

“IN-SILICO CHARACTERIZATION OF *PECTATE LYASE* GENE FAMILY AND FUNCTIONAL ANALYSIS OF *SLPL* ASSOCIATED WITH FRUIT SOFTENING IN TOMATO”

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ABSTRACT

Pectate lyases (PLs) are cell-wall-modifying enzymes that contribute to pectin disassembly and fruit softening. However, the evolutionary relationships and functional characteristics of the tomato *PL* gene family remain incompletely resolved. The present study performed an integrated *in silico* analysis of 22 tomato PL proteins, with particular emphasis on Solyc03g111690 (*SIPL*), a ripening-associated gene previously implicated in fruit firmness and shelf life. Phylogenetic reconstruction revealed distinct evolutionary clusters and strongly supported paralogous relationships within tomato, while comparative analysis with *Arabidopsis thaliana*, strawberry and banana indicated conserved evolutionary relationships among plant PLs. Sequence and physicochemical characterization, conserved-domain and motif analyses, and three-dimensional structural modelling supported the classification of *SIPL* as a pectate lyase. The predicted protein structure exhibited a β -helix-rich architecture with localized flexible regions, consistent with features of this enzyme family. Computational profiling revealed developmental and tissue-dependent expression patterns, including increased *SIPL* transcript abundance during fruit maturation and ripening. Additionally, the protein association analysis indicated relationships between *SIPL* and other proteins involved in cell-wall modification, and *SIPL* could be part of a network of cell wall-modifying proteins. Taken together, these findings support Solyc03g111690 as a candidate gene involved in pectin remodeling during tomato fruit ripening and softening, providing a computational framework for its further functional validation and genome-engineering applications.

KEYWORDS: Pectate lyase, Fruit softening, domain analysis, In silico, Transcriptome.

1. INTRODUCTION

Tomato (*Solanum lycopersicum*) ranks as the world's most widely cultivated vegetable after potatoes, accounting for approximately 14% of global vegetable production output (Bauchet and Causse, 2012) and global tomato production was approximately 188.5 million tonnes in 2024, according to the Food and Agriculture Organization Statistics (FAOSTAT, 2025). Despite its high production and economic importance, tomato is highly perishable, resulting in substantial postharvest losses occurring between harvest and consumption. Among the major factors contributing to these losses, rapid fruit softening and limited shelf life are particularly significant, as they affect fruit firmness, marketability, and storage potential (Li et al., 2021, 2022). Fruit ripening is a complex, genetically regulated process involving coordinated transcriptional, hormonal, metabolic and structural changes. These processes contribute to the development of characteristic fruit colour, sweetness, aroma, and texture through pigment accumulation, sugar metabolism, volatile compound production, and extensive cell wall modification (Giovannoni, 2004; Klee & Giovannoni, 2011). Among these changes, cell wall disassembly plays a central role in determining fruit texture and the progression of softening. Thus, understanding the molecular mechanism of cell wall degradation becomes very important in improving the quality of tomatoes.

Plant cell walls are composed mainly of cellulose, hemicellulose, and pectic polysaccharides that affect cell adhesion, wall stiffness, and tissue mechanical characteristics (Cosgrove, 2024). As fruits ripen, alterations in pectin solubilization, degradation, and arrangement reduce the integrity of the middle lamella and facilitate cell separation, leading to fruit softening (Brummell, 2006). Among the different cell-wall-related genes, *pectate lyase* (*PL*) has emerged as a particular focus because of its direct biochemical association with pectin disassembly. *PL* belongs to polysaccharide lyase family 1 (PL1) and cleaves polymers of α -1,4-galacturonic acid polysaccharides through a β -elimination mechanism, contributing to the disassembly of pectin-rich regions of the middle lamella and primary cell wall (Marin-Rodriguez et al., 2002). This enzymatic activity is particularly relevant to the degradation of de-esterified homogalacturonan, a major component of pectin that contributes to cell-to-cell adhesion and tissue integrity. Beyond fruit ripening, plant *PL* genes have been associated with several developmental processes, including pollen development, organ abscission, and cell wall remodelling in different tissues (Uluisik and Seymour, 2020; Gayathri et al., 2026). These diverse functions highlight the importance of

understanding *PL* gene diversity, structural characteristics, and expression patterns to clarify their contributions to plant development and fruit texture.

Tomato possesses a relatively large *PL* gene family comprising 22 members, but transcriptomic analysis identified Solyc03g111690 (*SIPL*) as a predominantly fruit-associated *PL* with strong expression during maturation and softening. Suppression of *SIPL* resulted in firmer fruits, altered pericarp cellular properties, prolonged shelf life, and increased resistance to postharvest decay. These effects distinguish *SIPL* from several other cell-wall-modifying genes and indicate that it represents a particularly promising molecular target for improving tomato postharvest quality. Experimental evidence has established a strong association between pectate lyase activity and tomato fruit texture. Uluisik et al. (2016) demonstrated that suppression of a tomato *PL* gene increased fruit firmness and extended shelf life without substantially affecting major ripening characteristics. Subsequent genome-wide analysis identified 22 *PL* genes in tomato, among which Solyc03g111690 (*SIPL*) showed predominant expression during fruit maturation. RNA interference-mediated suppression of *SIPL* enhanced fruit firmness and shelf life, altered pericarp cell-wall characteristics, and reduced susceptibility to *Botrytis cinerea* (Yang et al., 2017). These findings establish *SIPL* as an important component of ripening-associated cell-wall remodeling and highlight its potential relevance to improving tomato postharvest quality.

The increasing availability of genomic sequences and transcriptomic datasets has expanded the scope of comparative studies aimed at understanding the diversity and potential functions of plant gene families. For pectate lyase (*PL*) genes, combining sequence analysis with physicochemical properties, conserved domains and motifs, phylogenetic relationships, structural prediction, protein-association networks and tissue-specific expression profiling can provide complementary insights into *PL* family evolution and potential function. Such integrated approaches are particularly valuable for multigene families whose members may differ in sequence conservation, structural features, and expression patterns. Integrating these data with developmental and fruit-ripening expression profiles may help identify candidate *PL* genes associated with cell-wall modification and tomato fruit softening (Klee & Giovannoni, 2011; Gapper et al., 2013; Giovannoni et al., 2017). Among the identified tomato *PL* genes, Solyc03g111690 (*SIPL*) represents a particularly relevant candidate for integrated characterization because its expression is associated with fruit maturation, while previous experimental studies have demonstrated that its silencing can improve fruit firmness and prolong postharvest shelf life (Yang et al., 2017).

Therefore, the present study aimed to comprehensively characterize the tomato *PL* gene family, with particular emphasis on *SIPL* (Solyc03g111690). Physicochemical properties, conserved domains and motifs, sequence relationships, phylogeny, structural characteristics, protein-association networks and transcriptome expression patterns were examined to define its evolutionary and functional features. Particular attention was given to its developmental and ripening-associated expression to assess its potential relationship with cell-wall remodeling and fruit softening. This integrated analysis provides a computational basis for understanding the potential contribution of *SIPL* to pectin remodeling and for prioritizing *PL* genes for subsequent functional validation and genome-engineering studies aimed at improving tomato fruit quality and postharvest longevity.

2. MATERIALS AND METHODS

2.1. Sequence Retrieval and Phylogenetic Analysis

Pectate lyase (*PL*) protein sequences of *Solanum lycopersicum*, *Arabidopsis thaliana*, *Fragaria × ananassa*, and *Musa acuminata* were retrieved from the National Center for Biotechnology Information (NCBI) and UniProt using the corresponding gene or protein identifiers. The retrieved sequences were converted to FASTA format and subjected to multiple sequence alignment (MSA) using the Clustal Omega sequence alignment tool with MUSCLE (<https://www.ebi.ac.uk/jdispatcher/msa/clustalo>). and MEGA 12 software (Kumar et al., 2024) with the Cluster Method: UPGMA (for computationally less intensive establishment of initial branching order). After that, the alignments were used to make the maximum likelihood (ML) tree with MEGA X (1000 bootstraps; model: Jones-Taylor-Thornton (JTT), the gold standard of nuclear proteins) phylogenetic tree only for tomato pectate lyase proteins. For comparative interspecific analysis, *PL* protein sequences from tomato, *A. thaliana*, strawberry and banana were analyzed using the same sequence-alignment and phylogenetic framework. The resulting phylogenetic tree was subsequently imported into the software of iTOL (<https://itol.embl.de/>) platform for visualization and annotation.

2.2 Physicochemical properties

The physicochemical properties of the tomato pectate lyase (*SIPL*) proteins were analysed using the amino acid sequences retrieved from the Sol Genomics Network (SGN) and National Center for Biotechnology Information (NCBI) databases. The protein sequences were subjected to physicochemical characterization using the ProtParam tool of the ExPASy server (<http://web.expasy.org/protparam>) which was used to determine the number of amino acids, molecular weight, theoretical isoelectric point (pI), instability index, aliphatic index, grand average of hydropathicity (GRAVY), and amino acid composition. These parameters were calculated from the primary amino acid sequences to assess the biochemical characteristics, predicted stability, hydrophobicity, and general physicochemical nature of the *SIPL* protein family. The obtained values were compiled and compared among the identified *SIPL* proteins to determine variations in their physicochemical characteristics and to support subsequent comparative and functional bioinformatic analyses (Gasteiger et al., 2005; Tsirigos et al., 2015).

2.3 Conserved motif analysis

The conserved domain architecture of the tomato pectate lyase (SIPL) protein family was analysed using the NCBI Conserved Domain Database (CDD) (<https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>) through the CD-Search tool and SMART software (<https://smart.embl.de/>). The deduced amino acid sequences of the identified SIPL proteins were submitted in FASTA format to identify conserved protein domains, domain boundaries, and functionally important regions, thereby enabling the identification of conserved domains and their corresponding functional regions (Marchler-Bauer et al., 2017; Marchler-Bauer et al., 2011). The detected domains were recorded based on their domain name, accession number, start and end positions, E-value, and sequence coverage. Particular emphasis was placed on the conserved pectate lyase domain, consistent with the classification of plant PLs within polysaccharide lyase family 1. The conserved domain information obtained for all SIPL proteins was subsequently organized into a conserved domain matrix to compare the presence, absence, position, and relative extent of conserved domains among family members.

2.4 PL interaction analysis

Protein–protein interaction (PPI) analysis was performed to identify the potential functional association of the tomato pectate lyase (SIPL) proteins with other proteins involved in fruit development, ripening, cell-wall modification, and related biological processes. The predicted interaction networks were generated using the STRING database (<https://string-db.org/>), which integrates experimental evidence, curated database information, co-expression, genomic context, and literature-based text mining to construct protein association networks (Szklarczyk et al., 2023). For constructing the protein interaction network of SIPL, *Solanum lycopersicum* protein identifiers were taken as query sequences, and the protein interaction network was generated based on the existing tomato protein interaction networks. The protein interaction network was analyzed based on the combined confidence score, and the major interacting proteins for each SIPL member were noted. Since STRING takes into account both direct and indirect protein interactions, the generated network was considered a protein association network and not as an experimentally proven protein interaction network (Szklarczyk et al., 2023).

2.5 Transcriptome Mapping / Expression Profiling

Transcriptome mapping and expression profiling of the SIPL gene family were performed using tomato transcriptomic datasets, particularly the Tomato Expression Atlas (TEA) (<https://tea.solgenomics.net/>). TEA provides a searchable platform for examining tomato gene expression across different tissues, developmental stages, and fruit developmental and ripening conditions using RNA-seq-derived expression data (Fernandez-Pozo et al., 2017). The corresponding *SIPL* gene identifiers were queried individually in the database, and their expression profiles were retrieved across the available tomato tissues and developmental stages. Expression values were compared to determine the spatial and temporal expression patterns of Solyc03g111690 (SIPL), with particular emphasis on fruit development and ripening stages. The obtained transcript abundance data were represented graphically to visualize differential expression patterns of SIPL and to identify definite regions potentially associated with fruit development, ripening, and cell-wall-related processes. (Fernandez-Pozo et al., 2017).

3. RESULTS

3.1 Phylogenetic Analysis of Tomato Pectate Lyase Gene Family

To determine the evolutionary position of *Solyc03g111690*, phylogenetic trees were constructed from the tomato (*Solanum lycopersicum*) gene family members (Fig. 1a) and from a broader dataset that also included orthologs from *Arabidopsis thaliana* (AT), strawberry (*FaPEL*) and apple (*MdPEL*) (Fig. 1b). The tomato-only tree (Fig. 1a) resolved the 22 family members into several well-supported clades, with bootstrap values from 54 to 100%. The largest clade, supported at 91%, contained eight genes in four sister pairs: Solyc03g113150/Solyc06g071020(92%), Solyc02g087670/Solyc03g058890(97%), Solyc03g058910/Solyc02g067450 (99%) and Solyc01g010740/Solyc05g007080 (95%). A second group comprised Solyc02g080910, Solyc04g010230 and Solyc09g005850, with Solyc04g010230 and Solyc09g005850 forming a strongly supported subclade (100%). A third clade (91%) contained Solyc09g091430, Solyc06g083580 and Solyc09g061890. The most basal clade (98%) comprised Solyc09g008380, Solyc03g071570, Solyc05g055510 and Solyc11g008140, with the last two forming a sister pair (78%). Solyc03g111690 (red box) was placed in a distinct clade supported at 86%, together with Solyc02g093580, Solyc05g014000 and Solyc06g071840. Within this clade, Solyc03g111690 grouped most closely with Solyc06g071840 (bootstrap 80%), suggesting that the two genes arose from a relatively recent duplication event or share a close common ancestor.

The circular tree (Fig. 1b) places *Solyc03g111690* (green label, red box) in the wider context of the family across species. The gene did not form a lineage of its own but clustered with *Arabidopsis* homologs (e.g., *AT3G53190*) and with tomato paralogs, indicating that it belongs to an evolutionarily conserved subfamily shared between monocot-free eudicot lineages. The tree also showed that *Solyc03g111690* is distinct from the clades containing the strawberry and apple genes (*FaPEL*, *MdPEL*), which grouped separately near the top of the tree. The tomato genes were distributed across multiple clades alongside *Arabidopsis* members, consistent with an ancient diversification of the family that predates the divergence of Solanaceae and Brassicaceae. Together, these results show that *Solyc03g111690* belongs to a well-supported, conserved clade, with *Solyc06g071840* as its closest paralog. Its relationship to *Arabidopsis* homologs provides a basis for inferring its function by comparative analysis.

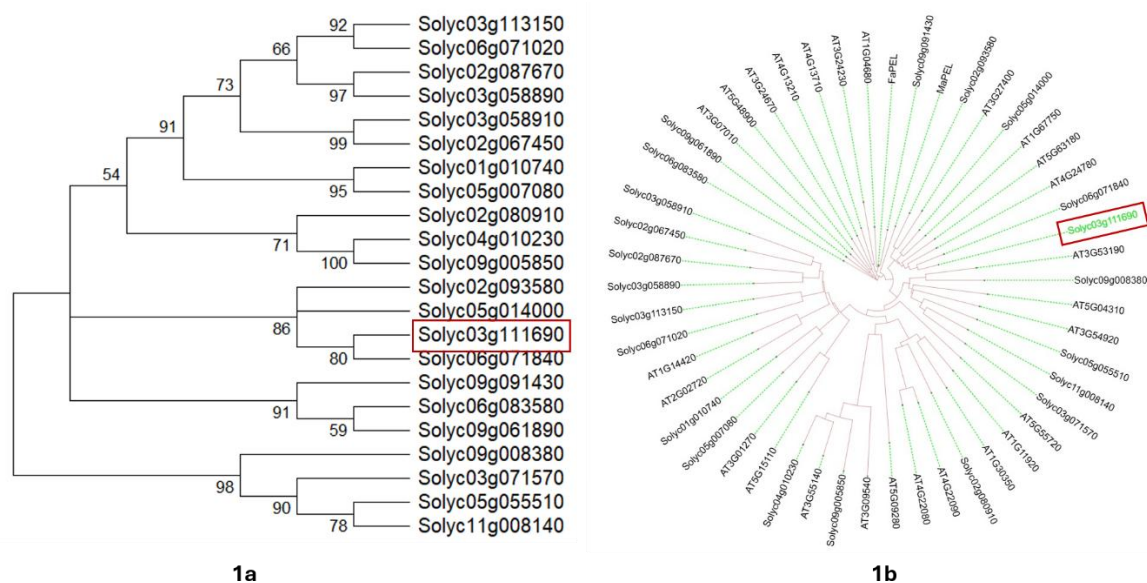


Figure 1. Phylogenetic analysis of *Solyc03g111690*. (a) Phylogenetic tree of tomato gene family members. Numbers at nodes are bootstrap values (%). (b) Circular phylogenetic tree including tomato (*Solyc*), *Arabidopsis* (*AT*), strawberry (*FaPEL*), and apple (*MdPEL*) sequences. *Solyc03g111690* is highlighted in red boxes in both panels.

3.2 Physicochemical characterization

The physicochemical characteristics of the PL protein were investigated using the ExPASy ProtParam server. The protein comprised 403 amino acids with a molecular mass of 44.31 kDa and a theoretical pI of 7.64, indicating a near-neutral to mildly basic character. The sequence contained 38 positively charged and 37 negatively charged residues, with a predicted extinction coefficient of $65,025 \text{ M}^{-1} \text{ cm}^{-1}$ for oxidized cysteine residues and $64,400 \text{ M}^{-1} \text{ cm}^{-1}$ under reducing conditions. The instability index (33.02) was below the ProtParam threshold of 40, predicting a relatively stable protein, while an aliphatic index of 78.86 indicated substantial representation of aliphatic side chains. The GRAVY value of -0.267 suggested an overall hydrophilic character. Serine (9.9%) and glycine (9.7%) were the most abundant residues, followed by leucine (7.2%), isoleucine (6.9%), aspartate (6.5%), asparagine (6.2%) and valine (6.2%). The predicted molecular formula was $\text{C}_{1939}\text{H}_{3018}\text{N}_{564}\text{O}_{586}\text{S}_{22}$, comprising 6,129 atoms. ProtParam additionally estimated half-lives of 30 h in mammalian reticulocytes in vitro, >20 h in yeast in vivo and >10 h in *E. coli* in vivo. Overall, the sequence-derived characteristics support a moderately sized, predominantly hydrophilic and predicted-stable SIPL protein with a near-neutral pI and appreciable aliphatic residue content.

3.3 3D Structural Modelling and Confidence Analysis

The three-dimensional structure of the SIPL (*Solyc03g111690*) protein was predicted using AlphaFold to investigate its overall folding architecture and structural characteristics. Structurally characterized pectate lyases commonly adopt a parallel β -helix fold, in which successive β -strands form a large right-handed helical scaffold with surface loops that can contribute to substrate recognition and catalytic activity (Figure 2). The AlphaFold model showed high confidence across the central structured region, whereas localized loop and terminal regions exhibited comparatively reduced confidence. Per-residue confidence evaluation (pLDDT) demonstrated that the central core is modeled with very high confidence ($\text{pLDDT} > 90$), adopting a prominent parallel β -helix/ β -barrel supersecondary structure (Figure 2b). Peripheral loop connections showed high confidence ($90 > \text{pLDDT} > 70$), while localized transition points displayed moderate-to-low confidence ($70 > \text{pLDDT} > 50$). In contrast, the terminal extended tail yielded a very low confidence score ($\text{pLDDT} < 50$), indicative of a highly flexible or intrinsically disordered protein region (IDR). The presence of a low-confidence terminal region should not automatically be interpreted as an intrinsically disordered region, although low pLDDT values can indicate structural flexibility, disorder, or context-dependent conformations. Overall, the predicted structure provided a reliable structural framework for subsequent analysis of the functional characteristics of SIPL (Figure 2a, 2b).

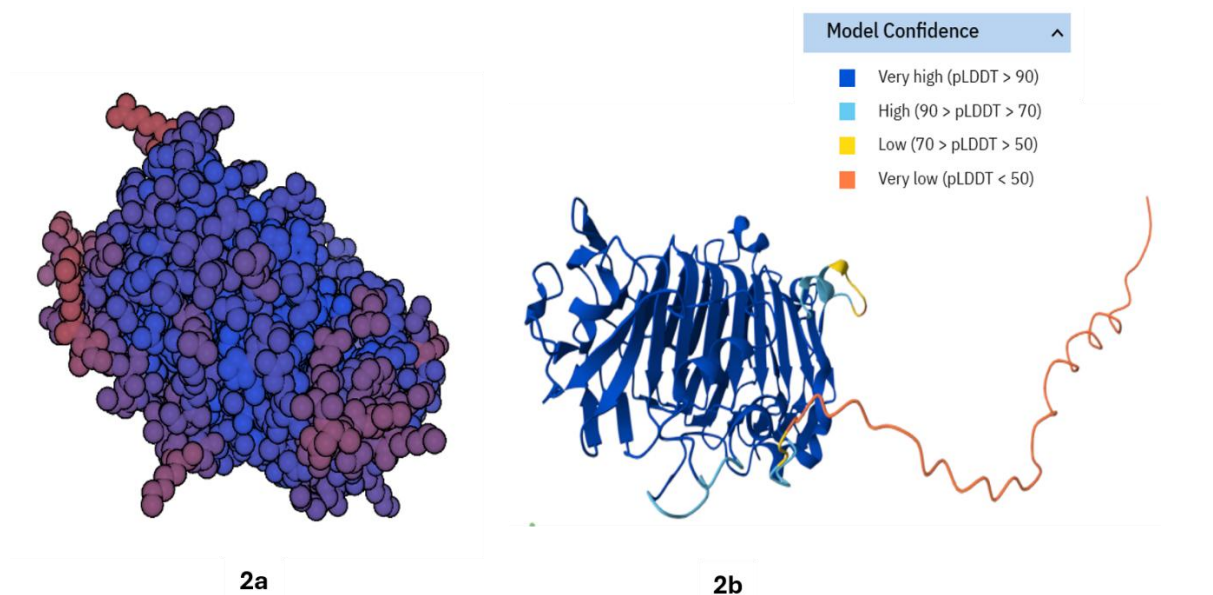


Figure. 2 The tertiary structure of pectate lyase was predicted. (Figure. 2a) Space-filling surface visualization revealed a compact globular structural core with localized surface projections. Figure. 2b Per-residue confidence evaluation (pLDDT) demonstrated that the central core is modeled with very high confidence (pLDDT > 90), adopting a prominent parallel β -helix/ β -barrel supersecondary.

3.4 Predicted Domain Architecture

Domain architecture analysis using the SMART database identified a prominent Amb_all (pectate lyase) domain spanning residues 128–325, with a highly significant E-value of 6.79×10^{-105} , supporting the assignment of this region as the principal conserved structural domain of SIPL. Within this primary domain region, two internal parallel β -helix repeats (PbH1) were mapped between residues 165–208 and 209–240. In addition, low-complexity regions were detected at the N-terminal residues 4–25 and C-terminal residues 368–379, indicating sequence regions with reduced compositional complexity. Collectively, the presence of a strongly conserved Amb_all domain together with internal PbH1 repeats supports the structural identity of SIPL as a pectate lyase and provides a molecular basis for its predicted role in pectin modification during tomato fruit development and ripening (Table 1).

Table 1: Confidently Predicted Domains, Repeats, and Motifs

Feature	Start Position	End Position	E-value	
low complexity	4	25	N/A	Region of low amino acid sequence complexity.
Amb_all	128	325	6.79×10^{-105}	Main structural domain detected in the protein.
PbH1	165	208	5.35×10^{-11}	Parallel β -helix repeat region within the main domain.
PbH1	209	240	1.72×10^{-13}	Secondary tandem parallel β -helix repeat region.
low complexity	368	379	N/A	C-terminal low-complexity segment.

3.5 PL interaction analysis

To investigate the putative functional associations of SIPL, a protein-association network was generated using the STRING database. The resulting network revealed a group of proteins predominantly associated with pectin modification and cell-wall remodeling. Among the prominent interactors, PG2 (polygalacturonase-2) showed a high STRING association score (0.899), while PG, K4CJ84_SOLLC, A0A3Q7J6B7, and A0A3Q7JS77 were also predicted as associated proteins. Importantly, STRING also identified SIPL as an associated protein with PME1.9 (Pectin Methyltransferase), supporting a potential functional relationship between pectin de-esterification and subsequent pectate degradation during fruit development and ripening. The predicted association of PME1.9 and PG2 with pectate lyase (PL) is particularly relevant to cell-wall remodeling during tomato fruit ripening. PME1.9 may regulate pectin methylesterification, thereby influencing the accessibility of pectic substrates to downstream degradation processes, while PG2 contributes to pectin depolymerization through the hydrolysis of homogalacturonan. The network also included several uncharacterized tomato proteins, suggesting potential additional components of the cell-wall remodeling machinery whose functions remain to be experimentally

validated. Additional uncharacterized and accession-specific tomato proteins (e.g., Q9ZT65_SOLLC, K4CJ84_SOLLC, A0A3Q7J6B7, and A0A3Q7JS77) were mapped as direct interactors. Together with PL-mediated pectin cleavage, these activities may contribute to cell-wall disassembly and changes in fruit firmness during ripening. The multiple edge connections between SIPL and these target enzymes highlight a co-functional network primarily involved in pectinesterase/pectin-degrading activities during fruit softening and floral tissue development (Figure. 3).

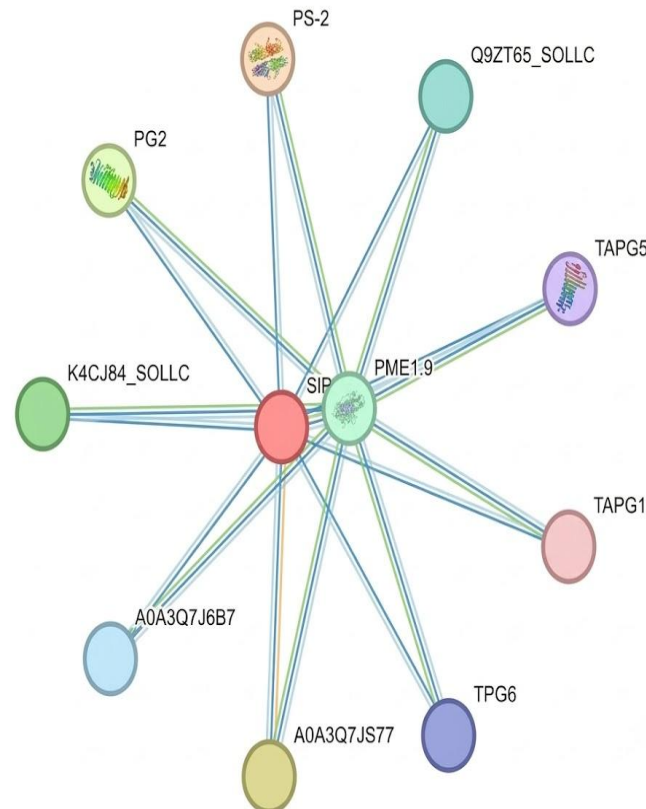


Figure. 3 Predicted protein-protein interaction (PPI) network of SIPL in tomato. The central SIPL node is connected with predicted functional partners involved predominantly in pectin and cell-wall modification, including PG2, PGcat, PME 1.9, K4CJ84_SOLLC, A0A3Q7J6B7, and A0A3Q7JS77. Lines connecting the nodes represent STRING-derived associations based on different evidence sources, including experimental evidence, curated databases, co-expression, text mining, and computational prediction. The network highlights the predicted association of SIPL with polygalacturonase and PME, suggesting coordinated involvement in pectin remodeling during tomato fruit development and ripening.

3.6 Transcriptome Mapping of Tomato Fruit Development

The spatiotemporal expression profile of *SIPL* (Soly03g111690) was examined using the tomato transcriptome resources available through the Tomato Expression Atlas (TEA), which provides high-resolution RNA-seq expression data across developmental stages, fruit tissues and pericarp cell layers. The expression pattern showed distinct developmental and tissue-specific variation, with relatively low transcript accumulation during the early stages of fruit development and increased expression toward the ripening transition. Across the mature-green and breaker stages, spatial differences were evident among the stem-end, equatorial and styler regions, indicating that *SIPL* expression is not uniform throughout the fruit. The pericarp-specific laser microdissection dataset further resolved this pattern at the cellular level, encompassing 5, 10 and 20 days post-anthesis (DPA) and the mature-green, breaker, pink, light-red and red-ripe stages. During ripening, *SIPL* transcript accumulation became progressively more pronounced within the pericarp, with localized expression in specific cell layers during the mature-green stage followed by broader distribution toward the inner pericarp during subsequent ripening stages. This spatial and temporal increase is consistent with the involvement of pectate lyase activity in ripening-associated cell-wall remodeling and provides further support for *SIPL* as a candidate gene associated with tomato fruit softening. The TEA framework specifically represents expression as reads per million mapped reads (RPM), enabling direct comparison of transcript abundance across the developmental and tissue-specific datasets (Figure 4).

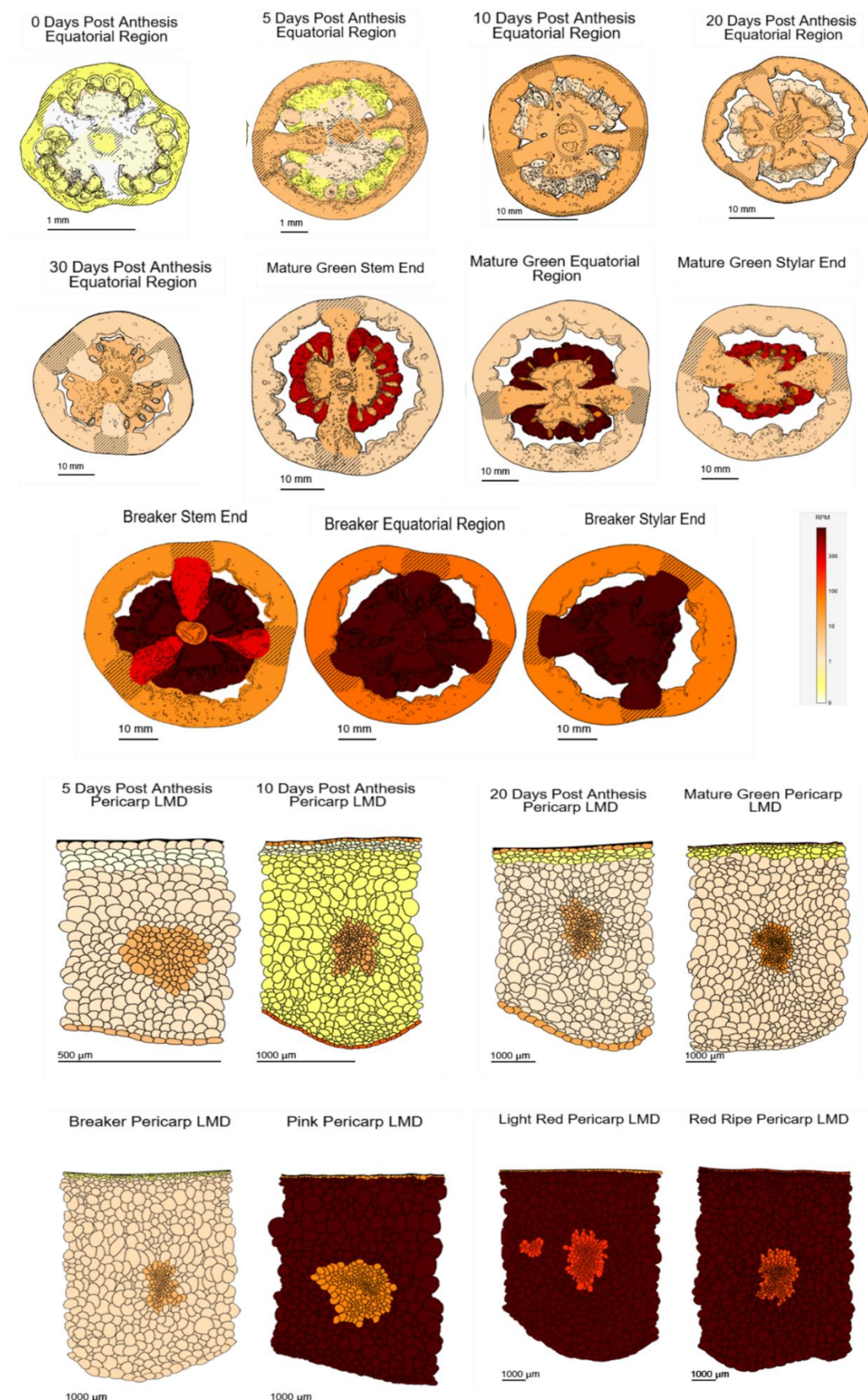


Figure. 4 Predicted spatiotemporal expression pattern of SIPL (Soly03g111690) during tomato fruit development and ripening. Gene expression is represented as reads per million mapped reads (RPM) using data from the Tomato Expression Atlas. The top panel shows expression across equatorial sections at 0, 5, 10, 20 and 30 days post-anthesis (DPA). The middle panel depicts expression in the stem-end, equatorial and stylar regions at the mature-green (MG) and breaker (Br) stages. The bottom panel presents laser microdissection-based expression profiles in pericarp cell/tissue layers at 5, 10 and 20 DPA and MG, Br, pink (Pk), light red (LR) and

red ripe (RR) stages. The colour scale represents relative transcript abundance, from low expression (<1 RPM; white/yellow) to very high expression (>300 RPM; dark red/maroon). The spatially resolved pattern illustrates developmental- and tissue-specific variation in SIPL expression, with increased transcript accumulation during the ripening transition.

4. DISCUSSION

This study presents a comprehensive in silico analysis of the tomato *PL* gene family, with particular focus on Solyc03g111690 (*SIPL*). In the phylogenetic analysis of 22 tomato PL proteins, several clusters emerged with well-supported evolutionary relationships, suggesting extensive diversification of the tomato *PL* gene family. The presence of SIPL among conserved PL lineages, along with other PL proteins from Arabidopsis, strawberry, and banana, indicates that pectate lyases are evolutionarily conserved in fruit-bearing species. This finding agrees with previous genome-wide analysis that identified 22 tomato *PL* genes and divided them into five subfamilies based on phylogeny (Yang et al., 2017). Diversification observed here fits well with the variety of biological roles ascribed to plant PLs, such as cell wall remodeling, pollen development, and fruit ripening (Marín-Rodríguez et al., 2002; Uluisik and Seymour, 2020).

The Sequence-level analysis further supported the functional identity of *SIPL* as a pectate lyase. The protein possesses conserved PL-associated sequence features and a characteristic pectate lyase domain architecture, while its predicted physicochemical properties indicate a moderately sized, near-neutral and predominantly hydrophilic protein with a favourable stability profile. Structural prediction additionally supports a conserved PL fold, providing a molecular framework for its interaction with pectic substrates. Importantly, the expression profile of *SIPL* showed strong association with fruit maturation and ripening, linking its molecular characteristics with a biologically relevant developmental process. This observation agrees closely with Yang et al. (2017), who reported that Solyc03g111690 exhibited dominant expression during tomato fruit maturation and that suppression of *SIPL* increased fruit firmness and extended shelf life. The CRISPR/Cas9 mediated knock out *SIPL*, *Solyc03g111690* decreased the *PL* expression there by led to fruit softening (Fumelli et al., 2025). It is also consistent with broader evidence that degradation of de-esterified pectin contributes substantially to cell-wall disassembly during fleshy-fruit ripening (Uluisik et al., 2016; Wang and Seymour, 2022).

Collectively, the phylogenetic conservation, characteristic sequence and structural features, and ripening-associated expression of SIPL strengthen its functional association with tomato fruit softening. The computational expression studies showed that pectate lyase proteins are prominently expressed on the pericarp cell wall layers of mature and ripened tomato fruits to loosen the cell wall, thereby leading to fruit softening. This is consistent with previous functional studies demonstrating that SIPL proteins were highly expressed in the exocarp and sarcocarp of tomato fruit cell walls, especially at fruit breaker stage 4, and that suppression of *SIPL* enhances fruit firmness, alters cell-wall composition, and reduces susceptibility to *Botrytis cinerea*, indicating that PL activity extends beyond simple pectin degradation and may influence broader cell-wall and stress-response processes (Yang et al., 2017). The present computational framework therefore provides a coherent molecular basis for prioritizing Solyc03g111690 as a candidate target for genome engineering aimed at modifying fruit texture and postharvest longevity. Nevertheless, computational predictions and expression associations should be interpreted as supportive evidence; definitive functional attribution requires experimental validation through targeted genome editing, independent mutant characterization, enzymatic assays and fruit cell-wall phenotyping.

5. CONCLUSION

This current study offers an integrated in silico analysis of the tomato *Pectate Lyase (PL)* gene family, specifically focusing on Solyc03g111690 (*SIPL*). This study integrates phylogenetic, sequence, physicochemical, structural, protein-association and transcriptomic analyses to characterize the tomato *Pectate Lyase (PL)* gene family, with particular emphasis on *Solyc03g111690 (SIPL)*. The evolutionary relationships among 22 tomato PL proteins, together with comparisons across Arabidopsis, strawberry and banana, revealed conserved and diversified features of the PL family. Conserved domain architecture, predicted protein structure and fruit-ripening-associated expression patterns collectively support the potential involvement of *SIPL* in pectin remodeling during tomato ripening. Predicted associations with other cell-wall-modifying proteins further suggest its participation in a broader network regulating pectin disassembly. These findings establish *SIPL* as a promising candidate for functional investigation and molecular improvement of tomato fruit firmness and shelf life. Future studies integrating targeted genome editing, enzyme activity assays, cell-wall compositional analysis and postharvest phenotyping are needed to establish its precise biochemical function and determine the effects of *SIPL* modification on fruit quality, storage performance and resistance to postharvest deterioration.

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