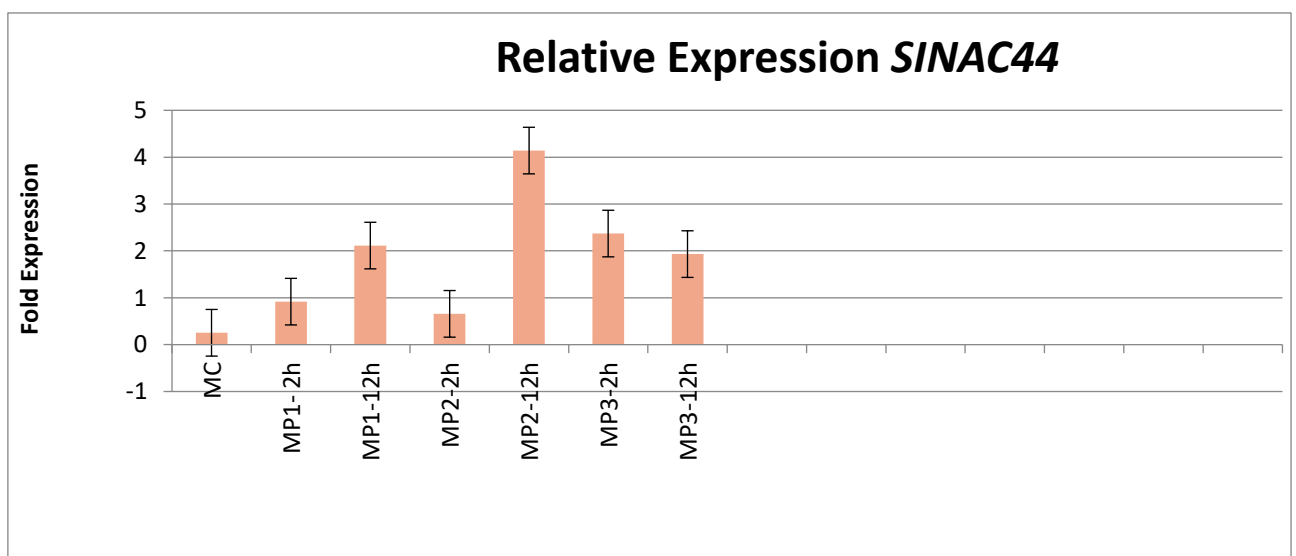


Figure 3.51. The Relative expression profile of *SINAC44* in root

The gene *SINAC44* was analyzed for its expression levels in tomato plants subjected to different treatments and time points. In the control group, the gene exhibited a moderate level of expression. However, following MP1 treatment, there was a substantial increase in gene expression at both the 2-hour and 12-hour time points compared to the control. Similarly, MP2-12h treatment resulted in a significant upregulation of the gene. MP2-2h treatment showed a moderate increase in expression, while MP3-2h and MP3-12h treatments exhibited relatively higher expression levels. These findings indicate that the expression of the *SINAC44* gene is highly responsive to the applied treatments and suggests its potential involvement in plant responses to these conditions (Fig.3.51).

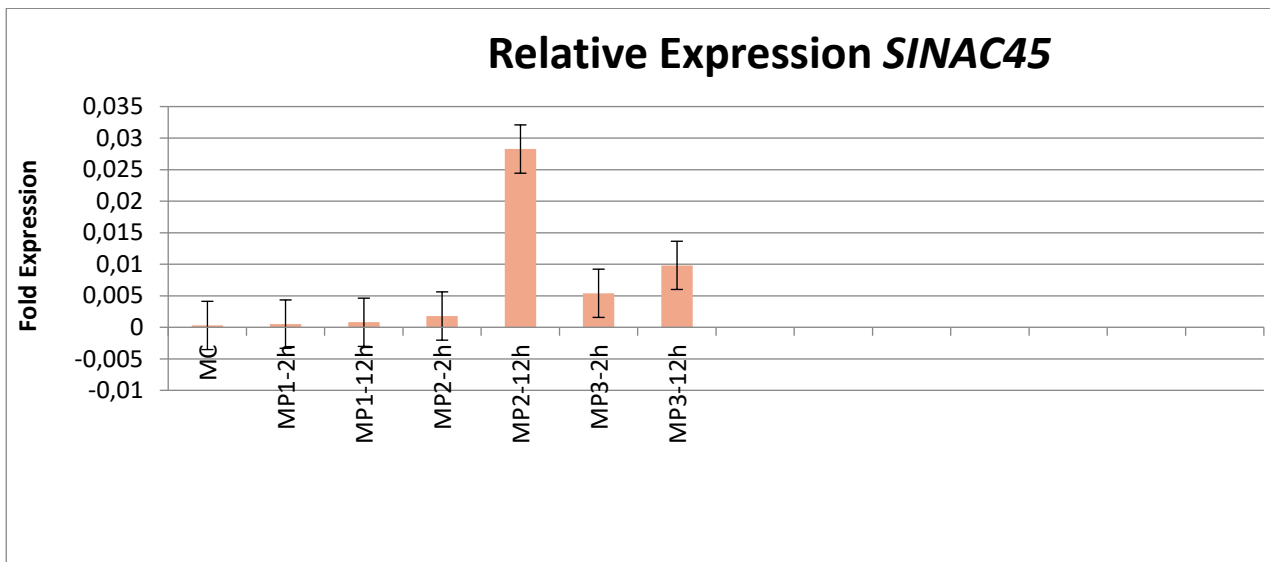


Figure 3.52. The Relative expression profile of *SINAC45* in root

In the control group (MS1 Control), the gene exhibited a low level of expression. Following MPS1 treatment, there were slight increases in gene expression at the 2-hour and 12-hour time points. Similarly, MP2-2h treatment showed a small increase in expression. However, it was the MP2-12h treatment that resulted in a significant upregulation of the gene. MP3-2h and MP3-12h treatments also showed increased expression levels, although to a lesser extent. These results suggest that the expression of the *SINAC45* gene is responsive to the applied treatments, particularly at the 12-hour time point (Fig.3.52).

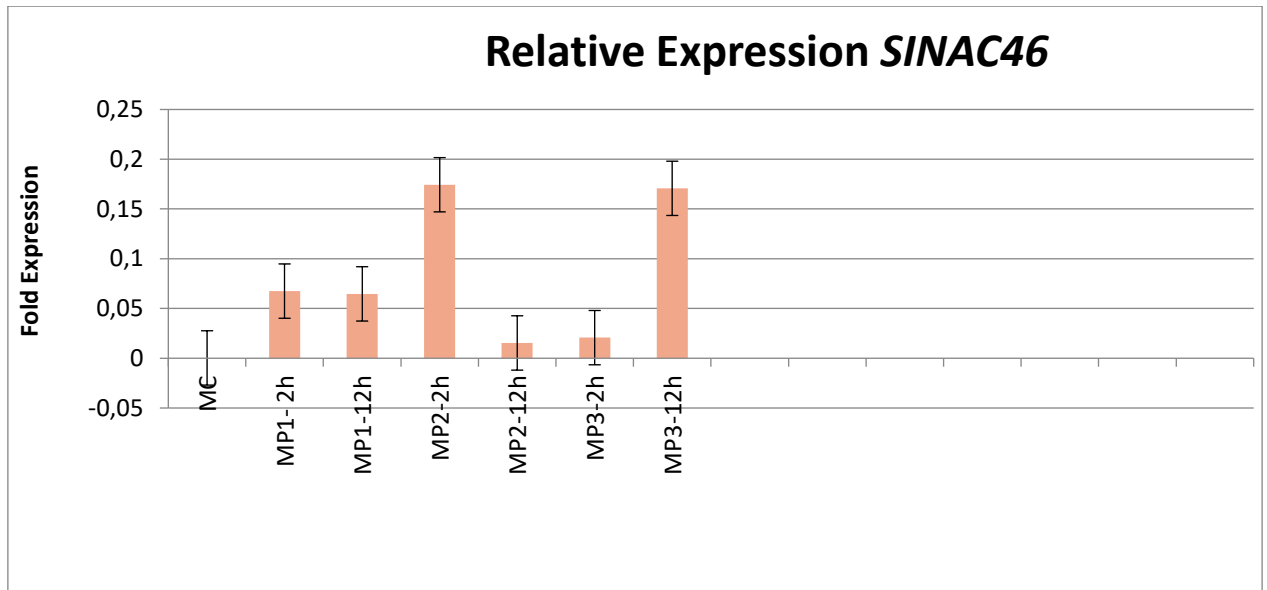


Figure 3.53. The Relative expression profile of *SINAC46* in root

The gene *SINAC46* was studied for its expression patterns in tomato plants under different treatment conditions. In the control group, the gene showed a low level of expression. Following MP1 treatment, there were slight increases in gene expression at the 2-hour and 12-hour time points. Notably, MP2-2h treatment resulted in a significant upregulation of the gene, while MP2-12h treatment showed a relatively lower expression level. MP3-2h and MP3-12h treatments also exhibited increased gene expression, although to a lesser extent. These findings suggest that the expression of the *SINAC46* gene is influenced by the applied treatments, particularly at the 2-hour and MP2-2h time points (Fig.3.53).

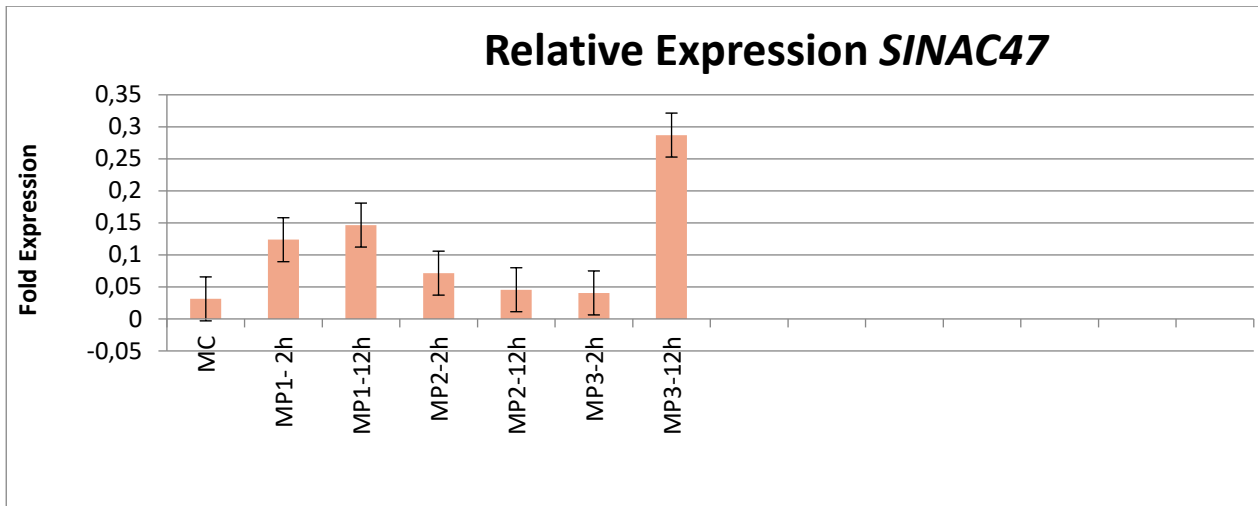


Figure 3.54. The Relative expression profile of *SINAC47* in root

In the control group, the gene exhibited a moderate level of expression. Following MP1 treatment, there were increases in gene expression at both the 2-hour and 12-hour time points. Similarly, MP2-2h and MP2-12h treatments resulted in upregulated gene expression, albeit to a lesser extent. MP3-2h treatment showed a modest increase in gene expression, while MP3-12h treatment showed a significant elevation. These results suggest that the expression of the *SINAC47* gene is influenced by the applied treatments, with notable changes observed at the 12-hour time point (Fig.3.54)

3.4. Expression Profile of *NHX* Genes (Root Samples)

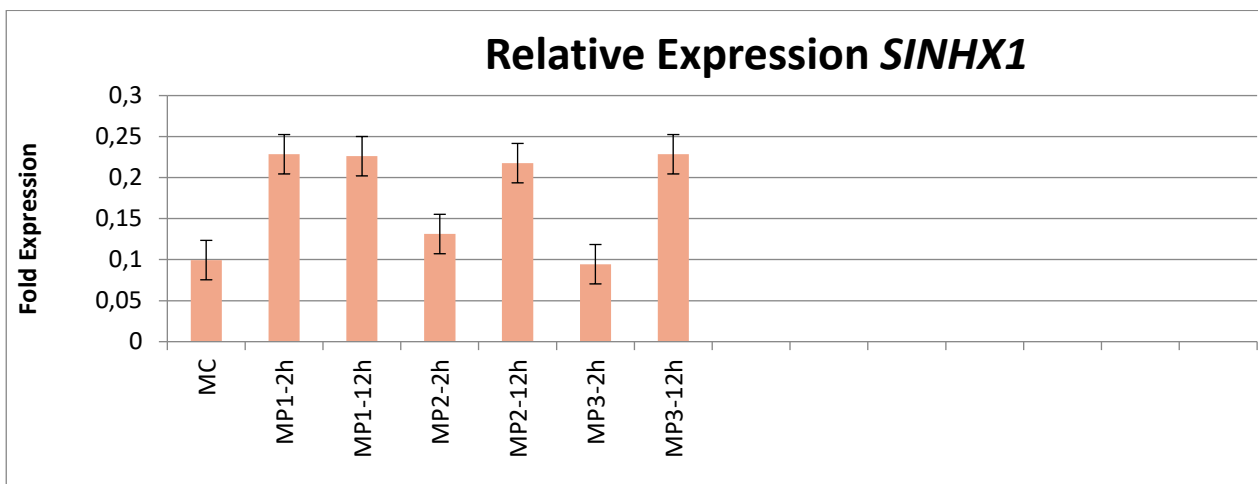


Figure 3.55. The Relative expression profile of *NHX1* in root

The gene *SINHX1* was analyzed for its expression levels in response to various treatment conditions. In the control group (MC), the gene exhibited a moderate level of expression. Following MPS1 treatment, there were slight increases in gene expression at both the 2-hour and 12-hour time points. MS2-2h and MS2-12h treatments also led to elevated gene expression, with the latter showing a higher level. MS3-2h and MS3-12h treatments resulted in modest increases in gene expression. Overall, these findings suggest that the expression of the *SINHX1* gene is influenced by the applied treatments, with varying degrees of upregulation observed across different time points (Fig.3.55).

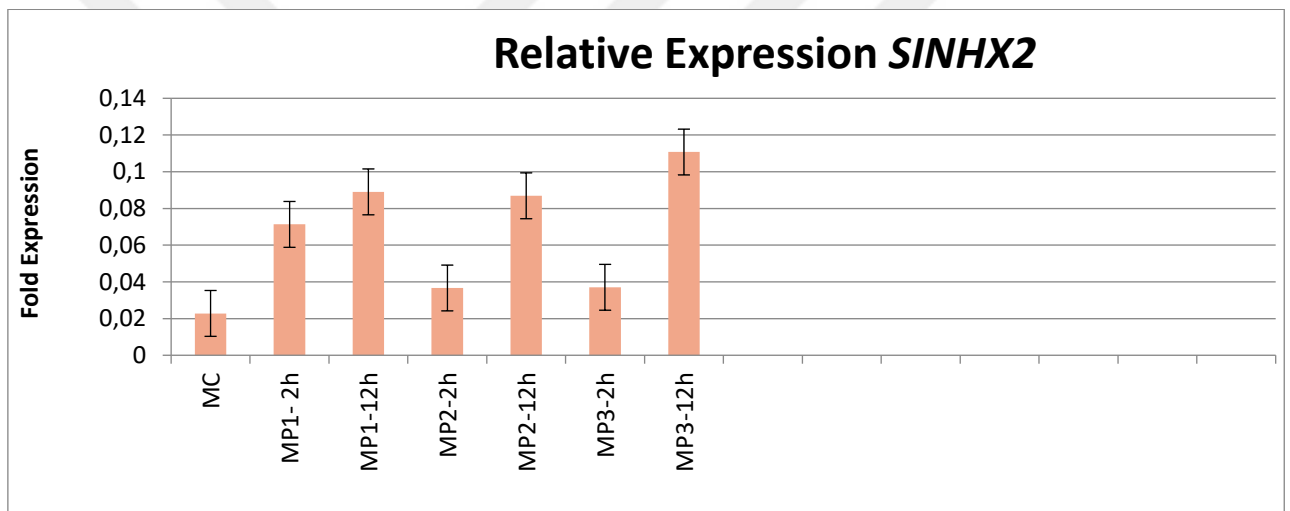


Figure 3.56. The Relative expression profile of *SINHX2* in root

In the control group, the gene exhibited a low level of expression. Following MS1 treatment, there were slight increases in gene expression at both the 2-hour and 12-hour time points. MP2-2h treatment also led to a modest increase in gene expression, while MP2-12h treatment showed a higher level. MP3-2h and MP3-12h treatments resulted in slightly elevated gene expression. These results suggest that the expression of the *SINHX2* gene is influenced by the applied treatments, with varying degrees of upregulation observed across different time points (Fig.3.56).

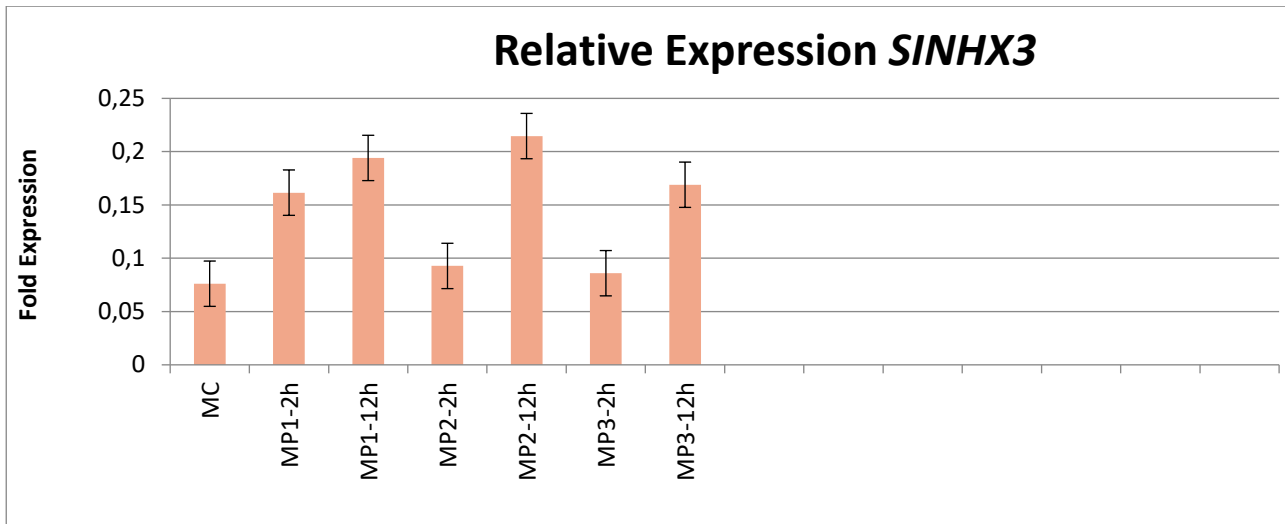


Figure 3.57. The Relative expression profile of *SINHX3* in root.

The transcript abundance of the gene *SINHX3* were investigated under different treatment conditions. In the control group (MC), the gene exhibited a moderate level of expression. Upon MP1 treatment, there was a noticeable increase in gene expression at both the 2-hour and 12-hour time points. MP2-2h treatment also led to an upregulation of gene expression, while MP2-12h treatment showed a higher level. Similarly, MP3-2h and MP3-12h treatments resulted in increased gene expression levels. These findings indicate that the expression of the *SINHX3* gene is influenced by the applied treatments, with notable variations observed across different time points. (Fig.3.57).

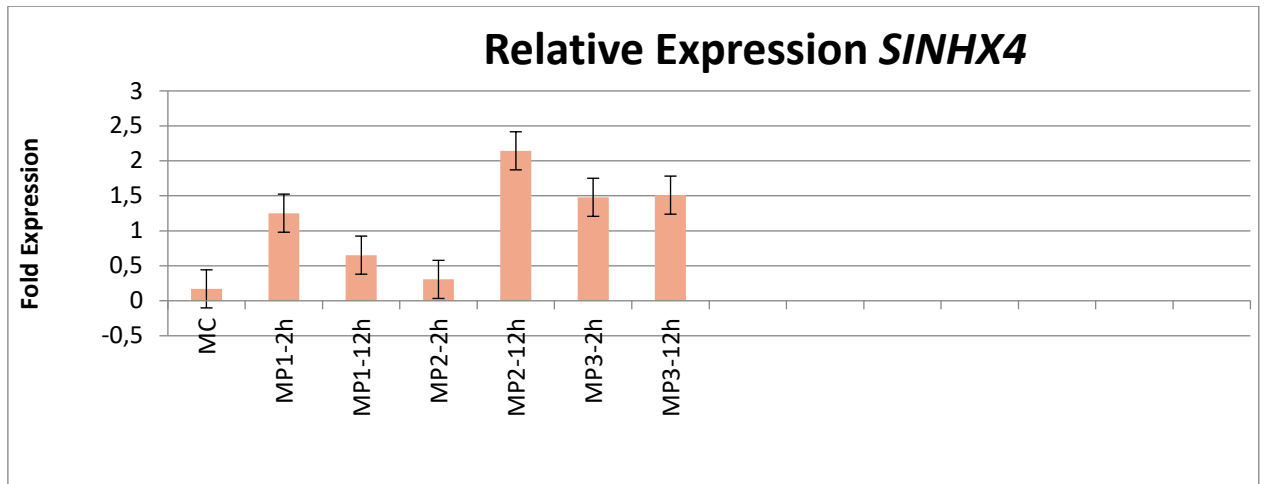


Figure 3.58. The Relative expression profile of *SINHX4* in root

In the control group, the gene exhibited a moderate expression level. Upon MS1 treatment, there was a significant increase in gene expression at the 2-hour time point, while the expression decreased at the 12-hour time point. MP2-2h treatment also resulted in an upregulation of gene expression, whereas MP2-12h treatment showed a substantial increase. Similarly, MP3-2h and MP3-12h treatments led to elevated gene expression levels. These findings suggest that the expression of the *SINHX4* gene is responsive to the applied treatments, with varying expression patterns observed at different time points (Fig.3.58).

3.5. Cis Regulatory Elements Analysis

3.5.1 SINAC CREs

Plant CARE program was used on 2,000 base pairs upstream region of 93 *SINAC* genes. In this analysis, the potential cis-regulatory elements that are responsible for different functions such as the transcriptome-related functions and response to environmental stressors in *S. lycopersicum* were detected. Based on this investigation, 86 types of motifs were detected in *SINAC* genes. These motifs were categorized into seven different groups according to their major roles. These groups were light-responsive elements (29%), hormone-responsive elements (14%), environmental stress-related elements (9%), developmental-related elements (13%), promoter-related elements (5%), site binding-related elements (5%), and other cis-regulatory elements (25%) that were shown through statistical analysis. The number of repetitions of the observed motifs is given separately in the below figures (Fig. 3.59).

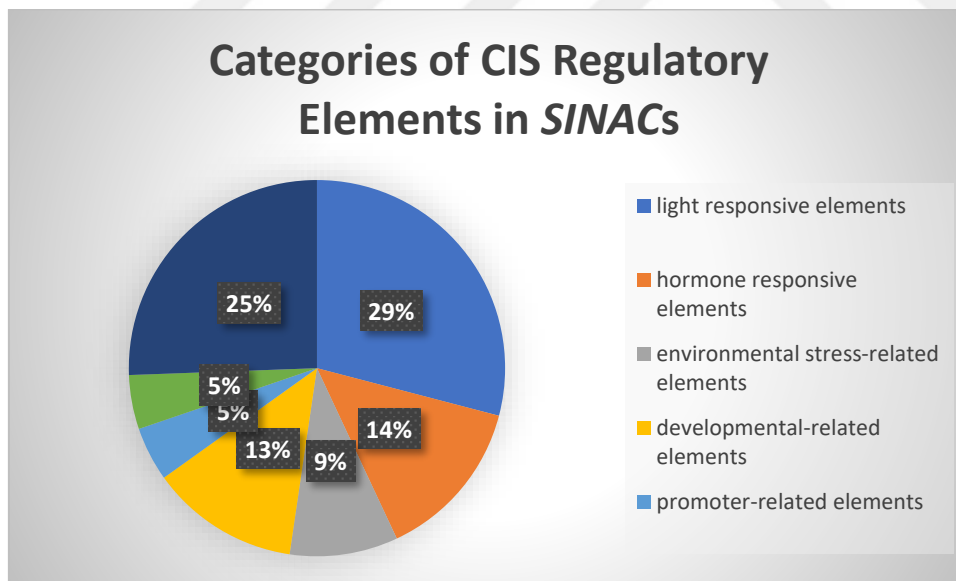


Figure 3.59. Categories of CIS Regulatory Elements in *SINACs*

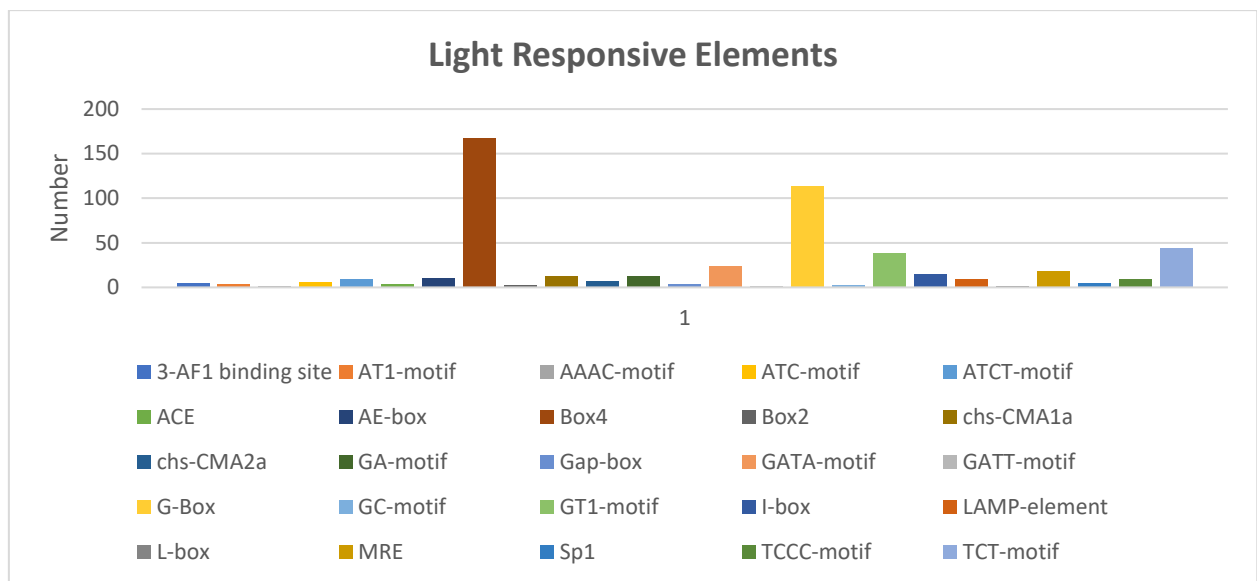


Figure 3.60. Light Responsive Elements in the *SINAC* genes

Box4, G-Box, GT1-motif, TCT-motif, and TCT-motif were the most important elements involved in plant light responses which were seen in the promoters of *SINAC* genes (Fig.3.60).

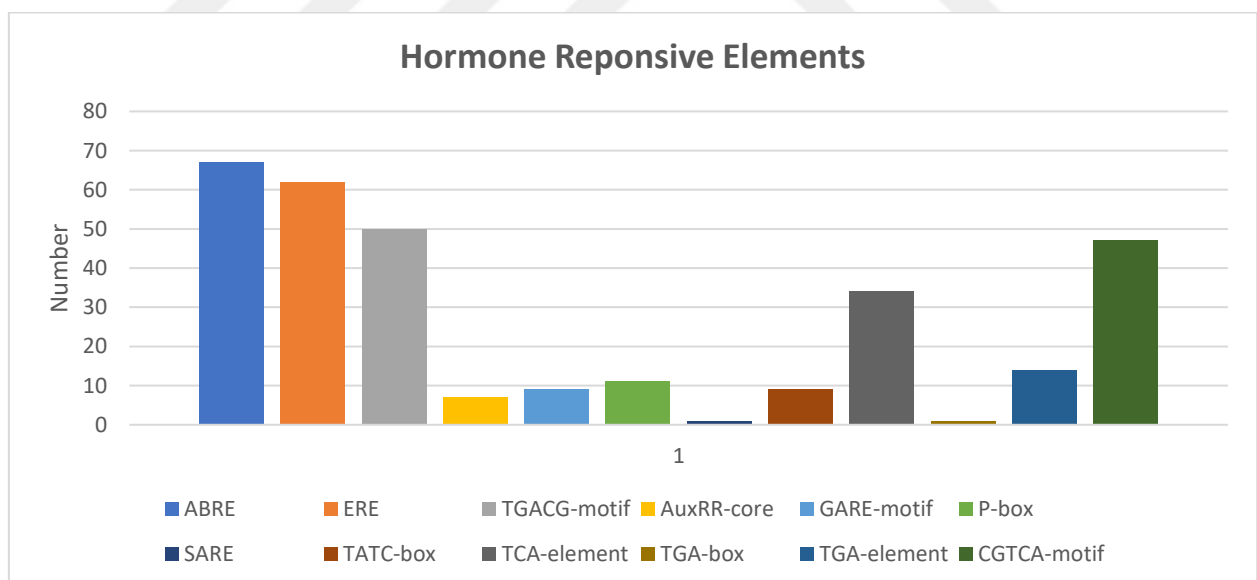


Figure 3.61. Hormone Responsive Elements in the *SINAC* genes

ABRE, ERE, TGACG-motif, TCA-element, and P-box were the most considerable elements involved in plant hormone responses which were found in this analysis (Fig. 3.61).

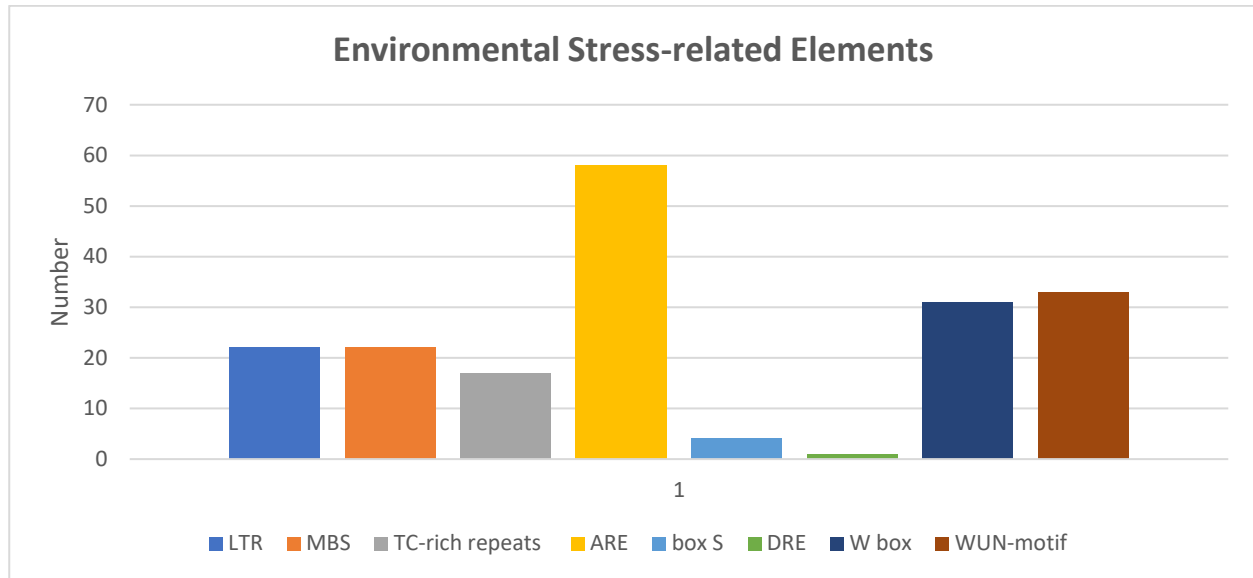


Figure 3.62. Environmental Stress-related Elements in the *SINAC* genes

Box4, ARE, W box and WUN-motif were the most prominent elements involved in environmental stress-related responses which were seen in the promoters of *SINAC* genes (Fig. 3.62).

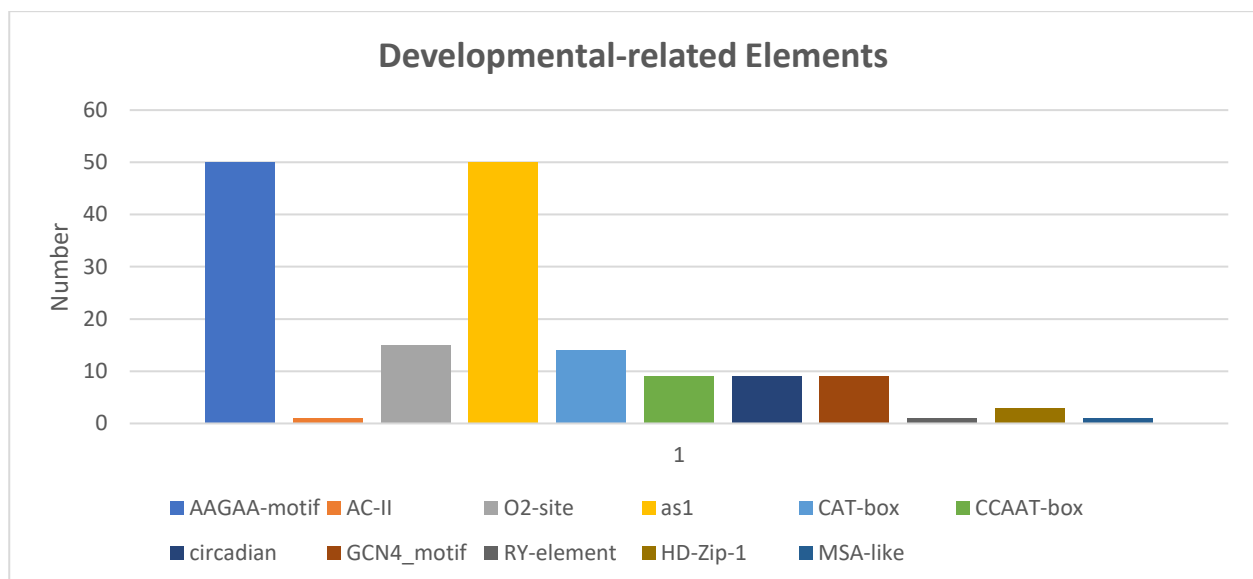


Figure 3.63. Developmental-related Elements in the *SINAC* genes

AAGAA-motif and as1 were the most important elements involved in developmental-related responses which were seen in the promoters of *SINAC* genes (Fig. 3.63).

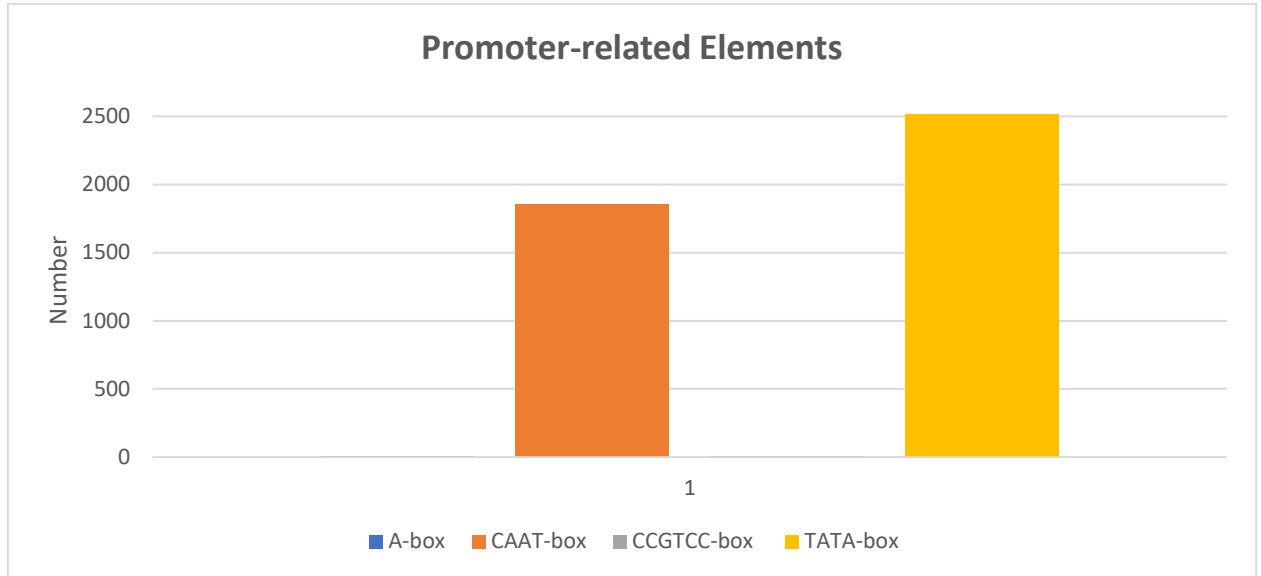


Figure 3.64. Promoter-related Elements in the *SINAC* genes

CAAT-motif and TATA-box were the most important elements related to promoters which were seen in the promoters of *SINAC* genes (Fig. 3.64).

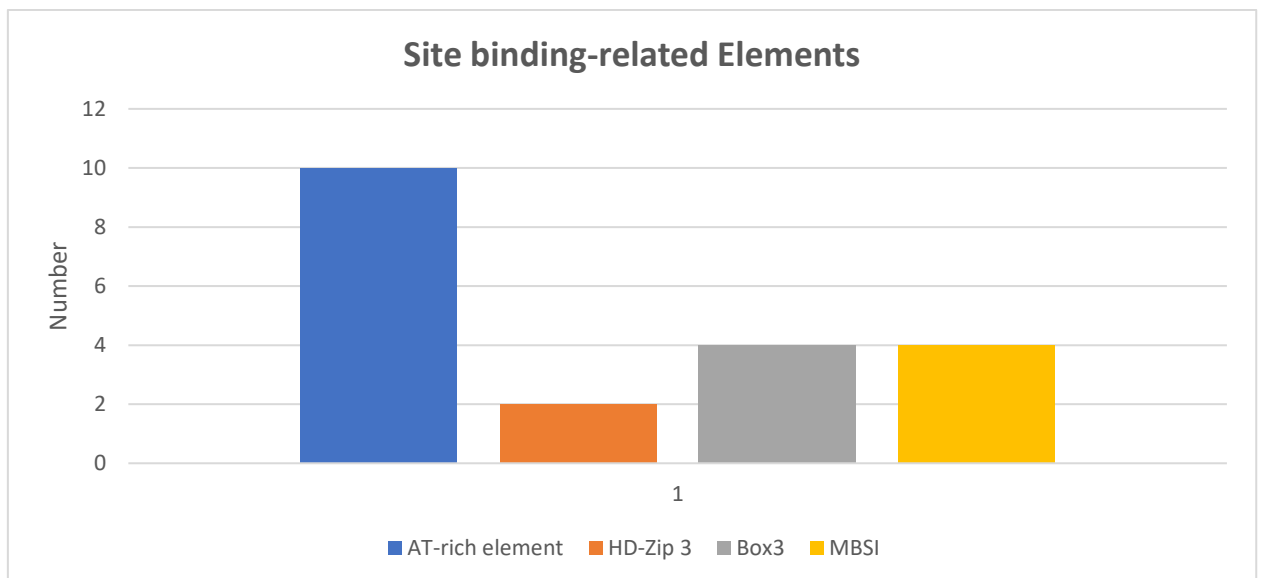


Figure 3.65. Site binding-related Elements in the *SINAC* genes

AT-rich elements were the most important elements related to site binding which were seen in the promoters of *SINAC* genes (Fig. 3.65).

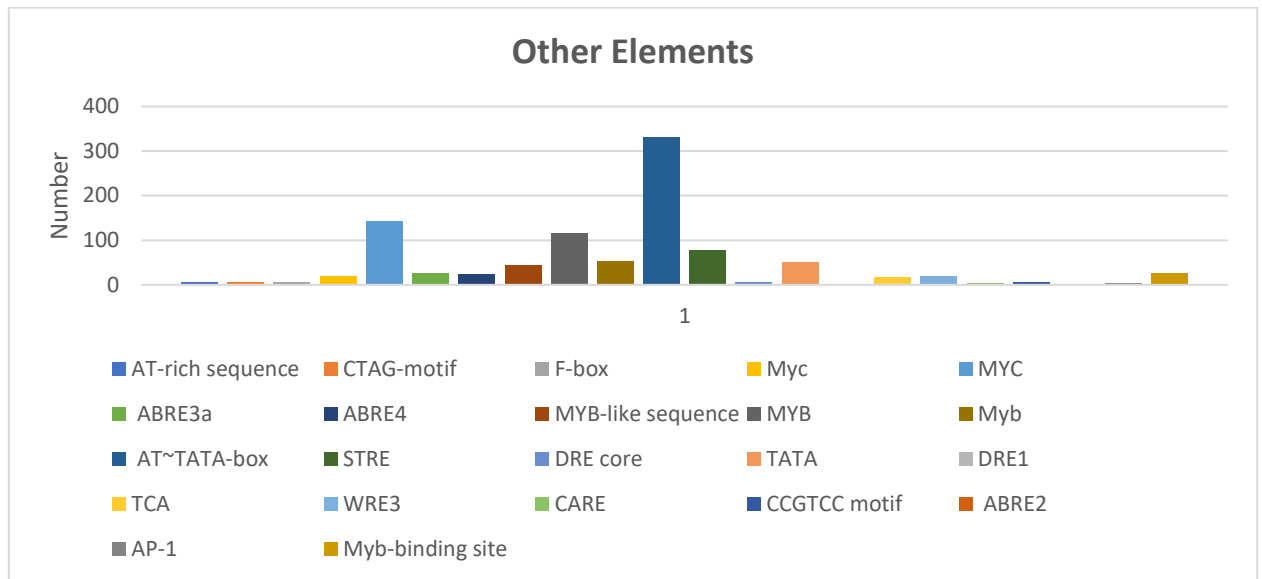


Figure 3.66. Other Elements in the *SINAC* genes

AT~TATA-box, MYC, MYB, and STRE elements were the most other important elements which were observed in the promoters of *SINAC* genes (Fig. 3.66).

3.5.2. *SINHX* CREs

The PLANTCARE program was used to investigate 2,000 base pairs upstream region of *NHX* genes. In this study, 4 *SINHX* genes for the analysis of potential cis-regulatory elements that are involved in the transcriptome-related functions and response to environmental stressors in *S. lycopersicum* were detected. According to the analysis, 39 types of motifs were found in *SINHX* genes. These motifs were grouped into seven different classes according to the major roles. These classes were light-responsive elements (26%), hormone-responsive elements (8%), environmental stress-related elements (13%), developmental-related elements (10%), promoter-related elements (10%), site binding-related elements (2%), and other cis-regulatory elements (31%) that were shown through statistical analysis. The number of repetitions of the observed motifs is given separately in the figures below (Fig. 3.67 to 3.73).

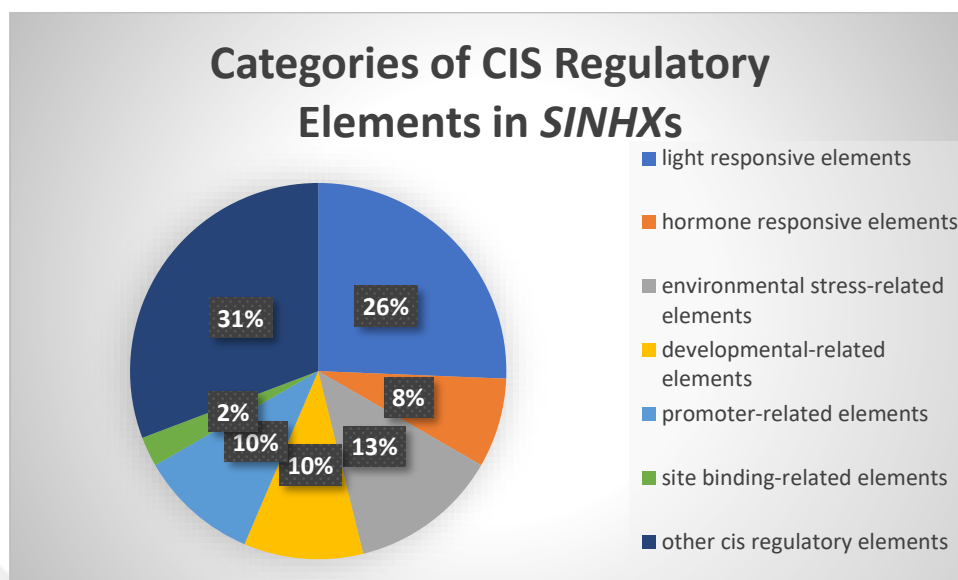


Figure 3.67. Categories of CIS Regulatory Elements in *SINHXs*

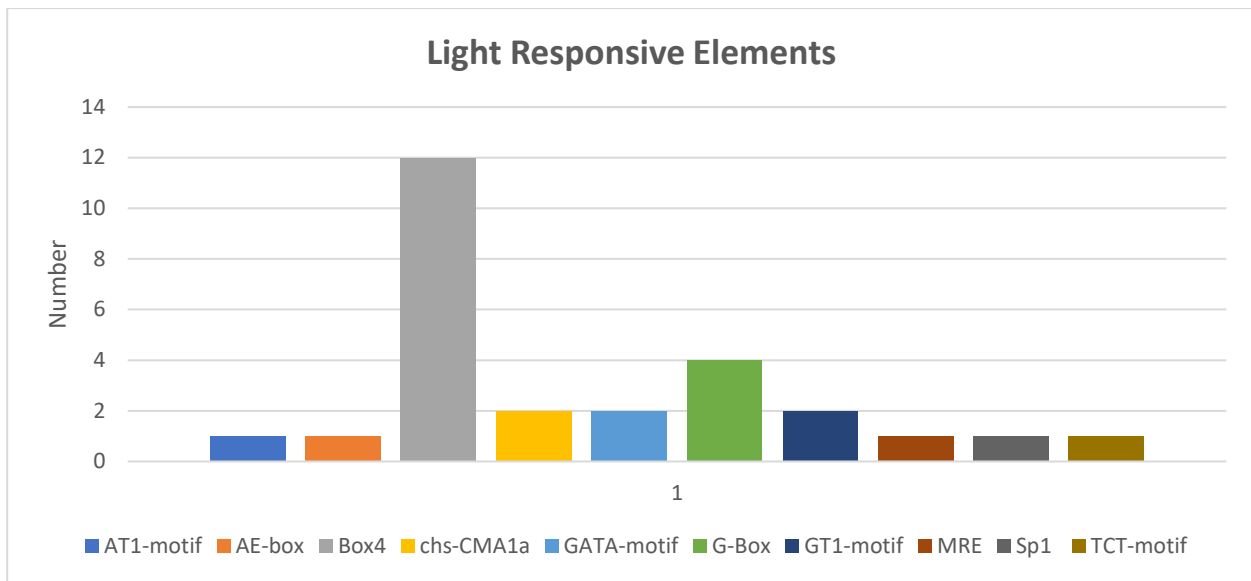


Figure 3.68. Light Responsive Elements in the *SINHX* genes

Box4, G-box, and GT1-motif were the most key elements involved in light responses which were seen in the promoters of *SINHX* genes (Fig. 3.69).

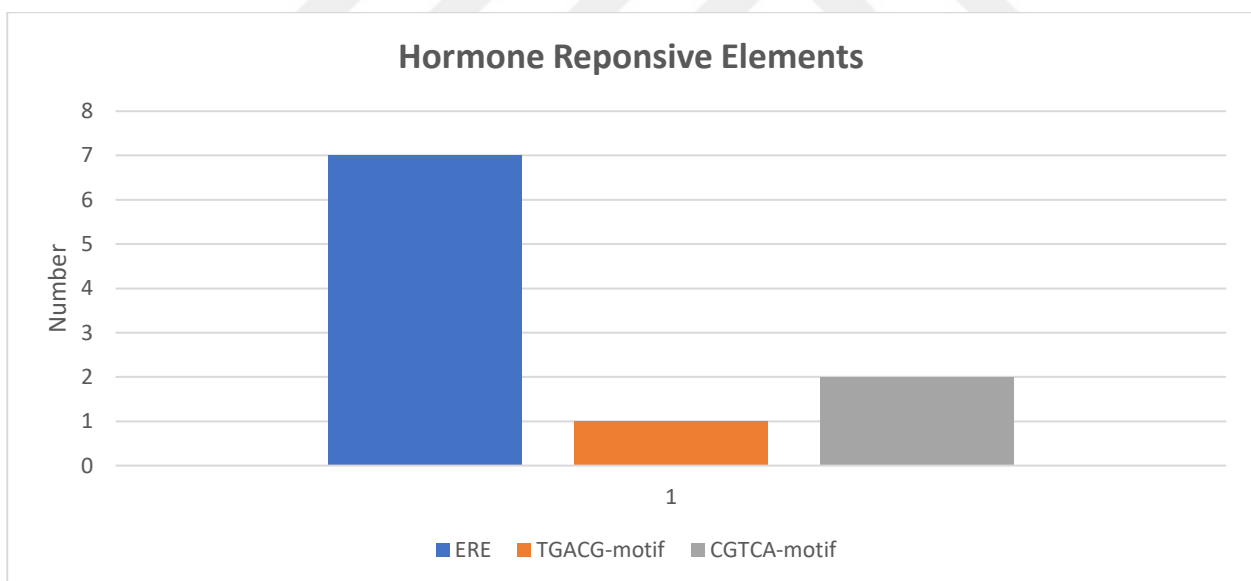


Figure 3.69. Hormone Responsive Elements in the *SINHX* genes

ERE, CGTCA-motif, and TGACG-motif were the most prominent elements involved in hormone-related responses which were seen in the promoters of *SINHX* genes (Fig. 3.69).

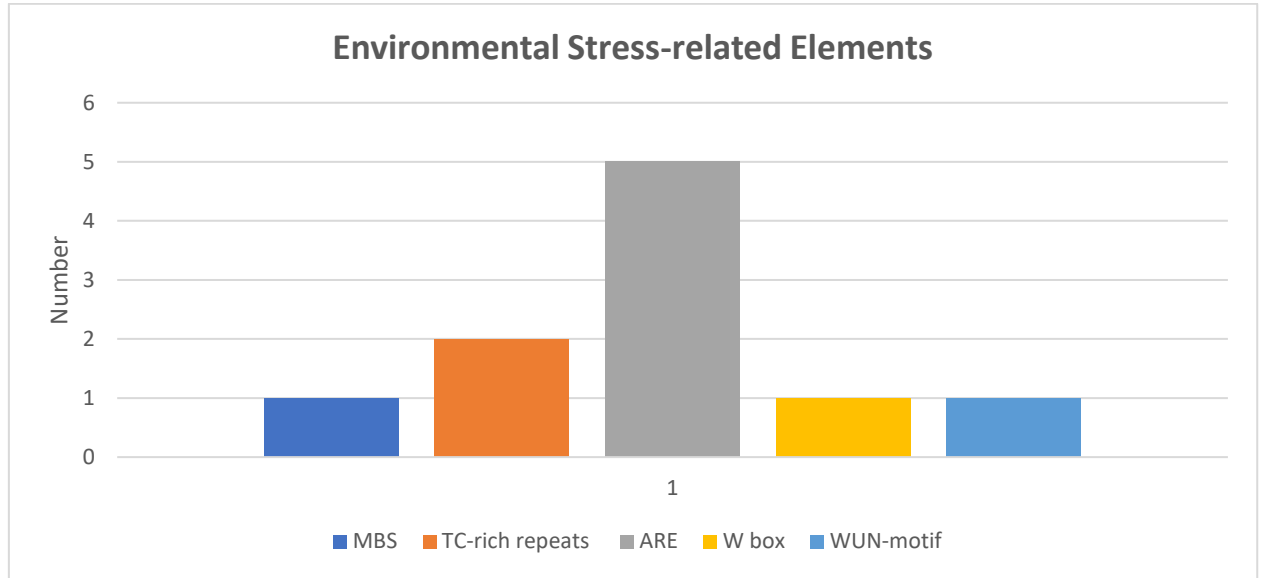


Figure 3.70. Environmental Stress-related Elements in the *SINHX* genes

ARE and TC-rich repeats were the most important elements involved in environmental stress-related responses which were seen in the promoters of *SINHX* genes (Fig. 3.70).

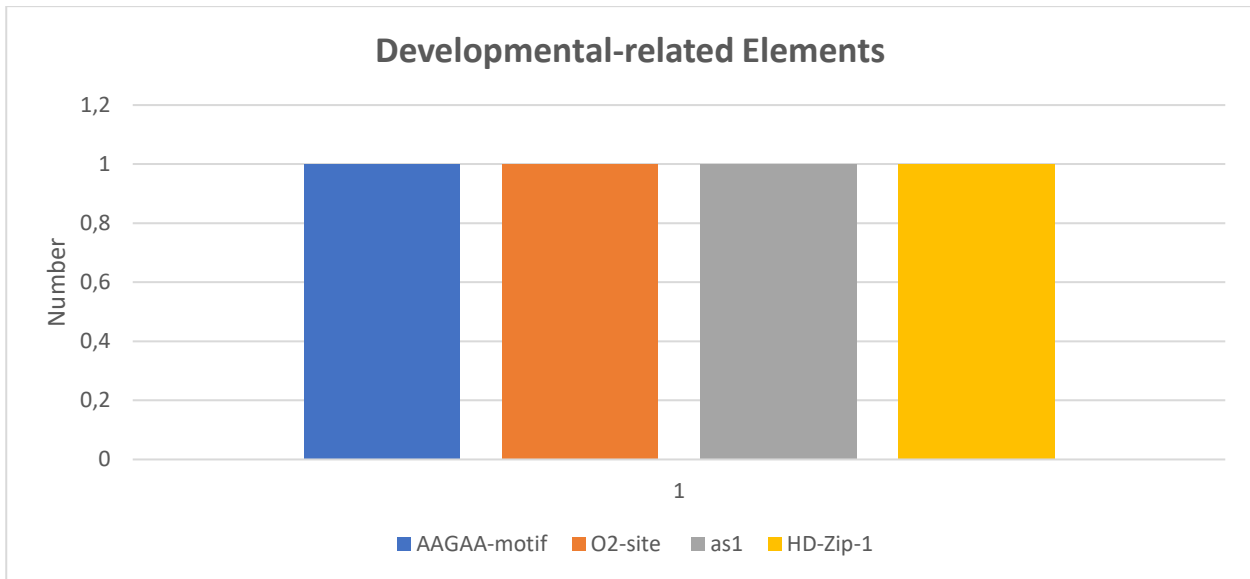


Figure 3.71. Developmental-related Elements in the *SINHX* genes

AAGAA-motif, O2-site, as1, and HD-Zip-1 were the most prominent elements involved in the development process which were seen in the promoters of *SINHX* genes (Fig. 3.71).

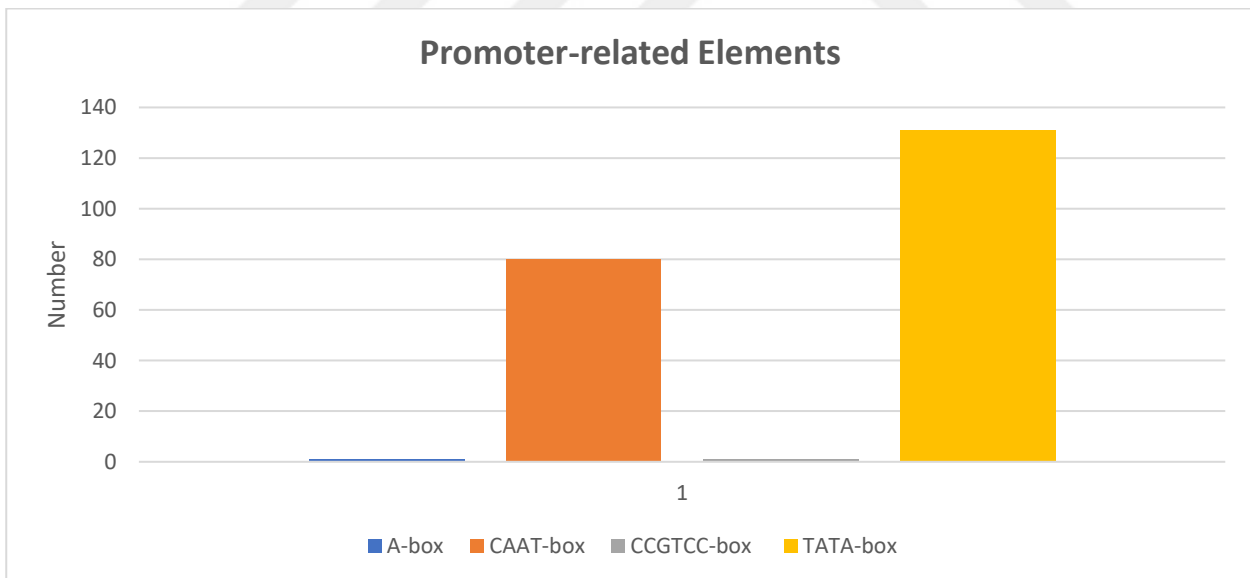


Figure 3.72. Promoter-related Elements in the *SINHX* genes

CAAT-box and TATA-box were the most key elements related to promoters which were seen in the promoters of *SINHX* genes (Fig. 3.72).

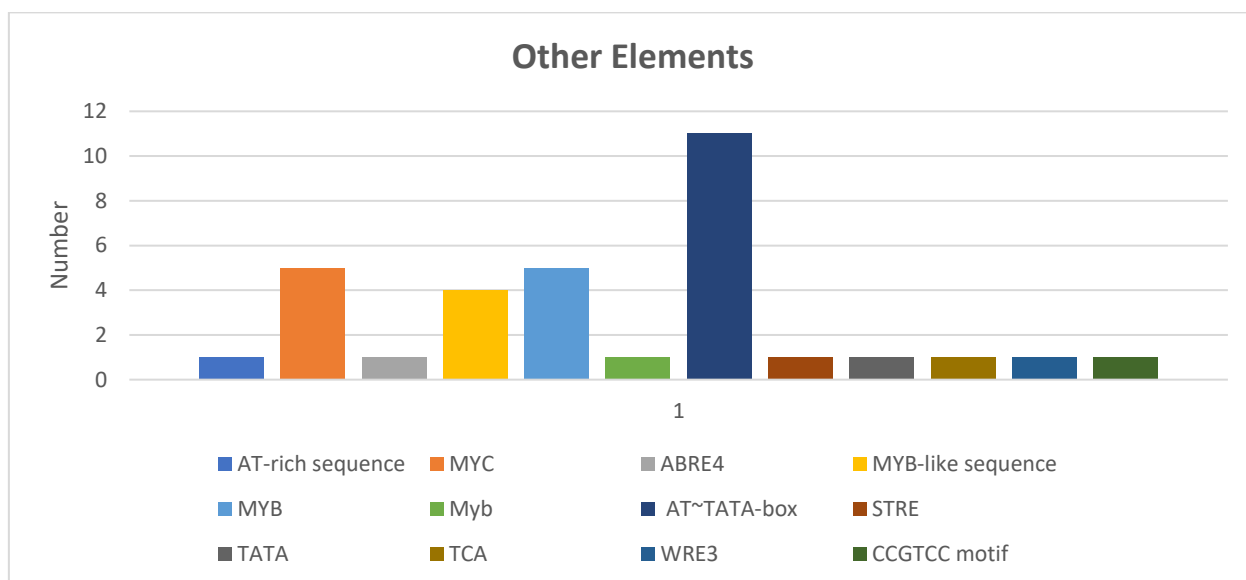


Figure 3.73. Other Elements in the *SINHX* genes

AT~TATA-box, MYC, MYB, and MYB-like elements were the most other important elements which were observed in the promoters of *SINHX* genes (Fig. 3.73).

4. DISCUSSION

Tomato cultivars responded to water restriction with a significant proportional fall in yield in semi-arid climate circumstances such as Turkey, also Water stress made plants more vulnerable to pathogenic diseases such as viruses, bacteria, and fungi (Celebi 2014). It is now widely known that several genes, including transcription factors (TFs) that help plants endure adverse conditions, regulate drought tolerance. These genes continue to be prospective genomic candidates for widespread crop breeding (Joshi et al., 2016). Also, globally, drought stress has an impact on plant development and productivity, and *NHX* genes, are well known for increasing drought tolerance in transgenic plants. Several plants have well-defined; nevertheless, nothing is known about *NHXs* in tomato plant (*S. lycopersicum*). Finally, the development of tomato cultivars that demonstrate tolerance to abiotic challenges highly depends on the identification of common and/or overlapping cis-regulatory elements since these elements are one of the main components of resistance protein interactions.

4.1. Expression Profile of *SINAC* Genes

In the current study, the expression profile of the tomato *NAC* gene family was systemically examined. Numerous researches have shown that *NAC* Transcription factors are present in a wide variety of plant species. and their ability to play a role in controlling plant growth, development, and stress responses (Puranik et al., 2012). Up until this point, this family appeared to be one of the biggest TFs. It was reported that Arabidopsis, rice, grape, apple, maize, chickpea, cassava, sesame, pears, and buckwheat have 117, 151, 79, 180, 152, 71, 96, 87, 185, and 80 *NAC* genes (Ooka et al., 2003; Nuruzzaman et al., 2010; Wang et al., 2013; Shiriga et al., 2014; Ha et al., 2014; Hu et al., 2015; Zhang et al., 2018; Ahmad et al., 2018; Liu et al., 2019).

According to previous studies in the *SolaNACeae* family, a considerable number of *NAC* genes were overexpressed under drought stress in *S. lycopersicum* (Al-Abdallat et al., 2015), *S. tuberosum* (Singh et al., 2013), *S. muricatum* (Yang et al., 2021), and sweet potato (Yan et al., 2021), and under other abiotic stresses such

as *S. lycopersicum* under Aluminum stress (Jin et al., 2020) or in the development process in *S. melongena* (Wan et al., 2021). This trend was consistent with the result of the present study where overexpression of a huge number of *NAC* genes under drought stress and PGPR inoculation has been approved.

Gene expression patterns can typically offer crucial clues for gene activity. Consequently, the expression levels of the 93 *SINAC* genes in the leaf and root of *S. lycopersicum* were determined using qRT-PCR data. A higher or lower expression level of the studied *SINAC*s under different conditions (drought stress and PGPR treatment) in the leaf and root tissues, compared to the control samples was found. These *SINAC*s demonstrated tissue- and stress-specific expression patterns. These genes may play significant roles in tomato stress tolerance. Additionally, different tissues and developmental phases showed distinct patterns of certain *SINAC* expressions, indicating that the *SINAC* TFs may perform a variety of tasks. Additionally, it was discovered that 36 *NAC* in root samples including *SINAC2*, *SINAC3*, *SINAC6*, *SINAC7*, *SINAC8*, *SINAC9*, *SINAC10*, *SINAC11*, *SINAC12*, *SINAC13*, *SINAC14*, *SINAC15*, *SINAC16*, *SINAC17*, *SINAC19*, *SINAC20*, *SINAC21*, *SINAC22*, *SINAC23*, *SINAC25*, *SINAC26*, *SINAC27*, *SINAC29*, *SINAC31*, *SINAC32*, *SINAC33*, *SINAC34*, *SINAC35*, *SINAC38*, *SINAC39*, *SINAC42*, *SINAC43*, *SINAC44*, *SINAC45*, *SINAC46*, *SINAC47* and four *NAC* genes in leaf samples including *SINAC37*, *SINAC40*, *SINAC43*, and *SINAC47* were highly expressed in all doses of drought stress treated samples, indicating that they may be involved in particular drought tolerance system in *S. lycopersicum*. The specific roles of the tomato *SINAC* genes will require further investigation in the future.

In the study conducted by Jin et al. (2020) on tomato (*S. lycopersicum*) root apex, 19 *SINAC* genes were found to exhibit both upregulation and downregulation. Among these genes, seven *SINAC*s showed an increase in expression levels under aluminum stress (5 μ M Al administered for 6 hours). Interestingly, three of these seven genes (*SINAC019*, *SINAC031*, and *SINAC033*) were also found to be upregulated in response to varying levels of drought stress. On the other hand, the expression levels of *SINAC28* and *SINAC41* were observed to decrease. Similarly, in the present study, these genes demonstrated downregulation following exposure

to a second dose of PEG for 2 hours. However, there were inconsistent results regarding the expression levels of *SINAC6*, *SINAC15*, *SINAC22*, and *SINAC39*. While these genes showed a decrease in expression under aluminum stress, an increase was observed at all levels of drought stress in the present research.

Under drought, plants may close their stomata to restrict the amount of water that goes out of their leaves. Homologous NAC family TFs of *ATNAC19*, *ATNAC55*, and *ATNAC72* in Arabidopsis leaf, can regulate JA/COR-mediated stomatal reopening (Zheng et al., 2012). *SINAC19* also showed a significant increase in the expression level under drought stress in root tissue in this study. This matter can show the importance of this gene in plant resistance under water deficiency pressure in different tissues.

4.2. Expression Profile of *SINHX* Genes

For many plants, including *A. thaliana* (Yokoi et al., 2002), rice (Basu et al., 2014), wheat (Yarra, 2019), sweet beet (Wu et al., 2019), cotton (Ma et al., 2020), and other plants, the importance of *NHX* gene families under drought and salt stresses have previously been discovered. However, the functionality of *NHX* genes in *S. lycopersicum* under drought stress using PGPR has not been studied yet. In this investigation, the genomic expression of four *NHX* genes in *S. lycopersicum* was examined. According to the research papers that have been mentioned earlier, the expression level of *NHX* genes changed significantly in drought and salt-stress-treated samples. Those results are completely consistent with the results of the *SINHX* gene expression profile in the present study.

Sodium-proton antiporters in tomato plants (*S. lycopersicum*) facilitate Na^+/H^+ and K^+/H^+ exchanges. This contributes to stress tolerance as well as K^+ nutrition. *NHXs* have also been found to increase salinity tolerance in leaves (Zhang and Blumwald, 2001). There was another research which was done by Rodriguez-Rosales et al. (2008) in this regard with the same approach. The *SINHXs* may also be a part of the responses to drought, according to the expression pattern for different genes and tissues. The current study found that the *NHX* genes express differently in two separate tissues of *S. lycopersicum*. The two tissues' diverse

expression patterns suggested that the *NHX* gene family offers options to breed this plant and overcome the functional restriction imposed by the original gene under drought stress. According to previous studies, it is known that there are many *NHX* protein isoforms present in tomato plants. Based on a study carried out by Rodríguez-Rosales et al. (2009) the majority of the *NHX* genes were activated by salt stress in the leaves of tomato (*lycopersicon esculentum*). Here it is proven that *NHX* genes can also be activated by drought stress in root tissue. It shows that *NHX* genes play a crucial role and have different functions in the defense system of *S. lycopersicum* in different tissues.

Regarding the effect of different durations of exposure to drought stress, it is reported that the expression level at different durations of PEG treatment was highly variable in leaf and root tissues of tea (*Camellia sinensis*) (Paul et al., 2021). This fold change variation was exactly what was observed in this study.

4.3. Plant growth-promoting rhizobacteria

The influence of plant growth-promoting rhizobacteria (PGPR) in bolstering host resilience during abiotic stress periods is well-documented, yet the molecular impact on tomato plants (*S. lycopersicum*), which frequently face drought conditions in Turkey, remains largely underexplored. *Bacillus megaterium* was found to stimulate tomato growth under both normal and salt-stressed environments. Regardless of the conditions, *B. megaterium* notably boosted the development of tomato plants, leading to more robust roots, shoots, and leaves (Nascimento et al., 2020). This study revealed that the inoculation of tomato seedlings with *B. megaterium* under normal conditions significantly increased the root and shoot dry weight, resulting in a pronounced augmentation in the overall dry biomass of the tomato plant. Similar observations were recorded under stress conditions, where *B. megaterium*-inoculated tomato seedlings displayed a substantially higher root and shoot dry weight, leading to an increase in total dry biomass compared to non-inoculated plants. These findings were complemented by the observed elevated *NAC* and *NHX* expression levels in PGPR-treated samples exposed to PEG, underpinning the beneficial role of PGPR in supporting tomato plants during drought stress.

While *B. megaterium* boosted expression levels of specific genes involved in the repair of damaged photosynthetic equipment and the preservation of redox equilibrium, it lowered the production of ROS and ethylene. Additionally, *B. megaterium* dramatically changed the metabolic profile to fix salinity-induced physiological disturbances in tomato (*L. esculentum*) (Akram et al., 2019). An observed increase in drought tolerance in this study is completely consistent with the higher level of expression in *NAC* and *NHX* genes in the leaf samples treated with X bacteria in this study.

Yang et al., (2022) also reported that *B. megaterium* could efficiently increase the tolerance of tomato (*S. lycopersicum*) under biotic stresses by affecting a number of functional resistance genes. In addition, Samaras et al. (2021) provided the same result in the transcription pattern of defense-related genes when this genus of rhizobacteria was inoculated into this plant. This rhizobacterium had the same impact as these two previous studies on *NAC* and *NHX* genes in this study.

4.4. CIS Regulatory Elements:

TFs play important roles in a variety of biological processes, including ripening, by precisely binding to cis-acting regions and activating or inhibiting the expression of downstream target genes (Okkema and Krause, 2005). (Karlova et al., 2014).

The important role of AAGAA-motif as drought stress-inducible cis-acting elements was pointed out previously in a study (Xu et al., 2020). Interestingly, 51 AAGAA motifs were seen in the *NAC* and *NHX* genes studied here. An overexpression was seen in the genes containing this motif under stress conditions in the present study.

Some genes contain a region named activation sequence-1 (as-1 promoter element) and are known to be salicylic acid and auxin-responsive. This promoter was seen to be involved in tolerance to reactive oxygen species (Garretón et al., 2002).

It was proven that reactive oxygen species were involved in drought stress tolerance (Cruz de Carvalho, 2008). Therefore, the high number of as-1 promoters in the structure of *NAC* and *NHX* genes studied in this research is another piece of evidence that can confirm the importance of these genes in drought stress tolerance.

OsSnRK2 genes, which were predicted to be involved in the stress responses in rice, contained CAT-box elements, and these elements were also seen frequently in *SINAC* and *SINHX* genes and their presence indicates the potential involvement of these elements in providing drought tolerance (Yu et al., 2022).



5. CONCLUSION

The expression profiles of the 46 *NAC* and 4 *NHX* genes in the root and 4 *NAC* and 4 *NHX* genes in tomato (*S. lycopersicum*) were examined in this research to determine how they respond to drought stress. The data obtained from this study will provide essential information for the functional characterization of these genes in other tissues of other species of tomato under other types of stressors. In general, a significant increase in the expression of all four investigated *SINAC* genes has been seen in the leaf tissue. This increase in expression at the highest level of PEG has been more considerable than other doses. Also, PGPR inoculation had a positive effect on increasing the expression of the mentioned genes, especially in the second and third doses of PEG. In relation to four *SINHX* genes, an increase in expression has been seen in the leaf tissue due to exposure to drought. This increase in expression in samples inoculated with PGPR has increased more in the second and third doses. In general, drought stress in all three levels of PEG caused an increase in the expression of all the studied *SINAC* genes. In some cases, the highest level of expression has been observed in the third doz and 12 hours of drought stress. Finally, drought stress in the root tissue caused an increase in the expression of all four *SINHX* genes, and this increase in expression in the twelve-hour treatments of all three doses had the highest level compared to the two-hour treatments.

REFERENCES

- Ahmad, M., Yan, X., Li, J., Yang, Q., Jamil, W., Teng, Y. and Bai, S., 2018. Genome wide identification and predicted functional analyses of NAC transcription factors in Asian pears. *BMC plant biology*, 18(1), pp.1-15.
- Aida, M., Ishida, T., Fukaki, H., Fujisawa, H. and Tasaka, M., 1997. Genes involved in organ separation in Arabidopsis: an analysis of the cup-shaped cotyledon mutant. *The plant cell*, 9(6), pp.841-857.
- Akram, W., Aslam, H., Ahmad, S.R., Anjum, T., Yasin, N.A., Khan, W.U., Ahmad, A., Guo, J., Wu, T., Luo, W. and Li, G., 2019. *Bacillus megaterium* strain A12 ameliorates salinity stress in tomato plants through multiple mechanisms. *Journal of Plant Interactions*, 14(1), pp.506-518.
- Al-Abdallat, A.M., Ali-Sheikh-Omar, M.A. and Alnemer, L.M., 2015. Overexpression of two ATNAC3-related genes improves drought and salt tolerance in tomato (*Solanum lycopersicum* L.). *Plant Cell, Tissue and Organ Culture (PCTOC)*, 120(3), pp.989-1001.
- Arefin, S., Bhuiyan, M.F.H., Akther, J., Prodhan, S.H. and Hoque, H., 2021. The dynamics of cis-regulatory elements in promoter regions of tomato sucrose transporter genes. *International Journal of Vegetable Science*, 27(2), pp.167-186.
- Aroca, R., 2012. Plant responses to drought stress. *From morphological to molecular features*, pp.1-5.
- Basu, S. and Roychoudhury, A., 2014. Expression profiling of abiotic stress-inducible genes in response to multiple stresses in rice (*Oryza sativa* L.) varieties with contrasting level of stress tolerance. *BioMed research international*, 2014.

REFERENCES (Cont.)

- Bhaskaran, S. and Savithramma, D.L., 2011. Co-expression of *Pennisetum glaucum* vacuolar Na⁺/H⁺ antiporter and *Arabidopsis* H⁺-pyrophosphatase enhances salt tolerance in transgenic tomato. *Journal of experimental botany*, 62(15), pp.5561-5570.
- BhattacharyyaPN, J., 2012. Plantgrowth-promotingrhizobacteria (PGPR): Emergencein agriculture. *World J. Microbiol. Biotechnol*, 28, pp.1327-1350.
- Blilou, I., Xu, J., Wildwater, M., Willemsen, V., Paponov, I., Friml, J., Heidstra, R., Aida, M., Palme, K. and Scheres, B., 2005. The PIN auxin efflux facilitator network controls growth and patterning in *Arabidopsis* roots. *Nature*, 433(7021), pp.39-44.
- Blum, A., 2005. Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive?. *Australian Journal of Agricultural Research*, 56(11), pp.1159-1168.
- Bohnert, H.J., Nelson, D.E. and Jensen, R.G., 1995. Adaptations to environmental stresses. *The plant cell*, 7(7), p.1099.
- Bray, E.A., 1988. Drought-and ABA-induced changes in polypeptide and mRNA accumulation in tomato leaves. *Plant Physiology*, 88(4), pp.1210-1214.
- Brini, F. and Masmoudi, K., 2012. Ion transporters and abiotic stress tolerance in plants. *International Scholarly Research Notices*, 2012.
- Calvo-Polanco, M., Sánchez-Romera, B., Aroca, R., Asins, M.J., Declerck, S., Dodd, I.C., Martínez-Andújar, C., Albacete, A. and Ruiz-Lozano, J.M., 2016. Exploring the use of recombinant inbred lines in combination with beneficial microbial inoculants (AM fungus and PGPR) to improve drought stress tolerance in tomato. *Environmental and Experimental Botany*, 131, pp.47-57.

REFERENCES (Cont.)

- Celebi, M., 2014. The effect of water stress on tomato under different emitter discharges and semi-arid climate condition. *Bulgarian Journal of Agricultural Science*, 20(5), pp.1151-1157.
- Chaves, M.M., Flexas, J. and Pinheiro, C., 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Annals of botany*, 103(4), pp.551-560.
- Chen, H., Li, Z. and Xiong, L., 2012. A plant microRNA regulates the adaptation of roots to drought stress. *FEBS letters*, 586(12), pp.1742-1747.
- Chen, X., Wang, Y., Lv, B., Li, J., Luo, L., Lu, S., Zhang, X., Ma, H. and Ming, F., 2014. The NAC family transcription factor OsNAP confers abiotic stress response through the ABA pathway. *Plant and Cell Physiology*, 55(3), pp.604-619.
- Cruz de Carvalho, M.H., 2008. Drought stress and reactive oxygen species: production, scavenging and signaling. *Plant signaling & behavior*, 3(3), pp.156-165.
- Dai, A.H., Yang, S.X., Zhou, H.K., Tang, K.Q., Li, G., Leng, J.T., Yu, H., Zhang, Y.H., Gao, J.S., Yang, X. and Guo, Y.J., 2018. Evolution and expression divergence of the CYP78A subfamily genes in soybean. *Genes*, 9(12), p.611.
- Davis, A. R., et al. 2023. Morphological, physiological, and biochemical adaptations of plant species to cope with drought stress. *Journal of Plant Physiology*, 245, 123-136.
- Deak, K.I. and Malamy, J., 2005. Osmotic regulation of root system architecture. *The Plant Journal*, 43(1), pp.17-28.

REFERENCES (Cont.)

- Demmig-Adams, B. and Adams III, W.W., 2006. Photoprotection in an ecological context: the remarkable complexity of thermal energy dissipation. *New phytologist*, 172(1), pp.11-21.
- Dixit, R., Agrawal, L., Gupta, S., Kumar, M., Yadav, S., Chauhan, P.S. and Nautiyal, C.S., 2016. Southern blight disease of tomato control by 1-aminocyclopropane-1-carboxylate (ACC) deaminase producing *Paenibacillus lentimorbus* B-30488. *Plant signaling & behavior*, 11(2), p.e1113363.
- Du, M., Zhai, Q., Deng, L., Li, S., Li, H., Yan, L., Huang, Z., Wang, B., Jiang, H., Huang, T. and Li, C.B., 2014. Closely related NAC transcription factors of tomato differentially regulate stomatal closure and reopening during pathogen attack. *The Plant Cell*, 26(7), pp.3167-3184.
- Duan, Y.B., Li, J., Qin, R.Y., Xu, R.F., Li, H., Yang, Y.C., Ma, H., Li, L., Wei, P.C. and Yang, J.B., 2016. Identification of a regulatory element responsible for salt induction of rice OsRAV2 through ex situ and in situ promoter analysis. *Plant molecular biology*, 90(1), pp.49-62.
- Duval, M., Hsieh, T.F., Kim, S.Y. and Thomas, T.L., 2002. Molecular characterization of AtNAM: a member of the Arabidopsis NAC domain superfamily. *Plant molecular biology*, 50(2), pp.237-248.
- Edwards, G. and Walker, D., 1983. C Three C Four: Mechanisms, Cellular and Environmental Regulation of Photosynthesis. Univ of California Press.
- Ermawati, N., Hong, J.C., Son, D. and Cha, J.Y., 2021. Isolation of Multi-Abiotic Stress Response Genes to Generate Global Warming Defense Forage Crops. *Journal of The Korean Society of Grassland and Forage Science*, 41(4), pp.242-249.

REFERENCES (Cont.)

- España, A.P., Santiago-Algarra, D., Pradel, L. and Spicuglia, S., 2017. High-throughput approaches to study cis-regulating elements. *Biologie Aujourd'hui*, 211(4), pp.271-280.
- FAO (2020) Food and Agriculture Organization of the United Nations. FAO, Rome
- Fukao, T., Xu, K., Ronald, P.C. and Bailey-Serres, J., 2006. A variable cluster of ethylene response factor-like genes regulates metabolic and developmental acclimation responses to submergence in rice. *The Plant Cell*, 18(8), pp.2021-2034.
- Gaff, D.F., 1987. Desiccation tolerant plants in South America. *Oecologia*, 74(1), pp.133-136.
- García-Abellan, J.O., Egea, I., Pineda, B., Sanchez-Bel, P., Belver, A., Garcia-Sogo, B., Flores, F.B., Atares, A., Moreno, V. and Bolarin, M.C., 2014. Heterologous expression of the yeast HAL5 gene in tomato enhances salt tolerance by reducing shoot Na⁺ accumulation in the long term. *Physiologia plantarum*, 152(4), pp.700-713.
- Garretón, V., Carpinelli, J., Jordana, X. and Holuigue, L., 2002. The as-1 promoter element is an oxidative stress-responsive element and salicylic acid activates it via oxidative species. *Plant physiology*, 130(3), pp.1516-1526.
- Gisbert, C., Rus, A.M., Bolarín, M.C., López-Coronado, J.M., Arrillaga, I., Montesinos, C., Caro, M., Serrano, R. and Moreno, V., 2000. The yeast HAL1 gene improves salt tolerance of transgenic tomato. *Plant physiology*, 123(1), pp.393-402.
- Goodstein, D.M., Shu, S., Howson, R., Neupane, R., Hayes, R.D., Fazo, J., Mitros, T., Dirks, W., Hellsten, U., Putnam, N. and Rokhsar, D.S., 2012. Phytozome: a comparative platform for green plant genomics. *Nucleic acids research*, 40(D1), pp.D1178-D1186.

REFERENCES (Cont.)

- Gowik, U. and Westhoff, P., 2011. The path from C3 to C4 photosynthesis. *Plant Physiology*, 155(1), pp.56-63.
- Guan, Y.S., Serraj, R., Liu, S.H., Xu, J.L., Ali, J., Wang, W.S., Venus, E., Zhu, L.H. and Li, Z.K., 2010. Simultaneously improving yield under drought stress and non-stress conditions: a case study of rice (*Oryza sativa* L.). *Journal of Experimental Botany*, 61(15), pp.4145-4156.
- Ha, C.V., Nasr Esfahani, M., Watanabe, Y., Tran, U.T., Sulieman, S., Mochida, K., Van Nguyen, D. and Tran, L.S.P., 2014. Genome-wide identification and expression analysis of the CaNAC family members in chickpea during development, dehydration and ABA treatments. *PLoS One*, 9(12), p.e114107.
- Higo, K., Ugawa, Y., Iwamoto, M. and Korenaga, T., 1999. Plant cis-acting regulatory DNA elements (PLACE) database: 1999. *Nucleic acids research*, 27(1), pp.297-300.
- Hu, W., Wei, Y., Xia, Z., Yan, Y., Hou, X., Zou, M., Lu, C., Wang, W. and Peng, M., 2015. Genome-wide identification and expression analysis of the NAC transcription factor family in cassava. *PLoS One*, 10(8), p.e0136993.
- Huang, L., Li, Q., Zhang, C., Chu, R., Gu, Z., Tan, H., Zhao, D., Fan, X. and Liu, Q., 2020. Creating novel Wx alleles with fine-tuned amylose levels and improved grain quality in rice by promoter editing using CRISPR/Cas9 system. *Plant Biotechnology Journal*, 18(11), p.2164.
- Huertas, R., Rubio, L., CagNAC, O., GARCÍA-SÁNCHEZ, M.J., Alché, J.D.D., Venema, K., Fernández, J.A. and RODRÍGUEZ-ROSALES, M.P., 2013. The K⁺/H⁺ antiporter *LeNHX2* increases salt tolerance by improving K⁺ homeostasis in transgenic tomato. *Plant, Cell & Environment*, 36(12), pp.2135-2149.

REFERENCES (Cont.)

- Jeong, J.S., Kim, Y.S., Redillas, M.C., Jang, G., Jung, H., Bang, S.W., Choi, Y.D., Ha, S.H., Reuzeau, C. and Kim, J.K., 2013. OsNAC5 overexpression enlarges root diameter in rice plants leading to enhanced drought tolerance and increased grain yield in the field. *Plant Biotechnology Journal*, 11(1), pp.101-114.
- Ji, X., Dong, B., Shiran, B., Talbot, M.J., Edlington, J.E., Hughes, T., White, R.G., Gubler, F. and Dolferus, R., 2011. Control of abscisic acid catabolism and abscisic acid homeostasis is important for reproductive stage stress tolerance in cereals. *Plant physiology*, 156(2), pp.647-662.
- Jian, W., Zheng, Y., Yu, T., Cao, H., Chen, Y., Cui, Q., Xu, C. and Li, Z., 2021. SINAC6, A NAC transcription factor, is involved in drought stress response and reproductive process in tomato. *Journal of Plant Physiology*, 264, p.153483.
- Jin, J.F., Wang, Z.Q., He, Q.Y., Wang, J.Y., Li, P.F., Xu, J.M., Zheng, S.J., Fan, W. and Yang, J.L., 2020. Genome-wide identification and expression analysis of the NAC transcription factor family in tomato (*Solanum lycopersicum*) during aluminum stress. *BMC genomics*, 21(1), pp.1-14.
- Johnson, M., 2021. Escalating frequency of severe drought conditions due to global climate change. *Environmental Science & Policy*, 25(1), 45-52.
- Jones, M.M., Turner, N.C. and Osmond, C.B., 1981. Mechanisms of drought resistance. In 'The physiology and biochemistry of drought resistance in plants'.(Eds LG Paleg, D Aspinall) pp. 15–37.
- Jongdee, B., Fukai, S. and Cooper, M., 2002. Leaf water potential and osmotic adjustment as physiological traits to improve drought tolerance in rice. *Field Crops Research*, 76(2-3), pp.153-163.

REFERENCES (Cont.)

- Joshi, R., Wani, S.H., Singh, B., Bohra, A., Dar, Z.A., Lone, A.A., Pareek, A. and Singla-Pareek, S.L., 2016. Transcription factors and plants response to drought stress: current understanding and future directions. *Frontiers in Plant Science*, 7, p.1029.
- Karaba, A., Dixit, S., Greco, R., Aharoni, A., Trijatmiko, K.R., Marsch-Martinez, N., Krishnan, A., Nataraja, K.N., Udayakumar, M. and Pereira, A., 2007. Improvement of water use efficiency in rice by expression of HARDY, an Arabidopsis drought and salt tolerance gene. *Proceedings of the National Academy of Sciences*, 104(39), pp.15270-15275.
- Kloepper, J.W. and Schroth, M.N., 1981. Relationship of in vitro antibiosis of plant growth-promoting rhizobacteria to plant growth and the displacement of root microflora. *Phytopathology*, 71(10), pp.1020-1024.
- Koul, A., Sharma, D., Kaul, S. and Dhar, M.K., 2019. Identification and in silico characterization of cis-acting elements of genes involved in carotenoid biosynthesis in tomato. *3 Biotech*, 9(7), pp.1-11.
- Lawlor, D.W. and Cornic, G., 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant, cell & environment*, 25(2), pp.275-294.
- Lawlor, D.W., 2002. Limitation to photosynthesis in water-stressed leaves: stomata vs. metabolism and the role of ATP. *Annals of botany*, 89(7), pp.871-885.
- Leidi, E.O., Barragán, V., Rubio, L., El-Hamdaoui, A., Ruiz, M.T., Cubero, B., Fernández, J.A., Bressan, R.A., Hasegawa, P.M., Quintero, F.J. and Pardo, J.M., 2010. The AtNHX1 exchanger mediates potassium compartmentation in vacuoles of transgenic tomato. *The Plant Journal*, 61(3), pp.495-506.

REFERENCES (Cont.)

- Lescot, M., 2002. D'haes P, Thijs G, Marchal K, Moreau Y, Van de Peer Y, et al. PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucl. Acids Res*, 30, pp.325-327.
- Li, H., Ding, X., Wang, C., Ke, H., Wu, Z., WANG, Y., Liu, H. and Guo, J., 2016. Control of tomato yellow leaf curl virus disease by *Enterobacter asburiae* BQ9 as a result of priming plant resistance in tomatoes. *Turkish Journal of Biology*, 40(1), pp.150-159.
- Li, M., Li, Y., Li, H. and Wu, G., 2011. Overexpression of *AtNHX5* improves tolerance to both salt and drought stress in *Broussonetia papyrifera* (L.) Vent. *Tree physiology*, 31(3), pp.349-357.
- Li, Q., Feng, Q., Snouffer, A., Zhang, B., Rodríguez, G.R. and Van Der Knaap, E., 2022. Increasing Fruit Weight by Editing a Cis-Regulatory Element in Tomato KLUH Promoter Using CRISPR/Cas9. *Frontiers in Plant Science*, 13.
- Lippmann, R., Babben, S., Menger, A., Delker, C. and Quint, M., 2019. Development of wild and cultivated plants under global warming conditions. *Current Biology*, 29(24), pp.R1326-R1338.
- Liu, M., Ma, Z., Sun, W., Huang, L., Wu, Q., Tang, Z., Bu, T., Li, C. and Chen, H., 2019. Genome-wide analysis of the NAC transcription factor family in Tartary buckwheat (*Fagopyrum tataricum*). *BMC genomics*, 20(1), pp.1-16.
- Lu, Z., Ricci, W.A., Schmitz, R.J. and Zhang, X., 2018. Identification of cis-regulatory elements by chromatin structure. *Current opinion in plant biology*, 42, pp.90-94.

REFERENCES (Cont.)

- Ma, W., Ren, Z., Zhou, Y., Zhao, J., Zhang, F., Feng, J., Liu, W. and Ma, X., 2020. Genome-wide identification of the *Gossypium hirsutum* *NHX* genes reveals that the endosomal-type Gh*NHX4A* is critical for the salt tolerance of cotton. *International journal of molecular sciences*, 21(20), p.7712.
- Mahato, A., 2014. Climate change and its impact on agriculture. *International Journal of Scientific and Research Publications*, 4(4), pp.1-6.
- Maqsood, H., Munir, F., Amir, R. and Gul, A., 2022. Genome-wide identification, comprehensive characterization of transcription factors, cis-regulatory elements, protein homology, and protein interaction network of DREB gene family in *Solanum lycopersicum*. *Frontiers in Plant Science*, 13.
- Martínez-Viveros, O., Jorquera, M.A., Crowley, D.E., Gajardo, G.M.L.M. and Mora, M.L., 2010. Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. *Journal of soil science and plant nutrition*, 10(3), pp.293-319.
- Meng, F., Zhao, H., Zhu, B., Zhang, T., Yang, M., Li, Y., Han, Y. and Jiang, J., 2021. Genomic editing of intronic enhancers unveils their role in fine-tuning tissue-specific gene expression in *Arabidopsis thaliana*. *The Plant Cell*, 33(6), pp.1997-2014.
- Mishra, P., Singh, N., Jain, A., Jain, N. and Mishra, V., 2018. Identification of cis-regulatory elements associated with salinity and drought stress tolerance in rice from co-expressed gene interaction networks. *Bioinformation*, 14(3), p.123.
- Miyazawa, S.I., Yoshimura, S., Shinzaki, Y., Maeshima, M. and Miyake, C., 2008. Deactivation of aquaporins decreases internal conductance to CO₂ diffusion in tobacco leaves grown under long-term drought. *Functional Plant Biology*, 35(7), pp.553-564.

REFERENCES (Cont.)

- Molla, A.H., Haque, M., Haque, A. and Ilias, G.N.M., 2012. Trichoderma-enriched biofertilizer enhances production and nutritional quality of tomato (*Lycopersicon esculentum* Mill.) and minimizes NPK fertilizer use. *Agricultural Research*, 1(3), pp.265-272.
- Morgan, J.M., 1984. Osmoregulation and water stress in higher plants. *Annual review of plant physiology*, 35(1), pp.299-319.
- Nascimento, F.X., Hernández, A.G., Glick, B.R. and Rossi, M.J., 2020. Plant growth-promoting activities and genomic analysis of the stress-resistant *Bacillus megaterium* STB1, a bacterium of agricultural and biotechnological interest. *Biotechnology reports*, 25, p.e00406.
- Nikonorova, N., Van den Broeck, L., Zhu, S., Van De Cotte, B., Dubois, M., Gevaert, K., Inzé, D. and De Smet, I., 2018. Early mannitol-triggered changes in the *Arabidopsis* leaf (phospho) proteome reveal growth regulators. *Journal of experimental botany*, 69(19), pp.4591-4607.
- Niu, D.D., Zheng, Y., Zheng, L., Jiang, C.H., Zhou, D.M. and Guo, J.H., 2016. Application of PSX biocontrol preparation confers root-knot nematode management and increased fruit quality in tomato under field conditions. *Biocontrol Science and Technology*, 26(2), pp.174-180.
- Nuruzzaman, M., Manimekalai, R., Sharoni, A.M., Satoh, K., Kondoh, H., Ooka, H. and Kikuchi, S., 2010. Genome-wide analysis of *NAC* transcription factor family in rice. *Gene*, 465(1-2), pp.30-44.
- Olias, R., Eljakaoui, Z., Li, J.U.N., De Morales, P.A., Marin-Manzano, M.C., Pardo, J.M. and Belver, A., 2009. The plasma membrane Na^+/H^+ antiporter SOS1 is essential for salt tolerance in tomato and affects the partitioning of Na^+ between plant organs. *Plant, cell & environment*, 32(7), pp.904-916.

REFERENCES (Cont.)

- Ooka, H., Satoh, K., Doi, K., Nagata, T., Otomo, Y., Murakami, K., Matsubara, K., Osato, N., Kawai, J., Carninci, P. and Hayashizaki, Y., 2003. Comprehensive analysis of NAC family genes in *Oryza sativa* and *Arabidopsis thaliana*. *DNA research*, 10(6), pp.239-247.
- Osakabe, Y., Osakabe, K., Shinozaki, K. and Tran, L.S.P., 2014. Response of plants to water stress. *Frontiers in plant science*, 5, p.86.
- Paul, A., Chatterjee, A., Subrahmanya, S., Shen, G. and Mishra, N., 2021. *NHX* Gene Family in *Camellia sinensis*: In-silico Genome-Wide Identification, Expression Profiles, and Regulatory Network Analysis. *Frontiers in plant science*, 12.
- Peleg, Z. and Blumwald, E., 2011. Hormone balance and abiotic stress tolerance in crop plants. *Current opinion in plant biology*, 14(3), pp.290-295.
- Pineda, B., García-Abellán, J.O., Antón, T., Pérez, F., Moyano, E., García Sogo, B., Campos, J.F., Angosto, T., Morales, B., Capel, J. and Moreno, V., 2012. Tomato: genomic approaches for salt and drought stress tolerance. Improving crop resistance to abiotic stress, pp.1085-1120.
- Pinheiro, C. and Chaves, M.M., 2011. Photosynthesis and drought: can we make metabolic connections from available data?. *Journal of experimental botany*, 62(3), pp.869-882.
- Plant, Á.L., Cohen, A., Moses, M.S. and Bray, E.A., 1991. Nucleotide sequence and spatial expression pattern of a drought-and abscisic acid-induced gene of tomato. *Plant Physiology*, 97(3), pp.900-906.
- Porembski, S. and Barthlott, W., 2000. Granitic and gneissic outcrops (inselbergs) as centers of diversity for desiccation-tolerant vascular plants. *Plant Ecology*, 151(1), pp.19-28.

REFERENCES (Cont.)

- Puranik, S., Sahu, P.P., Srivastava, P.S. and Prasad, M., 2012. *NAC* proteins: regulation and role in stress tolerance. *Trends in plant science*, 17(6), pp.369-381.
- Puranik, S., Sahu, P.P., Srivastava, P.S. and Prasad, M., 2012. *NAC* proteins: regulation and role in stress tolerance. *Trends in plant science*, 17(6), pp.369-381.
- Puranik, S., Sahu, P.P., Srivastava, P.S. and Prasad, M., 2012. *NAC* proteins: regulation and role in stress tolerance. *Trends in plant science*, 17(6), pp.369-381.
- Ray, S., Dansana, P.K., Giri, J., Deveshwar, P., Arora, R., Agarwal, P., Khurana, J.P., Kapoor, S. and Tyagi, A.K., 2011. Modulation of transcription factor and metabolic pathway genes in response to water-deficit stress in rice. *Functional & integrative genomics*, 11(1), pp.157-178.
- Reynolds, M. and Tuberosa, R., 2008. Translational research impacting on crop productivity in drought-prone environments. *Current opinion in plant biology*, 11(2), pp.171-179.
- Robinson, T., 2020. Understanding drought tolerance in plant species. *Plant Biology*, 12(3), 235-248.
- Rodríguez-Leal, D., Lemmon, Z.H., Man, J., Bartlett, M.E. and Lippman, Z.B., 2017. Engineering quantitative trait variation for crop improvement by genome editing. *Cell*, 171(2), pp.470-480.
- Rodríguez-Rosales, M.P., Gálvez, F.J., Huertas, R., Aranda, M.N., Baghour, M., CagNAC, O. and Venema, K., 2009. Plant *NHX* cation/proton antiporters. *Plant signaling & behavior*, 4(4), pp.265-276.

REFERENCES (Cont.)

- Sablowski, R.W. and Meyerowitz, E.M., 1998. A homolog of NO APICAL MERISTEM is an immediate target of the floral homeotic genes APETALA3/PISTILLATA. *Cell*, 92(1), pp.93-103.
- Samaras, A., Roumeliotis, E., Ntasiou, P. and Karaoglanidis, G., 2021. *Bacillus subtilis* MBI600 promotes growth of tomato plants and induces systemic resistance contributing to the control of soilborne pathogens. *Plants*, 10(6), p.1113.
- Sengupta, D. and Reddy, A.R., 2011. Water deficit as a regulatory switch for legume root responses. *Plant signaling & behavior*, 6(6), pp.914-917.
- Seo, P.J. and Park, C.M., 2009. Auxin homeostasis during lateral root development under drought condition. *Plant signaling & behavior*, 4(10), pp.1002-1004.
- Seo, P.J., Kim, S.G. and Park, C.M., 2008. Membrane-bound transcription factors in plants. *Trends in plant science*, 13(10), pp.550-556.
- Shao, H., Wang, H. and Tang, X., 2015. NAC transcription factors in plant multiple abiotic stress responses: progress and prospects. *Frontiers in plant science*, 6, p.902.
- Shi, H. and Zhu, J.K., 2002. Regulation of expression of the vacuolar Na⁺/H⁺ antiporter gene *AtNHX1* by salt stress and abscisic acid. *Plant molecular biology*, 50(3), pp.543-550.
- Shiriga, K., Sharma, R., Kumar, K., Yadav, S.K., Hossain, F. and Thirunavukkarasu, N., 2014. Genome-wide identification and expression pattern of drought-responsive members of the NAC family in maize. *Meta gene*, 2, pp.407-417.

REFERENCES (Cont.)

- Singh, A.K., Sharma, V., Pal, A.K., Acharya, V. and Ahuja, P.S., 2013. Genome-wide organization and expression profiling of the NAC transcription factor family in potato (*Solanum tuberosum* L.). *DNA research*, 20(4), pp.403-423.
- Slama, I., Ghnaya, T., Hessini, K., Messedi, D., Saviouré, A. and Abdelly, C., 2007. Comparative study of the effects of mannitol and PEG osmotic stress on growth and solute accumulation in *Sesuvium portulacastrum*. *Environmental and Experimental Botany*, 61(1), pp.10-17.
- Smith, J., 2022. Impacts of drought stress on crop yield. *Agriculture and Food Security*, 9(1), 32.
- Souer, E., van Houwelingen, A., Kloos, D., Mol, J. and Koes, R., 1996. The no apical meristem gene of *Petunia* is required for pattern formation in embryos and flowers and is expressed at meristem and primordia boundaries. *Cell*, 85(2), pp.159-170.
- Spollen, W.G. and Sharp, R.E., 1991. Spatial distribution of turgor and root growth at low water potentials. *Plant Physiology*, 96(2), pp.438-443.
- Su, H., Zhang, S., Yuan, X., Chen, C., Wang, X.F. and Hao, Y.J., 2013. Genome-wide analysis and identification of stress-responsive genes of the NAM–ATAF1, 2–CUC2 transcription factor family in apple. *Plant physiology and biochemistry*, 71, pp.11-21.
- Swinnen, G., Goossens, A. and Pauwels, L., 2016. Lessons from domestication: targeting cis-regulatory elements for crop improvement. *Trends in plant science*, 21(6), pp.506-515.

REFERENCES (Cont.)

- Tahiri, A.I., Meddich, A., Raklami, A., Alahmad, A., Bechtaoui, N., Anli, M., Göttfert, M., Heulin, T., Achouak, W. and Oufdou, K., 2022. Assessing the potential role of compost, PGPR, and AMF in improving tomato plant growth, yield, fruit quality, and water stress tolerance. *Journal of Soil Science and Plant Nutrition*, 22(1), pp.743-764.
- Tahiri, A.I., Meddich, A., Raklami, A., Alahmad, A., Bechtaoui, N., Anli, M., Göttfert, M., Heulin, T., Achouak, W. and Oufdou, K., 2022. Assessing the potential role of compost, PGPR, and AMF in improving tomato plant growth, yield, fruit quality, and water stress tolerance. *Journal of Soil Science and Plant Nutrition*, 22(1), pp.743-764.
- Takahashi, N., Yamazaki, Y., Kobayashi, A., Higashitani, A. and Takahashi, H., 2003. Hydrotropism interacts with gravitropism by degrading amyloplasts in seedling roots of *Arabidopsis* and radish. *Plant physiology*, 132(2), pp.805-810.
- Tallapragada, P., Dikshit, R. and Seshagiri, S., 2016. Influence of *Rhizophagus* spp. and *Burkholderia seminalis* on the growth of tomato (*Lycopersicon esculatum*) and bell pepper (*Capsicum annuum*) under drought stress. *Communications in Soil Science and Plant Analysis*, 47(17), pp.1975-1984.
- Tezara, W.M.V.J., Mitchell, V.J., Driscoll, S.D. and Lawlor, D.W., 1999. Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. *Nature*, 401(6756), pp.914-917.
- Tian, F., Chang, E., Li, Y., Sun, P., Hu, J. and Zhang, J., 2017. Expression and integrated network analyses revealed functional divergence of *NHX*-type Na⁺/H⁺ exchanger genes in poplar. *Scientific reports*, 7(1), pp.1-17.

REFERENCES (Cont.)

- Tomas, M., Beekwilder, J., Hall, R.D., Sagdic, O., Boyacioglu, D. and Capanoglu, E., 2017. Industrial processing versus home processing of tomato sauce: Effects on phenolics, flavonoids and in vitro bioaccessibility of antioxidants. *Food Chemistry*, 220, pp.51-58.
- Tosens, T., Niinemets, U., Vislap, V., Eichelmann, H. and Castro Diez, P., 2012. Developmental changes in mesophyll diffusion conductance and photosynthetic capacity under different light and water availabilities in *Populus tremula*: how structure constrains function. *Plant, cell & environment*, 35(5), pp.839-856.
- Uddin, M.N., Hossain, M.A. and Burritt, D.J., 2016. Salinity and drought stress: similarities and differences in oxidative responses and cellular redox regulation. *Water stress and crop plants: a sustainable approach*, 1, pp.86-101.
- Uga, Y., Sugimoto, K., Ogawa, S., Rane, J., Ishitani, M., Hara, N., Kitomi, Y., Inukai, Y., Ono, K., Kanno, N. and Inoue, H., 2013. Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. *Nature genetics*, 45(9), pp.1097-1102.
- Venuprasad, R., Lafitte, H.R. and Atlin, G.N., 2007. Response to direct selection for grain yield under drought stress in rice. *Crop Science*, 47(1), pp.285-293.
- Wan, F.X., Gao, J., Wang, G.L., Niu, Y., Wang, L.Z., Zhang, X.G., Wang, Y.Q. and Pan, Y., 2021. Genome-wide identification of NAC transcription factor family and expression analysis of ATAF subfamily members under abiotic stress in eggplant. *Scientia Horticulturae*, 289, p.110424.

REFERENCES (Cont.)

- Wang, C., Guo, Y., Wang, C., Liu, H., Niu, D., Wang, Y. and Guo, J., 2012. Enhancement of tomato (*Lycopersicon esculentum*) tolerance to drought stress by plant-growth-promoting rhizobacterium (PGPR) *Bacillus cereus* AR156. *Journal of Agricultural Biotechnology*, 20(10), pp.1097-1105.
- Wang, C., Yang, A., Yin, H. and Zhang, J., 2008. Influence of water stress on endogenous hormone contents and cell damage of maize seedlings. *Journal of integrative plant biology*, 50(4), pp.427-434.
- Wang, D.C., Jiang, C.H., Zhang, L.N., Chen, L., Zhang, X.Y. and Guo, J.H., 2019. Biofilms positively contribute to *Bacillus amyloliquefaciens* 54-induced drought tolerance in tomato plants. *International journal of molecular sciences*, 20(24), p.6271.
- Wang, N., Zheng, Y., Xin, H., Fang, L. and Li, S., 2013. Comprehensive analysis of NAC domain transcription factor gene family in *Vitis vinifera*. *Plant cell reports*, 32(1), pp.61-75.
- Wilkinson, S. and Davies, W.J., 2010. Drought, ozone, ABA and ethylene: new insights from cell to plant to community. *Plant, cell & environment*, 33(4), pp.510-525.
- Wilkinson, S., Kudoyarova, G.R., Veselov, D.S., Arkhipova, T.N. and Davies, W.J., 2012. Plant hormone interactions: innovative targets for crop breeding and management. *Journal of experimental botany*, 63(9), pp.3499-3509.
- Wu, G.Q., Wang, J.L. and Li, S.J., 2019. Genome-wide identification of Na⁺/H⁺ antiporter (*NHX*) genes in sugar beet (*Beta vulgaris* L.) and their regulated expression under salt stress. *Genes*, 10(5), p.401.
- Xiong, L. and Zhu, J.K., 2002. Molecular and genetic aspects of plant responses to osmotic stress. *Plant, Cell & Environment*, 25(2), pp.131-139.

REFERENCES (Cont.)

- Xu, Y., Jin, Z., Xu, B., Li, J., Li, Y., Wang, X., Wang, A., Hu, W., Huang, D., Wei, Q. and Xu, Z., 2020. Identification of transcription factors interacting with a 1274 bp promoter of MaPIP1; 1 which confers high-level gene expression and drought stress Inducibility in transgenic *Arabidopsis thaliana*. *BMC Plant Biology*, 20(1), pp.1-14.
- Xu, Z. and Zhou, G., 2008. Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *Journal of experimental botany*, 59(12), pp.3317-3325.
- Xu, Z.Z. and Zhou, G.S., 2005. Effects of water stress and nocturnal temperature on carbon allocation in the perennial grass, *Leymus chinensis*. *Physiologia Plantarum*, 123(3), pp.272-280.
- Yan, H., Ma, G., Teixeira da Silva, J.A., Qiu, L., Xu, J., Zhou, H., Wei, M., Xiong, J., Li, M., Zhou, S. and Wu, J., 2021. Genome-wide identification and analysis of NAC transcription factor family in two diploid wild relatives of cultivated sweet potato uncovers potential NAC genes related to drought tolerance. *Frontiers in genetics*, p.2378.
- Yang, J.C., Zhang, J.H., Ye, Y.X., Wang, Z.Q., Zhu, Q.S. and Liu, L.J., 2004. Involvement of abscisic acid and ethylene in the responses of rice grains to water stress during filling. *Plant, cell & environment*, 27(8), pp.1055-1064.
- Yang, S., Zhu, H., Huang, L., Zhang, G., Wang, L., Jiang, X. and Zhong, Q., 2021. Transcriptome-wide and expression analysis of the NAC gene family in pepino (*Solanum muricatum*) during drought stress. *PeerJ*, 9, p.e10966.

REFERENCES (Cont.)

- Yang, W., Zhao, Y., Yang, Y., Zhang, M., Mao, X., Guo, Y., Li, X., Tao, B., Qi, Y., Ma, L. and Liu, W., 2022. A Genomic Analysis of *Bacillus megaterium* HT517 Reveals the Genetic Basis of Its Abilities to Promote Growth and Control Disease in Greenhouse Tomato. *International Journal of Genomics*, 2022.
- Yarra, R., 2019. The wheat *NHX* gene family: potential role in improving salinity stress tolerance of plants. *Plant Gene*, 18, p.100178.
- Yarra, R., He, S.J., Abbagani, S., Ma, B., Bulle, M. and Zhang, W.K., 2012. Overexpression of a wheat Na^+/H^+ antiporter gene (*TaNHX2*) enhances tolerance to salt stress in transgenic tomato plants (*Solanum lycopersicum* L.). *Plant Cell, Tissue and Organ Culture (PCTOC)*, 111(1), pp.49-57.
- Yildirim, E., Karlidag, H., Turan, M., Dursun, A. and Goktepe, F., 2011. Growth, nutrient uptake, and yield promotion of broccoli by plant growth promoting rhizobacteria with manure. *HortScience*, 46(6), pp.932-936.
- Yokoi, S., Quintero, F.J., Cubero, B., Ruiz, M.T., Bressan, R.A., Hasegawa, P.M. and Pardo, J.M., 2002. Differential expression and function of *Arabidopsis thaliana* *NHX* Na^+/H^+ antiporters in the salt stress response. *The Plant Journal*, 30(5), pp.529-539.
- Yordanov, I., Velikova, V. and Tsonev, T., 2000. Plant responses to drought, acclimation, and stress tolerance. *Photosynthetica*, 38(2), pp.171-186.
- Yu, T., Cen, Q., Kang, L., Mou, W., Zhang, X., Fang, Y., Zhang, X., Tian, Q. and Xue, D., 2022. Identification and expression pattern analysis of the *OsSnRK2* gene family in rice. *Frontiers in Plant Science*, 13.

REFERENCES (Cont.)

- Zhang, H., Kang, H., Su, C., Qi, Y., Liu, X. and Pu, J., 2018. Genome-wide identification and expression profile analysis of the NAC transcription factor family during abiotic and biotic stress in woodland strawberry. *PLoS One*, 13(6), p.e0197892.
- Zhao, J., Lu, Z., Wang, L. and Jin, B., 2020. Plant responses to heat stress: physiology, transcription, noncoding RNAs, and epigenetics. *International journal of molecular sciences*, 22(1), p.117.
- Zheng, X.Y., Spivey, N.W., Zeng, W., Liu, P.P., Fu, Z.Q., Klessig, D.F., He, S.Y. and Dong, X., 2012. Coronatine promotes *Pseudomonas syringae* virulence in plants by activating a signaling cascade that inhibits salicylic acid accumulation. *Cell host & microbe*, 11(6), pp.587-596.
- Zhong, R., Lee, C. and Ye, Z.H., 2010. Functional characterization of poplar wood-associated NAC domain transcription factors. *Plant Physiology*, 152(2), pp.1044-1055.