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Exploring the Role of the AtNPR1 Gene in Mechanisms of Salt Stress Tolerance

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Abstract: Salt stress is one of the major abiotic factors limiting plant growth and productivity worldwide. Understanding the molecular mechanisms underlying salt tolerance is crucial for developing stress-resilient crops. The AtNPR1 (Nonexpressor of Pathogenesis-Related Genes 1) gene, widely recognized for its pivotal role in systemic acquired resistance and defense signaling in Arabidopsis thaliana, has recently been implicated in abiotic stress responses, including salinity. This study aims to investigate the functional role of AtNPR1 in modulating salt stress tolerance mechanisms. Through a combination of genetic, physiological, and molecular analyses, we explore how AtNPR1 influences plant responses under high salinity conditions. Preliminary findings suggest that AtNPR1 may regulate reactive oxygen species (ROS) scavenging, ion homeostasis, and stress-responsive gene expression, thereby enhancing plant tolerance to salt-induced oxidative damage. The study also examines cross-talk between biotic and abiotic stress pathways mediated by AtNPR1, highlighting its potential as a key regulatory hub. These insights could contribute to the development of genetically engineered crops with improved resilience to salt stress, offering a promising approach for sustainable agriculture under changing environmental conditions.

Keywords: AtNPR1, Salt Stress Tolerance, Arabidopsis thaliana, Abiotic Stress Response, Gene Regulation

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INTRODUCTION

Natural plant life is severely constrained in terms of development, yield, and even survival due to the many environmental challenges that plants encounter on a daily basis. Drought, excessive temperatures, heavy metals, and salt are examples of abiotic stressors that may have a devastating impact. Salt stress, in particular, poses a formidable challenge to global agriculture by affecting approximately one-fifth of irrigated land and leading to significant declines in crop yield. The increasing problem of soil salinization, exacerbated by climate change and poor irrigation practices, underscores the urgent need to understand the underlying mechanisms of salt stress tolerance in plants. Salt stress adversely impacts plants by creating a hyperosmotic environment and ionic imbalance, which hampers water uptake and leads to toxic accumulation of sodium (Na^+) and chloride (Cl^-) ions in the cytosol. This results in oxidative stress, membrane destabilization, disruption of photosynthesis, inhibition of enzymatic activity, and ultimately cell death. To adapt to and thrive in situations with high salt, plants have developed intricate and carefully controlled physiological, biochemical, and molecular systems.

One of the most critical aspects of plant response to salt stress lies in gene regulation. Numerous genes, including those encoding for ion transporters, osmolyte biosynthesis enzymes, transcription factors, and antioxidant proteins, are activated under salt stress. Over the last two decades, molecular studies have identified key players involved in salt tolerance, such as the Salt Overly Sensitive (SOS) pathway, dehydration-responsive element-binding (DREB) transcription factors, and Na^+/H^+ antiporters like NHX1.

Yet, emerging research reveals that the mechanisms governing salt stress responses often overlap with those involved in pathogen defense, particularly through redox-sensitive regulatory proteins and hormone signaling networks. This intersection between biotic and abiotic stress signaling pathways has become a critical focus area in plant stress physiology.

The *Nonexpressor of Pathogenesis-Related Genes 1* (*AtNPR1*) is a master regulatory gene in *Arabidopsis thaliana* traditionally associated with systemic acquired resistance (SAR) and salicylic acid (SA)-dependent responses to pathogen infection. Functioning as a redox-sensitive co-activator, *AtNPR1* exists as an oligomer in the cytoplasm under non-stressed conditions. Upon SA accumulation, disulfide bonds are reduced, leading to monomerization and translocation of NPR1 into the nucleus, where it interacts with transcription factors such as TGA to activate pathogenesis-related (PR) genes like PR1. Despite AtNPR1's well-known function in defence signalling, new research points to its potential participation in responses to abiotic stresses, such as drought, oxidative stress, salt, and drought. Several studies have shown that overexpression of *AtNPR1* or its orthologs in transgenic plants enhances tolerance to multiple abiotic stresses, likely through modulation of antioxidant enzyme activity, stress-responsive gene expression, and hormonal cross-talk.

This evolving view of *AtNPR1* as a multifunctional regulator brings forth compelling questions: How does *AtNPR1* mediate salt stress tolerance? Does it function through traditional SA-related defense pathways or engage in novel molecular circuits under abiotic conditions? What are the downstream genes regulated by NPR1 during salt exposure, and how do these contribute to physiological resilience? These questions are crucial, especially considering the convergence of ROS signaling in both pathogen and salt stress responses. Damage to cells may occur if reactive oxygen species (ROS) such hydrogen peroxides (H_kO_k) and superoxide radicals are not effectively eliminated when exposed to high salt. NPR1, being redox-sensitive, is ideally positioned to act as a sensor and regulator under these oxidative conditions, potentially linking environmental stress cues to transcriptional reprogramming.

The model plant *Arabidopsis thaliana* offers a powerful genetic platform to explore these mechanisms. With an extensively mapped genome, available T-DNA insertion mutants, overexpression lines, and gene-editing tools, *Arabidopsis* allows in-depth dissection of *AtNPR1* function under controlled conditions. Previous studies in *Arabidopsis* and other crops like rice and tobacco have demonstrated that manipulation of NPR1 levels can confer tolerance to both pathogens and abiotic stressors, further highlighting its potential as a genetic target for crop improvement. Notably, transgenic plants overexpressing *AtNPR1* exhibit improved survival under saline conditions, maintain higher chlorophyll content, better ion homeostasis, and upregulated expression of ROS-scavenging enzymes such as catalase, ascorbate peroxidase, and glutathione reductase.

Another layer of complexity in *AtNPR1* function arises from its interactions with hormonal pathways. Salt stress primarily induces the accumulation of abscisic acid (ABA), a key hormone regulating stomatal closure, gene expression, and stress adaptation. Interestingly, NPR1 is traditionally associated with SA signaling, and antagonistic or synergistic interactions between SA and ABA signaling pathways may influence NPR1's role in salt stress. Moreover, NPR1's involvement in crosstalk with jasmonic acid (JA), ethylene (ET), and nitric oxide (NO) pathways could further shape plant responses to salinity. These overlapping signaling modules create a dense network of regulatory interactions, in which *AtNPR1* may act as a central node. Understanding this integrative function could unlock new strategies for developing stress-resilient crops with broad-spectrum stress tolerance.

In this context, the present study aims to explore the regulatory role of *AtNPR1* in the salt stress tolerance mechanism of *Arabidopsis thaliana*. By utilizing both wild-type and *npr1* mutant lines, along with overexpression genotypes, we seek to evaluate physiological responses such as germination rate, chlorophyll content, electrolyte leakage, proline accumulation, and antioxidant enzyme activity under saline conditions. Additionally, quantitative RT-PCR analysis of key stress-responsive genes will be conducted to assess the transcriptional impact of *AtNPR1* under salt stress. The integration of physiological, biochemical, and molecular data will provide comprehensive insights into how *AtNPR1* contributes to plant resilience in high salinity environments. By elucidating these mechanisms, this study contributes to a broader understanding of plant stress biology and offers potential genetic strategies for engineering salt-tolerant crops in the face of global environmental challenges.

LITERATURE OF REVIEW

Induced defence responses against biotic and abiotic stressors are regulated by signalling networks, and one such network is the plant hormone salicylic acid (SA) (Mahmoud et al., 2021c). Non-expressor of pathogenesis-related genes 1 (NPR1) functions as a vital mediator in systemic acquired resistance (SAR), playing a key role in transmitting the salicylic acid (SA) signaling pathway, which subsequently induces the expression of pathogenesis-related (PR) genes. NPR1's role has recently been better understood. Cao et al. (1997) were the first to find NPR1, and they demonstrated that mutants lacking NPR1 were more susceptible to infections, had low expression of genes involved in pathogenesis (PR), and did not react to SAR-inducing therapies. It was first believed that the npr1 mutant was impaired only in defence mediated by SA. According to Dong (2004), NPR1 is involved in many defense-signaling pathways. No pathogenic rhizobacteria can generate induced systemic resistance (ISR) in the npr1 mutant (Choudhary and Johri 2009). Notably, this resistance response necessitates ETR1 and JAR1, two regulators of ethylene and jasmonic acid (JA) signalling, respectively; it is not reliant on SA (Pieterse et al., 1998).

The redox-controlled transition of NPR1 from an oligomeric to monomeric form governs its localization within the cell, determining whether it resides in the nucleus or cytoplasm. Additionally, this transformation enables NPR1 to associate with diverse interacting proteins across different tissue types (Dong, 2004). When plant defence responses are activated, certain WRKY genes positively control NPR1 expression, as was previously shown by Yu et al. (2001). Our results show that SA-induced WRKY gene expression was not reliant on NPR1, which is in agreement with this concept.

METHOD AND METHODOLOGY

Analysis of Salt Stress Effects on Transgenic Citrus Plants Expressing Arabidopsis NPR1 and Their Gene Expression Patterns

Plant Material and Growth Conditions

In this study, transgenic 'Hamlin' sweet orange (*Citrus sinensis*) lines, engineered to overexpress the

Arabidopsis thaliana NPR1 (AtNPR1; Gene ID: AT1G64280) cDNA, were employed. As described earlier by Dutt et al. (2015), gene expression was driven by the constitutive CaMV 35S promoter. To ensure uniform root structure and consistent nutrient uptake efficiency, both the genetically modified lines and the non-transgenic controls were clonally propagated using the same citrus rootstock, US-942.

All plantlets were grown in 3-gallon pots filled with a standard citrus soil mix and maintained in a controlled greenhouse facility at the University of Florida's Citrus Research and Education Center, Lake Alfred, FL, USA. Environmental conditions in the greenhouse were maintained at approximately 28±2°C during the day and 20±2°C at night, with 60–70% relative humidity and natural light supplemented by high-pressure sodium lamps providing 16-hour photoperiods. The plants were irrigated uniformly and fertilized with a balanced nutrient solution once weekly.

Salt Stress Treatments

Salt stress was imposed on one-year-old trees using sodium chloride (NaCl) solutions at final concentrations of 0, 100, and 200 mM. In order to avoid osmotic shock and allow gradual acclimatization, NaCl concentrations were increased incrementally over a 2-week period. Each plant received 500 mL of the respective NaCl solution thrice weekly for a total duration of 3 months. Salt treatments were carried out in a randomized complete block design (RCBD), with separate pots arranged per treatment group to avoid cross-contamination of salt.

Physiological Evaluation and Sampling

After continuous salt exposure for three months, physiological parameters, including visual symptom scoring, leaf chlorophyll content measured by SPAD values, relative water content (RWC), and electrolyte leakage, were assessed. These measurements allowed assessment of salt stress-induced injury and the relative performance of transgenic lines versus non-transgenic controls. Leaf samples were collected at the end of the treatment period, flash-frozen in liquid nitrogen, and stored at –80°C until RNA extraction was performed.

Gene Expression Analysis

RNA Isolation and cDNA Synthesis

Total RNA was extracted from treated and control leaf samples using a commercial plant RNA extraction kit (e.g., Qiagen RNeasy Plant Mini Kit) following the manufacturer's protocol. RNA quality and concentration were confirmed via NanoDrop spectrophotometry and agarose gel electrophoresis. First-strand cDNA was synthesized from 1 µg of total RNA using oligo(dT) primers and reverse transcriptase (e.g., SuperScript III, Invitrogen).

Quantitative Real-Time PCR (qRT-PCR)

Gene expression patterns were analyzed using quantitative real-time PCR (qRT-PCR) with gene-specific primers (Table 1). The reactions were set up using SYBR Green Master Mix (e.g., Bio-Rad or Applied Biosystems) on a real-time PCR detection system. Each reaction was performed in triplicate using technical and biological replicates. There was a 5-minute denaturation stage at 95°C, then 40 cycles of 95°C for 10 seconds and 60°C for 30 seconds under the thermal cycling conditions. Each amplification product's specificity was confirmed by melt curve analysis.

Using the 2^-ΔΔCt technique, relative expression levels were determined. The citrus *β-actin* gene (orange1.1g017124m) was used as an internal reference to normalize expression data. Genes analyzed included those involved in antioxidant defense (*Peroxidase*, *CAT1*, *APX2*, *CSD1*, *CSD2*, *GST*), ion transport (*SOS1*, *SOS2*, *SOS3*, *NHX1*), pathogenesis-related responses (*PR1–PR5*), transcriptional regulation (*WRKY70*, *WRKY33*), aquaporin function (*CsPIP1;1*, *CsPIP2;3*, *CsTIP4;1*), and hormonal modulation (*CYP707A3*).

Research Design and Data Analysis

The study followed a **two-way factorial design** involving two independent factors: (1) Plant genotype (Transgenic vs. Non-transgenic lines), and (2) NaCl concentration (0, 100, and 200 mM). Each treatment combination included four biological replicates (n=4), resulting in a total of 24 experimental units.

Statistical analyses were performed using **JMP Pro Software Version 14** (SAS Institute, Cary, NC, USA). Data were subjected to **two-way ANOVA** to determine the effects of plant genotype, salt concentration, and their interaction. Mean separation was conducted using Tukey's HSD test at a significance level of $p < 0.05$. Gene expression data were log-transformed where necessary to satisfy assumptions of normality and homogeneity of variances.

Table 1: Primers used in real-time polymerase chain reaction

No.	Gene	Common Name	Group	Forward Seq	Reverse Seq
1	orange1.1g020635m	Peroxidase	Antioxidant	TTCGGAAGCGAATAGGGATATG	CCAAGAGTATGTCCACCTGATAAA
2	orange1.1g020619m	Peroxidase		ACAGGAAGAAGGGATGGTAGA	GACCAGGTCATGAACAGTAAGG
3	orange1.1g027134m	GST-protein		GGCTTGACCAATTCAAACTACAC	GTTCATTGTCTCCTGGCTCTT
4	orange1.1g042356m	CAT1		CTTCTTCTCCCATCATCCTGAAA	TCCTTCCATGTGCCTGTAATC
5	orange1.1g025588m	APX2		CCACATGGGTCTGAGTGATAAG	GTTAGTCCAGGGTCCTTCAAAT
6	orange1.1g031837m	CSD1		CAACTGTATCAGGAAGCCTCTC	CCAGTAGACATGCAACCATTTG
7	orange1.1g026287m	CSD2		CGCTCTTCCTCTTCTTCTTCTT	CGGCGAGAGATAAGTTGAGAC
8	orange1.1g005031m	PAL1		CTCGATGGCAGCTCTTATGTTA	GGTGAAGTTCTCAGGGCATAA

9	orange1.1g001116m	SOS1	Na^+ co-transporter	GCCAAGTGGTATCTGGCTTAT	GCACCTCATAGAGACCCAAATTA
10	orange1.1g013421m	SOS2		GCGAGGAAGAGGAAGTGAAT	GAGAGGACCTCCGACTTTATTT
11	orange1.1g027657m	SOS3		TTCGATCATTGGGTGTCTTCC	AACTCCTCCCGCTCAATAAAC
12	orange1.1g009116m	NHX1		GAGCTTTGACCTCTCTCACATC	GCACTAAGCAGTCCAGCTATAA
13	orange1.1g048073m	PR1	Pathogenesis Related Proteins	GTGGCGGAGAAAGCTAACTATAA	AACCCTAGCACATCCAACAC
14	orange1.1g019014m	PR2		ACAACCCAGTACGTGTCTTTC	TGCCGTGGAAACTTTGATTTG
15	orange1.1g020187m	PR3		ACAAGGAAACCCTGGAGATTAT	GCTGGACCGTAGTTGTAGTT
16	orange1.1g032389m	PR4		GTATGGATGGACTGCCTTCTG	TTGAGCTCCTGTCCCTCTATTA
17	orange1.1g026001m	PR5		CTCCGTTGTGGCTTGTAAGA	CTGTGTCGGAGAACACGTATC
18	orange1.1g021598m	CsWRKY70	WRKY	CTGTGCTCGGTACTACTGTTAC	CGGCGATAGTCATCGGAATTA
19	orange1.1g013222m	CsWRKY33		CCGGATTGTCCGATGAAGAAA	GATGTAGGCTTGGGATGATTGT
20	orange1.1g018895	CsPIP1;1	Aquaporin Proteins	CATTCTCATCACAACATCAAACG	CTGCTAGTCCCTCAAAAACACAA
21	orange1.1g019681	CsPIP2;3		TGTTGTCATTTTGCTACTCGTTTC	GGCGTGCCATATTGCTTTTA
22	orange1.1g025864	CsTIP4;1		AAGCTGCTGTTTCTCTCTTGATG	CAAAATGACAGCAGCCAAAAA
23	orange1.1g012199m	CYP707A3	Abscisic Acid	AACCTTCTGGCATATACAGCTT	TTGCTTCTCTCCCAGTCATTATC
24	orange1.1g017124m	β-actin	House Keeping	GCTGCCTGATGGCCAGATC	AGTTGTAGGTAGTCTCATGAA

RESULT AND DISCUSSION

Results of salt stress on transgenic citrus plants carrying the Arabidopsis NPR1 gene and their relative performance and gene expression patterns

- **Photosynthesis:** Compared to the control, 'Hamlin' leaves subjected to NaCl stress had a lower total chlorophyll content. Transgenic plants subjected to salt stress showed a substantial increase in total chlorophyll content.

- **Modifications to free radical scavenging activity and MDA content:** Increasing the NaCl treatments resulted in an increase in MDA content in both the wild type and transgenic plants. A significant decrease in MDA content was observed in the transgenic line compared to wild-type plants, indicating reduced lipid peroxidation and improved membrane stability. However, under salt stress conditions, DPPH-radical scavenging activity declined markedly in both lines, suggesting that prolonged salinity adversely affected the plant's antioxidant defense capacity compared to non-saline conditions. Transgenic plants outperformed the saline control group in terms of DPPH radical scavenging activity.

Table 2: Transgenic hamlin overexpressing AtNPR1 shows altered chlorophyll capacity index (CCI), peroxide radical scavenging ability, and malondialdehyde (MDA) levels.

Variables	**SPAD (CCI)**		**MDA (nmol^{-1} MDA eq. g FW)**		**DPPH (mg Trolox g^{-1} FW)**	
Treatment/Lines	WT	NPR1-Line	WT	NPR1-Line	WT	NPR1-Line
0 mM NaCl	72.33 ± 2.69^{a*}	77.43 ± 1.45^{a}	0.83 ± 0.04^{c}	0.74 ± 0.08^{c}	0.231 ± 0.04^{a}	0.218 ± 0.03^{a}
100 mM NaCl	47.37 ± 1.15^{b}	75.00 ± 3.66^{a}	1.81 ± 0.11^{ab}	1.51 ± 0.06^{b}	0.135 ± 0.02^{bc}	0.189 ± 0.02^{ab}
200 mM NaCl	38.77 ± 0.88^{c}	73.33 ± 4.14^{a}	2.29 ± 0.14^{a}	2.09 ± 0.26^{ab}	0.083 ± 0.01^{c}	0.190 ± 0.01^{ab}

* Data is shown as the average plus or minus the standard deviation. Tukey-Kramer HSD test results show no significant difference at $p < 0.05$ when the identical letter appears in each column after the mean.

- **Changes in antioxidant enzyme activity:** Under different NaCl concentrations, the transgenic animals showed no discernible changes in CsPOD2 (peroxidase 2) expression, but the wild type animals showed a significant reduction. Under 100 mM NaCl treatment, the transgenic plants exhibited a marked upregulation in the transcript levels of **CsCSD1** (cytosolic Cu/Zn superoxide dismutase) when compared to the wild-type plants across various conditions. This indicates an enhanced antioxidant response in the transgenic line, contributing to improved detoxification of reactive oxygen species (ROS) under salt stress.

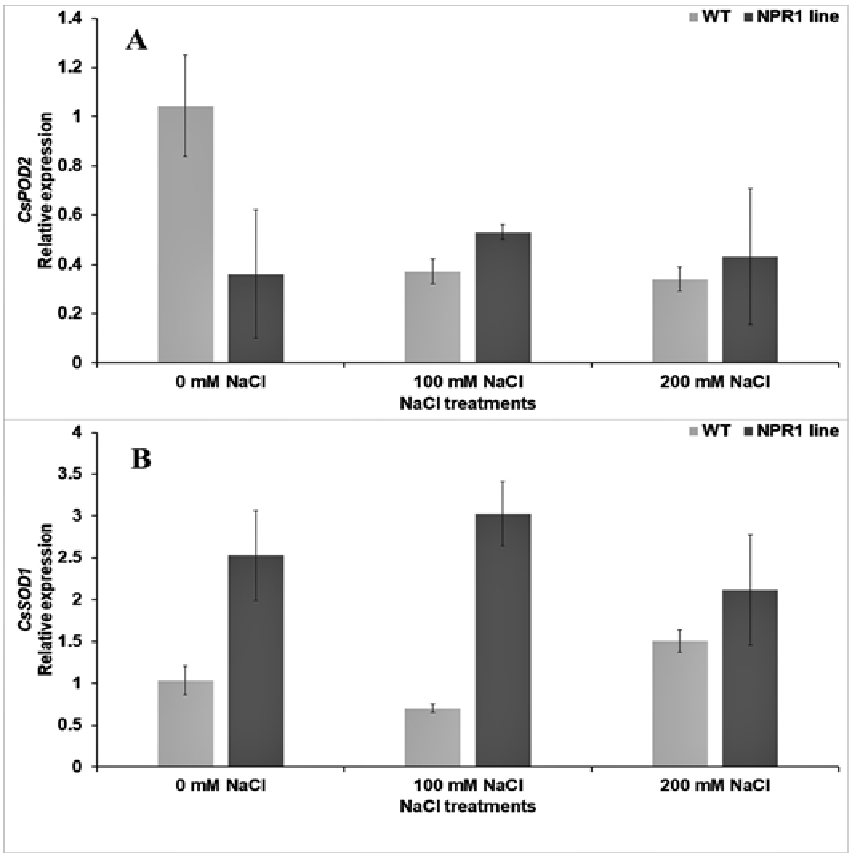

Figure 1: Changes in gene expression of *CsPOD2* (A) and *CsSOD1* (B) in NPR1 transgenic lines following NaCl treatment

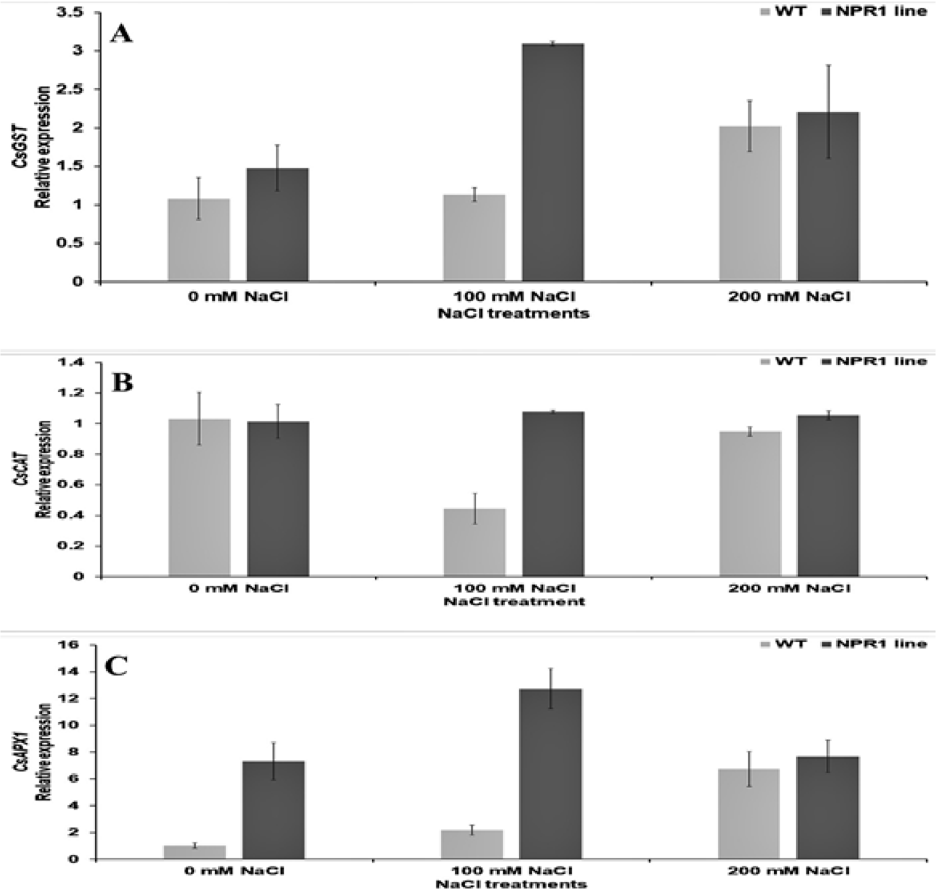

Figure 2: Changes in gene expression of Cs*GST* (A), *CsAPX1* (B) and *CsCAT* (C) in NPR1 transgenic lines following NaCl treatments.

The expression levels of **CsGST**, **CsCAT**, and **CsAPX1** were found to differ significantly between the transgenic and wild-type plants under both control (0 mM NaCl) and salt stress (100 mM NaCl) conditions. This indicates a distinct transcriptional response in the transgenic lines in relation to key antioxidant enzymes when exposed to salinity. A few subtle changes were observed between the wild type and transgenic plants when exposed to 200 mM NaCl.

- **Changes in activity of salt overly sensitive (*SOS*) pathway genes and the Na^+/H^+ antiporter *NHX1***

Compared to the wild type, the NPR1 transgenic plants showed a considerable downregulation of CsSOS1 and CsSOS2 transcript levels. In contrast to the wild type, NPR1 transgenic plants showed substantial overexpression of the salt overly sensitive 3 (CsSOS3) and Na+/H+-antiporter 1 (CsNHX1) genes.

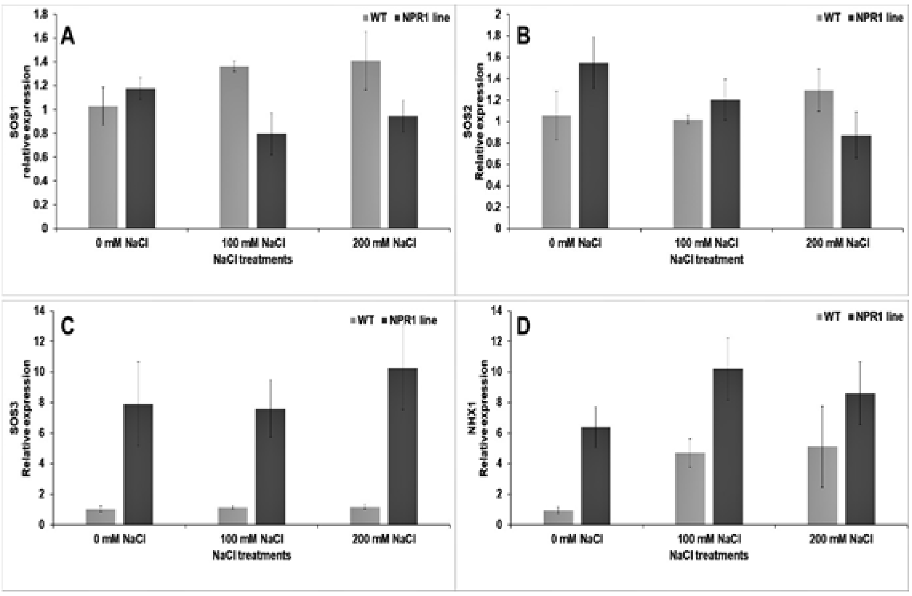

Figure 3: Changes in gene expression of Cs*SOS1* (A), *CsSOS2* (B), *CsSOS3* (C) and *CsNHX1* in NPR1 transgenic lines following NaCl treatment

- **Abscisic acid (ABA) signaling gene activity**

The expression of ABA 8'-hydroxylase (CsCYP707A3) was examined to better understand the role of the NPR1 gene in salt stress. Under 100 mM, the wild type showed a small rise in CsCYP707A3 expression, but overexpression of the AtNPR1 gene resulted in a decrease in expression relative to the control condition.

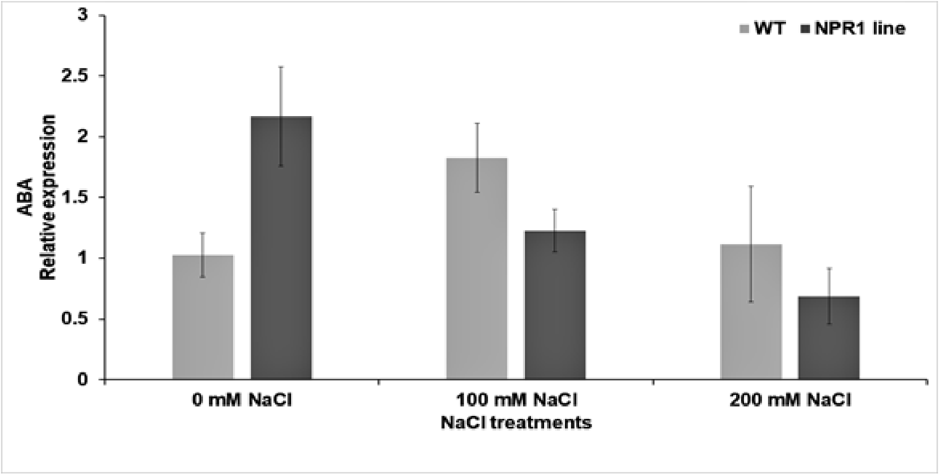

Figure 4: Changes in gene expression of ABA 8'-hydroxylase (*CsCYP707A3*) in NPR1 transgenic lines following NaCl treatments.

We found that NaCl stunted the development and chlorophyll content of 'Hamlin' delicious oranges. Nevertheless, these effects were mitigated by the exogenous overexpression of the AtNPR1 gene. Recent studies have provided new insights into the role of Non-expressor of Pathogenesis-Related Genes 1 (NPR1), a key regulator of Systemic Acquired Resistance (SAR) and a vital component in mediating salicylic acid (SA) signaling to trigger the expression of Pathogenesis-Related (PR) genes. These findings highlight NPR1's broader involvement not only in biotic stress responses but also in enhancing plant resilience to abiotic stresses such as salinity. Multiple investigations have shown that SA helps plants' defence mechanisms deal with oxidative stress. According to Sakhabutdinova et al. (2003), wheat growth was enhanced when 50 µM SA was applied, since it caused cell division in the apical meristem zone. It was previously thought that changes in photosynthesis, stomatal conductance, and transpiration status were responsible for SA's beneficial function (Stevens et al., 2006). We found that, as is often the case, plants grow better when their photosynthetic parameters are tweaked (Gururani et al., 2013).

Increased antioxidant activity effectively mitigated the toxic effects of reactive oxygen species (ROS) in plants, as evidenced by an elevated DPPH radical-scavenging capacity. Our findings revealed that salicylic acid (SA) notably enhanced DPPH-radical scavenging activity in okra, aligning with earlier research by Esan et al. (2017) and Golkar et al. (2019). Trees are able to react appropriately to environmental stress because many physiological reactions involving phytohormones share cellular processes. Evidence from a number of studies (Gossett et al., 1994; Gueta-Dahan et al., 1997; Dionisio-Sese and Tobita 1998; Hernandez et al., 2000; Shalata et al., 2001) points to the possibility that inducing genes for antioxidants might help lower salt stress.

One of the most important defence mechanisms in plants is superoxide dismutases (CSD), which convert O-2 to H2O2 very quickly. Hydrogen peroxide may be detoxicated by enzymatic antioxidants like POD, CAT, and APX, which convert it to water and stop the production of the dangerous hydroxyl radicals. One important mechanism in the mitigation of oxidative stress has been shown to be the action of phytohormones promoting enzymatic antioxidant synthesis (Gu et al., 2001; Guan and Scandalios 2002; Zhang et al., 2006). In most cases, the transgenic plants showed greater levels of CsCSD expression. Evidence like these suggested that the NPR1 gene helped reduce the harmful effects of increasing NaCl-induced oxidative stress.

Two key cellular components play crucial roles in maintaining low cytoplasmic Na^+ concentrations in plant cells: the Salt Overly Sensitive (SOS1) protein, located on the plasma membrane (Ji et al., 2013), and the Na^+/H^+ exchanger 1 (NHX1), situated on the tonoplast (Blumwald & Poole, 1985). As reported by Yang et al. (2009), the SOS gene family—SOS1, SOS2, and SOS3—is integral to a signaling cascade that regulates ion homeostasis in response to salt stress, enabling plants to adapt to high salinity conditions by modulating intracellular sodium levels. The transgenic plants exhibited expression of CsSOS1, CsSOS2, and CsSOS3, with CsSOS3 showing the greatest levels of expression. Thus, our results corroborate Shi et al. (2003) that these genes are essential for cytoplasmic Na+ pumping and ion homeostasis maintenance in the aftermath of Na+ poisoning. NHX1 is often activated by salicylic acid (SA) treatment (Cao et al., 2016) and plays a crucial role in ion homeostasis by facilitating the transport of Na^+ or K^+ into vacuoles in exchange for H^+ ions into the cytosol (Bassil et al., 2011b). In our study, the transgenic lines demonstrated a significant upregulation of CsNHX1, indicating enhanced vacuolar ion compartmentalization under salt stress. Additionally, salinity stress is known to elevate abscisic acid (ABA) levels, which subsequently triggers the expression of genes associated with defense responses and osmotic adjustment in plants (Gong et al., 2018). When osmotic stress is applied, ABA accumulates. After salt stress, this occurrence is seen as playing a crucial function. The impact of the NPR1 transgenic line on ABA biosynthesis was found to be minimal, according to our data. The study conducted by Liu et al. (2019b) found that SA and ABA interact antagonistically. SA suppresses ABA signalling and ABA synthesis, whereas ABA inhibits pathways upstream and downstream of SA signalling (Lee et al., 2019). Furthermore, SA prevents Phaseolus vulgaris leaves from dropping and stomatal closure induced by ABA (Rai et al., 1986; Apte and Laloraya 1982).

CONCLUSION

The overexpression of antioxidant genes and Na+ co-transporters triggered by NPR1 While the exact processes by which NPR1 improves citrus tree development and decreases oxidative damage in response to salt stress are unclear, our findings point to it as a promising option. In order to come up with specific plans to alleviate salt stress in transgenic citrus plants, additional field evaluations are necessary, since our present work just examined trees in a controlled environment.

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