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Overexpression of the tomato pathogenesis-related gene *SlPR-1.9* confers increased tolerance to salt stress in *Arabidopsis thaliana*

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ABSTRACT

Pathogenesis-related (PR) proteins are essential components of plant defense mechanisms, responding to both biotic and abiotic stresses. Among these, PR-1 proteins feature a CAP (Cysteine-rich secretory proteins, Antigen 5, and Pathogenesis-related 1) domain, which is crucial for immune responses and pathogen defense due to its ability to stabilize protein structures and interact with various molecules. This study investigated the role of the tomato PR-1 gene *SlPR-1.9* in enhancing salt tolerance in *Arabidopsis thaliana*. The gene's coding sequence was cloned and transferred into *Arabidopsis* to create *SlPR-1.9* overexpression lines. These transgenic lines, alongside wild-type plants, were exposed to salt stress (150 mM NaCl) to assess their tolerance. Morphological analysis revealed that the transgenic lines demonstrated greater resilience to salt stress compared to wild-type plants, with less severe leaf curling and color changes. Additionally, lower proline accumulation, a stress marker, in the transgenic lines indicated an enhanced adaptive response. Bioinformatics analysis of the protein encoded by *SlPR-1.9*, A0A3Q7HSC4, suggested a strong interaction with galactolipase. Expression analysis showed that SlPR-1.9 was mainly expressed in roots and during early fruit development, suggesting a significant role in root physiology and stress response. These findings indicate that overexpression of *SlPR-1.9* can improve plant tolerance to salt stress, offering potential applications for enhancing crop resilience to environmental challenges.

1. Introduction

Plants employ various strategies to manage environmental threats, including adapting growth habits and developing mechanisms to sustain essential functions (Singhal et al. 2016). Gene expression related to stress responses occurs at both the transcriptional and translational levels (Cushman and Bohnert 2000). Understanding these mechanisms is vital for safeguarding agricultural productivity and quality. Comprehensive analyses of stresses are facilitated by combining molecular biology with morphological, physiological, and biochemical methods (Roca Paixão et al. 2019; Liu et al. 2019). Advances in functional genetics and modern genetic technologies have greatly enhanced our ability to identify and characterize genes involved in stress responses (Wani et al. 2017; Khan et al. 2019; Prihatna et al. 2018).

Plant pathogenesis-related (PR) proteins are key components of the plant defense mechanism against microbial pathogens and insects, encompassing seventeen well-characterized families (van Loon et al. 2006; Sels et al. 2008). These proteins are typically low molecular weight, acid-soluble, and protease-resistant, attributes that enhance their stability and efficacy in the plant defense system. Notably, PR proteins can be synthesized in both infected and uninfected tissues, ensuring a broad-spectrum defensive response (Ahuja et al. 2012; Ali et al. 2017). Among these, PR-1 proteins were the first to be identified and remain the most abundantly produced, underscoring their pivotal role in plant immunity.

It has been shown that pathogenesis-related 1 (PR-1) proteins respond not only against biotic stress elements but also response in abiotic stress tolerance (Hong and Hwang 2005; Liu et al. 2022). PR-1 proteins consist of a CAP domain, found in proteins such as Cysteine-rich secretory proteins, Antigen 5, and Pathogenesis-related 1. It is characterized by its high cysteine content, which forms disulfide bonds, stabilizing the protein structure. This domain is involved in immune responses, pathogen defense, and other biological processes due to its ability to interact with various molecules. The PR-1 superfamily proteins are found in a wide range of organisms, including prokaryotes (Yeats et al. 2003) and non-vertebrate eukaryotes (Milne et al. 2003).

PR-1 genes are crucial for systemic acquired resistance (SAR) and respond to both biotic and abiotic stress factors (Almeida-Silva and Venancio 2022). Their expression increases in response to drought (Akbudak et al. 2020), freezing (Goyal et al. 2016), and salinity (Wang et al. 2019a). It has been shown that overexpressing PR-1 genes can enhance plant resistance to various harmful environmental conditions. For example, overexpression of the pepper CABPR1 gene, a basic PR-1

homologue, not only enhanced defense against pathogens such as *Ralstonia solanacearum*, *Phytophthora nicotianae*, and *Pseudomonas syringae* pv. Tabaci but also improved tobacco plants' tolerance to heavy metal stress (Sarowar et al. 2005).

The primary enzyme co-expressed with *SlPR-1.9* in the tomato genome, known as galactolipase or galactolipid acyl-hydrolase, plays a crucial role in lipid metabolism by catalyzing the hydrolysis of galactolipids. These galactolipids are integral components of thylakoid membranes in plants, particularly within chloroplasts (Douce and Joyard 1980). Belonging to the Lipolytic Acyl Hydrolases (LAH) family, galactolipases contribute to the remodeling of membrane lipids, a vital process for maintaining membrane fluidity and integrity, especially under challenging environmental conditions such as cold stress or drought (Yu et al. 2021). The enzymatic activity of galactolipase breaks down galactolipids into fatty acids and glycerol (Bhattacharya 2022). The fatty acids released by galactolipase activity serve, not only as energy sources but also as signaling molecules. For example, jasmonic acid, a plant hormone crucial for stress responses and developmental processes, is derived from linolenic acid, which is released through lipolytic activity. Additionally, during pathogen attacks, LAH enzymes play a role in the production of defense-related molecules, with free fatty acids acting as precursors for antimicrobial compounds (Lee and Park 2019; Wang et al. 2019b).

LAH enzymes are also involved in the synthesis of secondary metabolites such as cutin and suberin, which are components of the plant cuticle that help protect against environmental stresses. The expression and activity of LAH enzymes are tightly regulated by developmental cues and environmental factors, ensuring that lipid metabolism is precisely modulated to meet the plant's physiological needs. In plants, galactolipase activity is particularly important during stress conditions such as drought, freezing, or pathogen attack, where membrane remodeling and the release of fatty acids are crucial for survival. This enzyme also plays a significant role during leaf senescence and other developmental stages where lipid turnover is essential (Moellering and Benning 2011). *SlPR-1.9* (*Solyc08g068990*) is one of thirteen PR-1 genes identified in the tomato genome (Akbudak et al. 2020). Although its expression is notably upregulated under drought (Akbudak et al. 2020) and cold stress conditions (Kasap and Akbudak 2024), its specific role in these responses is still not fully understood. In this study, we further investigated *SlPR-1.9* through bioinformatics analyses, followed by its cloning and functional assessment in *Arabidopsis* under salt stress. Our findings reveal that *SlPR-1.9* enhances salt tolerance in transgenic *Arabidopsis* plants.

2. Material and Methods

2.1. Obtaining SlPR-1.9 OE arabidopsis lines

The coding sequence of *SlPR1.9* was amplified from the genomic DNA (gDNA) of tomato plants using PCR and subsequently cloned into the pIPKb004 vector (Himmelbach et al. 2007) through the Gateway cloning method. Successful integration of *SlPR1.9* into the plasmid was confirmed by PCR and restriction enzyme analysis. The resulting transformation vector, designated pPR1.9, was then introduced into *Arabidopsis thaliana* using a refined floral dip method. Floral-dipped plants were grown in a growth chamber at 23°C during the day and 21°C at night, with a 16-hour light/8-hour dark photoperiod and 60% relative humidity for one month. When the seed pods started turning brown, they were left to dry for about a week before the seeds were carefully harvested. After 3-4 days of drying, approximately ¼ of the seeds were surface sterilized and sown on ½ MS medium containing 25 mg l^{-1} Hygromycin B. The selection plates were placed in the growth chamber under the same conditions. Three plants which tested positive through antibiotic selection were transferred to a 2:1 mixture of peat and perlite. DNA was isolated from these plants and the presence of T-DNA having *SlPR1.9* gene was confirmed by PCR. The PCR-verified transgenic plants were grown normally, then dried, and their seeds collected.

2.2. Stress application

Three transgenic *SlPR1.9* OE plants and three wild-type (WT) plants were sown in a 2:1 mixture of peat and perlite and grown under standard conditions for three weeks, with watering every two days. After this period, the plants were subjected to a 150 mM NaCl treatment. During the two-week stress application, the same concentration of NaCl was applied at each watering, while the control plants received only tap water. Following the stress treatment, the plants were bulk harvested, flash-frozen in liquid nitrogen, and stored at -80°C for further analysis.

2.3. Proline analysis

Leaf tissue samples weighing 0.5 g from each plant group were ground in liquid nitrogen and then homogenized in 10 ml of 3% sulfosalicylic acid. The extracts obtained were centrifuged at 5000 rpm for 5 minutes. A 2 ml portion of the supernatant was combined with 2 ml of an acid ninhydrin solution (prepared by dissolving 1.25 g of ninhydrin in 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid) and 2 ml of glacial acetic acid. This mixture was then incubated at a temperature of 95-100°C for 1 hour. To stop the reaction, the samples were cooled on ice, and 4 ml of cold toluene was added. The phase containing the chromophore was measured at 520 nm using a spectrophotometer. Each measurement was conducted in triplicate. The proline content was determined as micromoles of proline per gram of fresh weight using the formula provided by Bates et al. (1973).

2.4. Analysis of conserved domain and protein interaction network

The amino acid sequences, conserved domains and the protein structures of the proteins were retrieved from UNIPROT (https://www.uniprot.org), InterPro (https://www.ebi.ac.uk/interpro/) and Phytozome (https://phytozome-next.jgi.doe.gov), respectively. The interaction networks were generated using the STRING 11 database and can be accessed at http://string-db.org/ (Szklarczyk et al. 2021).

2.5. *Digital expression pattern*

Data from the Tomato Genome Consortium (2012) was obtained to analyze the expression patterns of *SlPR-1.9* and tomato g*alactolipase* genes. The expression profiles of these genes were examined across different anatomical parts and developmental stages. The heatmap was drawn using the Heatmap program in TBtools software.

3. Results

3.1. Morphological analysis of SlPR1.9 OE lines

Under stress-free conditions, the WT (wild-type) and the *SlPR1.9* OE line (1, 2, and 3) plants displayed healthy, well-expanded leaves with symmetrical growth and a robust appearance (Fig. 1). Under salt stress conditions, WT plants experienced significant reduction in leaf size, noticeable color change, and pronounced curling, indicating severe stress symptoms. In contrast, the *SlPR-1.9* OE lines demonstrated better tolerance to salt stress. SlPR1.9 (1) maintained its green color better and exhibited less curling compared to WT. SlPR1.9 (2) experienced some curling but retained its leaf area and green color more effectively than WT. SlPR1.9 (3) showed leaf size reduction but less color change compared to WT. Overall, SlPR1.9 lines, especially SlPR1.9 (1) and SlPR1.9 (2), exhibited better resilience to salt stress compared to WT, with less severe morphological changes and better maintenance of leaf color.

3.2. Proline Accumulation in SlPR-1.9 OE Lines

Prolonged or excessive accumulation of proline could indicate that the plant is experiencing sustained or severe stress (Ghosh et al. 2022). In such cases, while the plant is managing stress, ongoing or severe conditions might still impact its overall health and growth. High proline levels in plants typically indicate that the plant is under stress. Proline accumulates in response to various environmental challenges such as drought, salinity, or extreme temperatures. This accumulation helps the plant manage stress by maintaining osmotic balance, protecting cellular structures from oxidative damage, and stabilizing proteins and membranes. While high proline levels do signal stress, they also reflect the plant's adaptive mechanisms to cope with and survive adverse conditions.

Under the salt treatment, the SlPR1.9 (1) and SlPR1.9 (2) lines showed lower proline levels compared to the wild-type (WT), indicating a potentially less robust stress response. In contrast, the SlPR1.9 (3) line exhibited proline similar to the WT, suggesting it was affected more by the salt stress compared to the other two lines (Fig. 2).

3.3. Analysis of A0A3Q7HSC4 encoded by SlPR-1.9

SlPR-1.9 (*Solyc08g068990*) encodes the A0A3Q7HSC4 protein, consisting of 181 amino acids (Fig. 3). This protein is localized extracellularly. Domain analysis using the InterPro tool revealed that A0A3Q7HSC4 contains a CAP (Cysteine-rich secretory proteins, Antigen 5, and Pathogenesis-related 1) domain. The PPI (Protein-Protein Interaction) network analysis revealed that protein A0A3Q7HSC4 (Solyc08g068990) in tomato had a strong interaction with protein A0A3Q7F1E6 (Solyc02g065090), with an interaction score of 0.720 (Fig. 4). A0A3Q7F1E6 was identified as galactolipase, a patatin-like protein, belonging to the Lipolytic Acyl Hydrolases (LAH) family. Patatin proteins are known for their lipid acyl hydrolase (lipase) activity, which allows them to hydrolyze fatty acids from membrane lipids, thus playing a key role in lipid metabolism in plants. Beyond their metabolic functions, patatin proteins are also crucial for plant defense. Their lipase activity can compromise the membrane integrity of pathogens, thereby contributing to the plant's immune response. In addition to A0A3Q7F1E6, A0A3Q7HSC4 interacts with two other uncharacterized proteins, A0A3Q7HKV2 and A0A3Q7HYQ6, with interaction scores of 0.441 and 0.452, respectively (represented by blue and green dots in Fig. 4). The UniProt BLAST analysis identified F3L17.40 (AT4G31470) as the primary ortholog of A0A3Q7HSC4 in *Arabidopsis thaliana*. This protein, F3L17.40, is another pathogenesis-related protein consisting of a CAP (Cysteine-rich secretory proteins, Antigen 5, and Pathogenesis-related 1) domain, similar to A0A3Q7HSC4. It is composed of 185 amino acids (Fig. 5). At high confidence level (0.700), STRING protein network analysis revealed that there were five predicted functional partners of F3L17.40 in *Arabidopsis* (Fig. 6, Table 1). CXE3 acts on esters with varying acyl chain lengths and is likely involved in the breakdown or modification of esters within the cell, potentially influencing metabolic pathways or detoxification

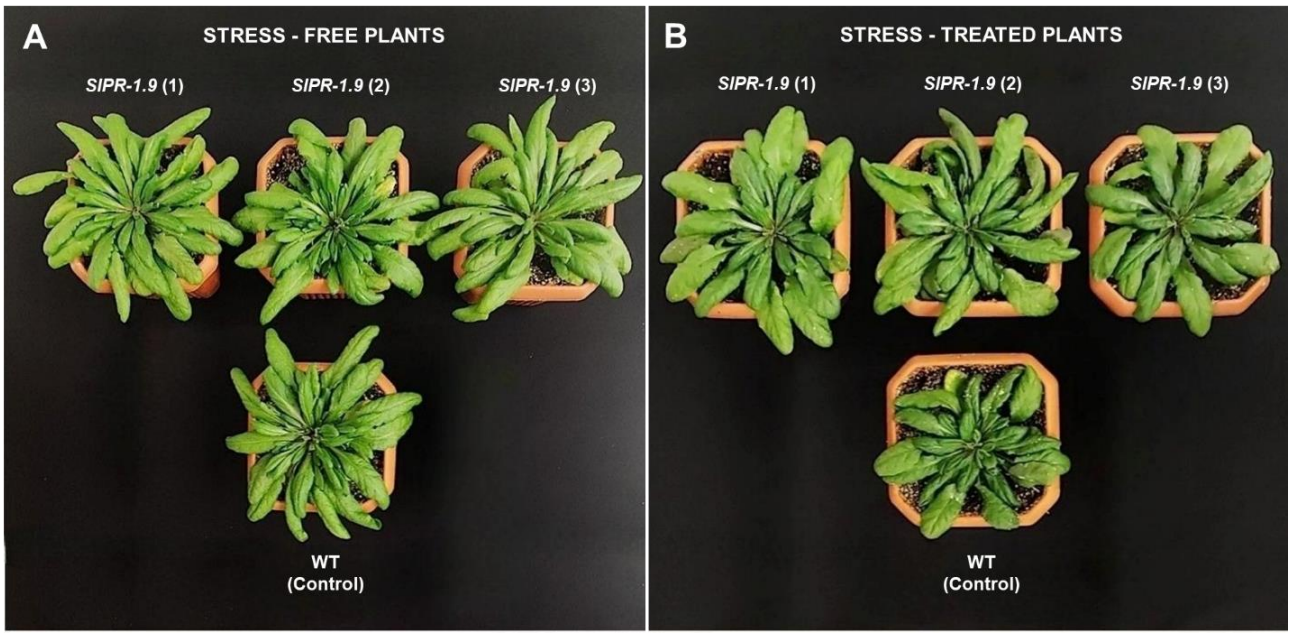

Figure 1. Increased NaCl stress tolerance in transgenic plants overexpressing *SlPR-1.9*. (A) Phenotypes of wild-type (WT) and T2 transgenic *SlPR-1.9* overexpression (OE) lines under stress-free conditions. (B) Phenotypes of WT and transgenic lines following NaCl treatment.

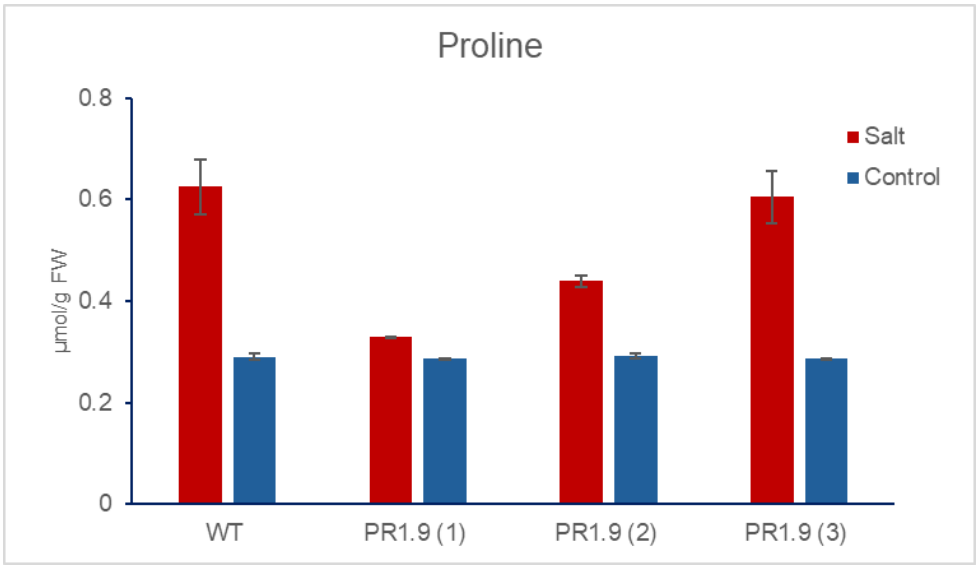

Figure 2. Proline accumulation in Arabidopsis leaves. Control: Stress-free, Salt: 150 mM NaCl treatment. Error bars represent the standard error of the mean (n= 3).

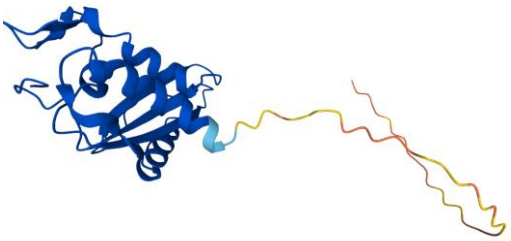

Figure 3. Protein structure of A0A3Q7HSC4. Modeled using data from the UniProt database (https://www.uniprot.org).

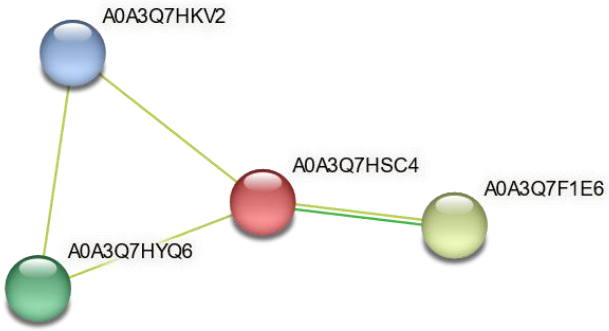

Figure 4. Protein-Protein Interaction network of A0A3Q7HSC4 in tomato. The dark green line represents evidence of direct protein–protein interactions and while the light green lines indicates associations identified through textmining.

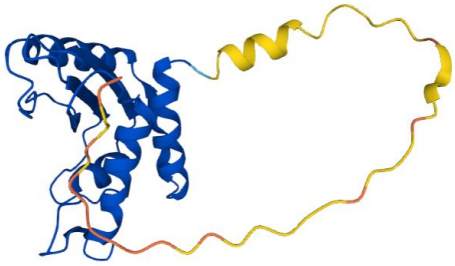

Figure 5. Protein structure of F3L17.40. Modeled using data from the UniProt database (https://www.uniprot.org).

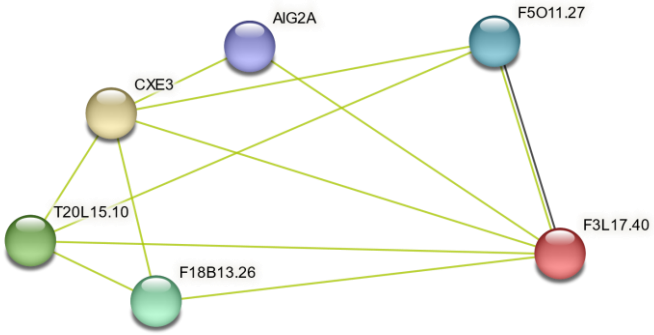

Figure 6. Protein–Protein Interaction Network of F3L17.40 in Arabidopsis. Green lines represent evidence of direct protein–protein interactions, while black lines denote co-expression relationships.

Table 1. Predicted functional partners of F3L17.40

Protein	Description	Conf. Score
CXE3	Probable carboxylesterase 3	0.794
T20L15.10	Nuclear transport factor 2 (NTF2) family protein	0.791
F18B13.26	MAPK kinase substrate protein At1g80180	0.779
F5O11.27	Uncharacterized protein	0.759
AIG2A	Protein AIG2 A; Putative gamma-glutamylcyclotransferase	0.718

processes. T20L15.10 is a Nuclear Transport Factor 2 Family Protein that plays a critical role in the transport of molecules between the nucleus and cytoplasm, affecting gene regulation and signal transduction. F18B13.26, also referred to as MAPK Kinase Substrate Protein (At1g80180), may play a role in the regulation of stomatal patterning and is likely involved in signaling pathways that regulate stomatal development, which is crucial for plant respiration and water regulation. AIG2A, is a putative gamma-glutamylcyclotransferase and belongs to the gamma-glutamylcyclotransferase family, potentially involved in the modification or degradation of peptides, impacting amino acid metabolism and cellular homeostasis. F3L17.40 is co-expressed with F5O11.27, an uncharacterized protein with an unknown function, highlighting a promising area for further investigation. The interaction network of F3L17.40 in *Arabidopsis* suggests a potential involvement in a complex, which could be crucial for understanding how pathogenesis response genes regulate the plant's response to environmental stresses.

3.4. Expression profiles of SlPR-1.9 and tomato galactolipase genes

Digital expression analysis showed that *SlPR-1.9* is highly expressed in root tissue (Fig. 7). Additionally, the gene exhibits significant expression during the early stages of fruit development, particularly in 1 cm and 2 cm fruits, where its expression is notably high. This expression pattern suggests that *SlPR-1.9* may play a crucial role in the initial phases of fruit formation. In contrast, the expression level of *SIPR-1.9* is substantially lower in other tissues, including the bud, flower, and leaf. The minimal expression in these tissues suggests that *SIPR-1.9* is less involved in the physiological processes occurring in these parts of the plant or at these stages of development. Overall, the expression profile of *SIPR-1.9* highlights its likely significance in root physiology and early fruit development. The expression analysis revealed that the galactolipase gene expressed across different plant tissues and developmental stages shows a highly specific pattern (Fig. 8). The most significant expression of galactolipase is found in the root tissue. This suggests that the galactolipase gene is highly active in the roots, likely contributing to its well-known roles in lipid metabolism and storage, which are critical for root function and energy reserves.

In addition to its high expression in the roots, moderate expression was observed in leaf tissues. This implies that while galactolipase's primary role might be in the root, it also has some level of activity in the leaves, possibly contributing to lipid metabolism or defense mechanisms within these tissues. In contrast, the expression of the galactolipase gene is minimal to negligible in other plant parts. Little to no activity of galactolipase indicates that the gene is not significantly involved in the physiological processes of these tissues, especially those related to reproductive development.

4. Discussion

The overexpression of the *SlPR-1.9* gene in *A. thaliana* significantly enhances tolerance to salt stress, as demonstrated by improved morphological characteristics and reduced proline accumulation in transgenic lines compared to wild-type (WT) plants. These findings contribute to the growing body of evidence supporting the role of PR-1 proteins in both biotic and abiotic stress tolerance, expanding our understanding of their functional diversity. The PR-1 protein family, to which *SlPR-1.9* belongs, is characterized by the presence of the CAP domain, known for its role in stabilizing protein structures and mediating immune responses (Gibbs et al. 2008). This domain is highly conserved across various species, indicating its fundamental role in defense mechanisms (Yeats et al. 2003; Milne et al. 2003). The ability of PR-1 proteins to interact with diverse molecules, as evidenced by their involvement in systemic acquired resistance (SAR) and responses to abiotic stresses like drought, freezing, and salinity (Akbudak et al. 2020; Wang et al. 2019a), underscores their multifaceted role in plant stress responses.

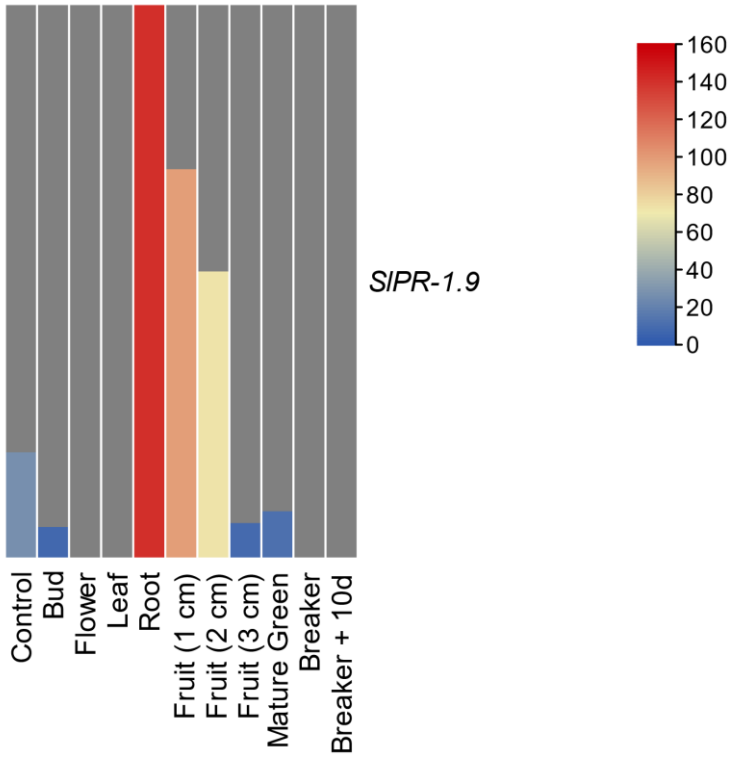

Figure 7. Heatmap of the expression profile of *SlPR-1.9* gene across various organs and developmental stages in tomato. In the heatmap, blue elements indicate low relative expression levels, while red elements indicate high relative expression levels.

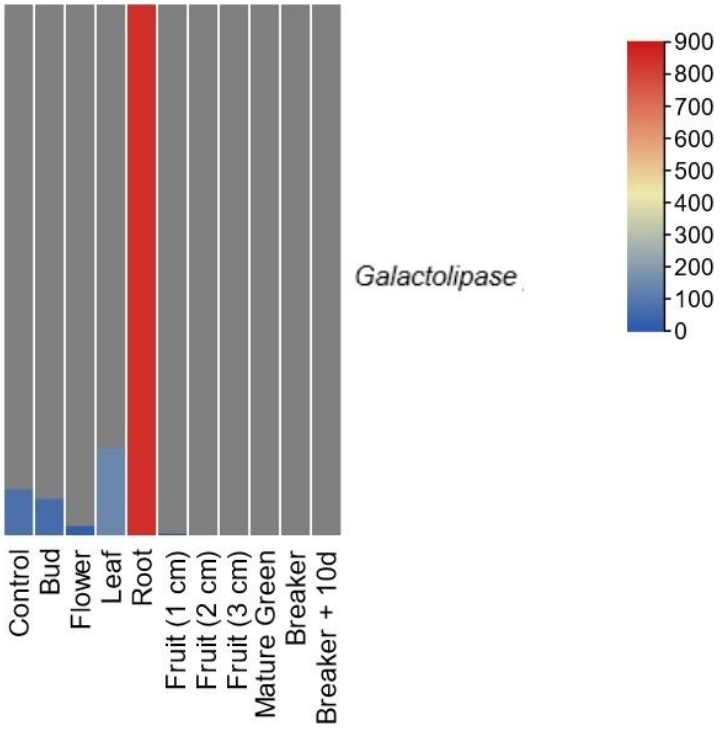

Figure 8. Heatmap of the expression profile of *Galactolipase* gene across various organs and developmental stages in tomato. In the heatmap, blue elements indicate low relative expression levels, while red elements indicate high relative expression levels.

The morphological analysis in this study revealed that *SlPR-1.9* overexpression lines exhibited less severe leaf curling and color changes under salt stress compared to WT plants, which is consistent with the role of PR-1 proteins in maintaining cellular integrity under stress conditions (Sarowar et al. 2005). The reduced proline accumulation in transgenic lines further supports the idea that *SlPR-1.9* helps mitigate the effects of osmotic stress, a common consequence of high salinity. Proline acts as an osmoprotectant and is a marker of stress severity; thus, lower levels in transgenic plants suggest a more efficient stress adaptation mechanism (Cushman and Bohnert 2000).

Bioinformatics analysis revealed a significant interaction between the protein encoded by *SlPR-1.9* and galactolipase, an enzyme involved in lipid metabolism (Moellering and Benning 2011). Galactolipase plays a critical role in the hydrolysis of galactolipids, which are major components of thylakoid membranes in chloroplasts. Under stress conditions, such as drought or salinity, membrane remodeling is essential for maintaining cellular homeostasis (Moellering and Benning 2011). The interaction between *SlPR-1.9* and galactolipase suggests that *SlPR-1.9* may enhance salt tolerance by influencing lipid metabolism and membrane stability, which are crucial for plant survival under adverse environmental conditions. Furthermore, the expression pattern of *SlPR-1.9* predominantly in root tissues and during early fruit development, as shown in this study, aligns with the findings of Almeida-Silva and Venancio (2022), who highlighted the tissue-specific expression of PR-1 genes under stress conditions. Roots, being in direct contact with soil, are particularly vulnerable to abiotic stresses such as salinity, making the high expression of *SlPR-1.9* in roots a likely adaptive response to such challenges. The involvement of PR-1 proteins in both biotic and abiotic stress responses, as reported by Ahuja et al. (2012) and Ali et al. (2017), further supports the notion that *SlPR-1.9* plays a critical role in the plant's defense mechanisms.

The ability of *SlPR-1.9* to interact with proteins involved in lipid metabolism, such as galactolipase, also highlights the potential for these interactions to influence metabolic pathways that are crucial for stress tolerance. This is particularly relevant in the context of systemic acquired resistance (SAR), where the coordination of multiple stress response pathways is essential for plant survival (Loon et al. 2006).

In conclusion, the overexpression of *SlPR-1.9* in *A. thaliana* enhances salt stress tolerance through mechanisms that likely involve both direct effects on stress-responsive pathways and interactions with key metabolic processes, such as lipid metabolism. These findings not only expand our understanding of the functional roles of PR-1 proteins but also suggest potential applications in developing crops with improved resistance to environmental stressors, thereby contributing to agricultural sustainability in challenging climates. Future research should focus on elucidating the detailed molecular pathways through which *SlPR-1.9* and its interacting partners, such as galactolipase, confer stress tolerance, as well as exploring the applicability of these findings to other economically important crops.

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Authors' Contribution

KY: Investigation and Data curation, MAA: Conceptualization, Writing- Original Draft, Writing- Review & Editing, Supervision.

References

Ahuja I, Kissen R, Bones AM (2012) Phytoalexins in defense against pathogens. Trends in Plant Science 17(2): 73-90.

Akbudak MA, Yildiz S, Filiz E (2020) Pathogenesis related protein-1 (PR-1) genes in tomato (*Solanum lycopersicum* L.): Bioinformatics analyses and expression profiles in response to drought stress. Genomics 112(6): 4089-4099.

Ali S, Mir ZA, Tyagi A, Bhat JA, Chandrashekar N, Papolu PK, Rawat S, Grover A (2017) Identification and comparative analysis of Brassica juncea pathogenesis-related genes in response to hormonal, biotic and abiotic stresses. Acta Physiol Plant 39(12): 268.

Almeida-Silva F, Venancio TM (2022) Pathogenesis-related protein 1 (PR-1) genes in soybean: Genome-wide identification, structural analysis and expression profiling under multiple biotic and abiotic stresses. Gene 809: 146013.

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39(1): 205-207.

Bhattacharya A (2022) Lipid Metabolism in Plants Under Low-Temperature Stress: A Review | SpringerLink. Physiological processes in plants under low temperature stress: 409-516.

Cushman JC, Bohnert HJ (2000) Genomic approaches to plant stress tolerance. Current Opinion in Plant Biology 3(2): 117-124.

Douce R, Joyard J (1980) 12 - Plant Galactolipids. In: Lipids: Structure and Function (Stumpf PK, Ed.). Academic Press, pp. 321-362.

Ghosh UK, Islam MN, Siddiqui MN, Cao X, Khan M a. R (2022) Proline, a multifaceted signalling molecule in plant responses to abiotic stress: understanding the physiological mechanisms. Plant Biology 24(2): 227-239.

Gibbs GM, Roelants K, O'Bryan MK (2008) The CAP Superfamily: Cysteine-Rich Secretory Proteins, Antigen 5, and Pathogenesis-Related 1 Proteins—Roles in Reproduction, Cancer, and Immune Defense. Endocrine Reviews 29(7): 865-897.

Goyal RK, Fatima T, Topuz M, Bernadec A, Sicher R, Handa AK, Mattoo AK (2016) Pathogenesis-Related Protein 1b1 (PR1b1) Is a Major Tomato Fruit Protein Responsive to Chilling Temperature and Upregulated in High Polyamine Transgenic Genotypes. Front Plant Sci 7: 901.

Himmelbach A, Zierold U, Hensel G, Riechen J, Douchkov D, Schweizer P, Kumlehn J (2007) A Set of Modular Binary Vectors for Transformation of Cereals. Plant Physiology 145(4): 1192-1200.

Hong JK, Hwang BK (2005) Functional Characterization of PR-1 Protein, β-1,3-Glucanase and Chitinase Genes During Defense Response to Biotic and Abiotic Stresses in Capsicum annuum. The Plant Pathology Journal 21(3): 195-206.

Kasap M, Akbudak MA (2024) Domates Pr-1 Genlerinin Soğuk Stresi Altındaki İfade Profilleri. In: 8th International Cukurova Agriculture and Veterinary Congress.

Khan R, Zhou P, Ma X, Zhou L, Wu Y, Ullah Z, Wang S (2019) Transcriptome Profiling, Biochemical and Physiological Analyses Provide New Insights towards Drought Tolerance in Nicotiana tabacum L. Genes 10(12): 1041.

Lee H-J, Park OK (2019) Lipases associated with plant defense against pathogens. Plant Science 279: 51-58.

Liu Y, Qi Y, Zhang A, Wu H, Liu Z, Ren X (2019) Molecular cloning and functional characterization of AcGST1, an anthocyanin-related glutathione S-transferase gene in kiwifruit (Actinidia chinensis). Plant Mol Biol 100(4): 451-465.

Liu T, Chen T, Kan J, Yao Y, Guo D, Yang Y, Ling X, Wang J, Zhang B (2022) The GhMYB36 transcription factor confers resistance to biotic and abiotic stress by enhancing PR1 gene expression in plants. Plant Biotechnology Journal 20(4): 722-735.

Loon LC van, Martijn Rep, Corné MJ Pieterse (2006) Significance of Inducible Defense-related Proteins in Infected Plants. Annu Rev Phytopathol 44(1): 135-162.

Milne TJ, Abbenante G, Tyndall JDA, Halliday J, Lewis RJ (2003) Isolation and Characterization of a Cone Snail Protease with Homology to CRISP Proteins of the Pathogenesis-related Protein Superfamily *. Journal of Biological Chemistry 278(33): 31105-31110.

Moellering ER, Benning C (2011) Galactoglycerolipid metabolism under stress: a time for remodeling. Trends in Plant Science 16(2): 98-107.

Prihatna C, Barbetti MJ, Barker SJ (2018) A Novel Tomato Fusarium Wilt Tolerance Gene. Front Microbiol 9.

Roca Paixão JF, Gillet F-X, Ribeiro TP, Bournaud C, Lourenço-Tessutti IT, Noriega DD, Melo BP de, Almeida-Engler J de, Grossi-de-Sa MF (2019) Improved drought stress tolerance in Arabidopsis by CRISPR/dCas9 fusion with a Histone AcetylTransferase. Sci Rep 9(1): 8080.

Sarowar S, Kim YJ, Kim EN, Kim KD, Hwang BK, Islam R, Shin JS (2005) Overexpression of a pepper basic pathogenesis-related protein 1 gene in tobacco plants enhances resistance to heavy metal and pathogen stresses. Plant Cell Rep 24(4): 216-224.

Sels J, Mathys J, De Coninck BMA, Cammue BPA, De Bolle MFC (2008) Plant pathogenesis-related (PR) proteins: A focus on PR peptides. Plant Physiology and Biochemistry 46(11): 941-950.

Singhal P, Jan AT, Azam M, Haq QMR (2016) Plant abiotic stress: a prospective strategy of exploiting promoters as alternative to overcome the escalating burden. Frontiers in Life Science 9(1): 52-63.

Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, Doncheva NT, Legeay M, Fang T, Bork P, Jensen LJ, von Mering C (2021) The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Research 49(D1): D605-D612.

Tomato Genome Consortium (2012) The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485(7400): 635-641.

Wang J, Mao X, Wang R, Li A, Zhao G, Zhao J, Jing R (2019a) Identification of wheat stress-responding genes and TaPR-1-1 function by screening a cDNA yeast library prepared following abiotic stress. Sci Rep 9(1): 141.

Wang K, Durrett TP, Benning C (2019b) Functional diversity of glycerolipid acylhydrolases in plant metabolism and physiology. Progress in Lipid Research 75: 100987.

Wani SH, Dutta T, Neelapu NRR, Surekha C (2017) Transgenic approaches to enhance salt and drought tolerance in plants. Plant Gene 11: 219-231.

Yeats C, Bentley S, Bateman A (2003) New Knowledge from Old: In silico discovery of novel protein domains in Streptomyces coelicolor. BMC Microbiol 3(1): 3.

Yu L, Zhou C, Fan J, Shanklin J, Xu C (2021) Mechanisms and functions of membrane lipid remodeling in plants. The Plant Journal 107(1): 37-53.