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RESEARCH ARTICLE

Genome-Wide Analysis of the Phenylalanine Ammonia-Lyase Gene Family in Strawberry (*Fragaria × ananassa*) under Stress Conditions

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Abstract

The phenylalanine ammonia-lyase (PAL) gene family plays a pivotal role in plant secondary metabolism, particularly within the phenylpropanoid pathway that produces essential metabolites contributing to plant growth, defense, and fruit quality. This study presents a comprehensive genome-wide analysis of the PAL gene family in *Fragaria × ananassa* Duch. (Strawberry) to elucidate their evolutionary relationships, chromosomal distribution, regulatory mechanisms, and potential involvement in abiotic and biotic stress responses. Eight PAL genes (designated *FaPAL1–FaPAL8*) were identified and systematically characterized. Gene duplication analysis revealed evolutionary relationships among *FaPAL* members and suggested possible functional redundancy. Phylogenetic analysis of *FaPAL* proteins with those from five other plant species clustered the genes into distinct clades, indicating both functional diversification and evolutionary conservation. Conserved motif analysis confirmed the presence of key domains required for PAL enzymatic activity, while promoter analysis uncovered numerous cis-regulatory elements associated with light responsiveness, hormonal regulation, and abiotic stress tolerance (including drought and temperature stress). Furthermore, microRNA (miRNA) interaction analysis indicated that *FaPAL3* and *FaPAL5* exhibited the highest miRNA targeting frequency (38.9%; score 7), highlighting their potential regulatory significance under stress conditions. Overall, this study provides valuable insights into the structural, evolutionary, and functional dynamics of the *PAL* gene family in strawberry. The findings lay a foundation for future genetic and biotechnological interventions aimed at enhancing fruit quality, disease resistance, and stress resilience for sustainable strawberry cultivation.

Keywords: Cis-regulatory elements, MicroRNA regulation, Phenylalanine ammonia-Lyase, Stress condition.

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Introduction

Phenylalanine ammonia-lyase (PAL) is a pivotal enzyme in plant secondary metabolism, catalyzing the deamination of *L*-phenylalanine to produce trans-cinnamic acid and ammonia. This reaction represents the initial step in the phenylpropanoid pathway, a crucial metabolic route responsible for synthesizing a variety of secondary metabolites, including lignins, flavonoids, and phenolic compounds. These metabolites play essential roles in plant growth, development, and defense mechanisms (Zhang et al. 2025).

PAL is encoded by a multigene family whose members exhibit diverse expression patterns and functions, depending on the tissue type and environmental conditions (Achnine et al. 2004, Olsen et al. 2008). This enzyme is of particular interest in plant biology because it bridges primary and secondary metabolism and plays a central role in plant responses to biotic and abiotic stresses (Cass et al. 2015, Gho et al. 2020). The versatility and adaptability of PAL in regulating various stress responses further emphasize its biological significance. As a gateway enzyme in the phenylpropanoid pathway, PAL governs the synthesis of metabolites involved in pathogen defense, ultraviolet (UV) protection, and structural reinforcement. Its activity is highly inducible under extreme environmental conditions such as drought, salinity, and high temperature (Vogt 2010, Rasmussen et al. 2013). Moreover, PAL-mediated metabolites with antioxidant properties, such as flavonoids and phytoalexins, mitigate oxidative damage caused by environmental stresses (Weisshaar and Jenkins 1998).

Studying PAL provides valuable insights into plant resilience mechanisms and offers avenues for developing stress-tolerant crop varieties through genetic manipulation and breeding programs. The evolutionary conservation and functional diversity of PAL genes have been demonstrated across numerous plant species. Four distinct but overlapping PAL genes (*AtPAL1–4*) have been identified in the model plant *Arabidopsis thaliana*, all of which participate in development and stress responses (Dong and Shang 2013). In crops such as rice (*Oryza sativa*) and maize (*Zea mays*), PAL genes have been implicated in improving resistance to environmental stress through

enhanced lignin biosynthesis and antioxidant production (Yu et al. 2018). Similarly, recent genome-wide analyses in cucumber (*Cucumis sativus*) identified 11 PAL genes, several of which exhibited significant upregulation under heat stress, highlighting their role in abiotic stress tolerance (Amjad et al. 2024).

Despite these advances, information on the PAL gene family in strawberry (*Fragaria × ananassa*) remains limited, particularly regarding their roles in biotic and abiotic stress responses. The phenylpropanoid pathway forms the cornerstone of plant secondary metabolism, with PAL functioning as its entry point. This pathway generates a wide range of metabolites that contribute to plant structure, defense, and environmental adaptation. For instance, lignins derived from this pathway provide mechanical strength and pathogen resistance, while flavonoids offer UV protection and antioxidative properties (Dixon et al. 2002). In addition, phytoalexins- antimicrobial compounds synthesized in response to pathogen attack- are also products of this pathway. PAL activity and regulation influence the metabolic flux through this pathway, thereby determining a plant's capacity to adapt to stress. Given its central role, understanding the genetic and functional dynamics of PAL is crucial for elucidating the complexity of plant stress adaptation.

Strawberries are economically valuable fruits renowned for their nutritional and medicinal properties, largely due to their high phenolic content. However, strawberry cultivation faces significant challenges from abiotic stresses such as drought, salinity, and temperature extremes, which adversely impact yield and fruit quality. PAL plays a vital role in mitigating these effects by regulating phenylpropanoid metabolism, thereby enhancing the plant's structural integrity and antioxidative defense mechanisms. Furthermore, phenolic compounds such as anthocyanins, whose biosynthesis is PAL-dependent, contribute to fruit color, flavor, and health benefits, underscoring the enzyme's importance in strawberry cultivation and postharvest quality (Chen et al. 2022, Ninkuu et al. 2025).

Although PAL genes have been characterized in several plant species, studies on their genome-wide identification, expression profiling, and functional analysis in strawberries remain scarce. The limited data on PAL's role in strawberry abiotic stress responses present a significant research gap. Previous investigations have primarily focused on PAL's involvement in fruit development and anthocyanin biosynthesis, with little emphasis on its potential contribution to stress tolerance. Additionally, the regulatory mechanisms controlling PAL gene expression under abiotic stress- such as cis-regulatory elements and microRNA (miRNA) interactions- are still poorly understood in strawberries.

This study aims to address these gaps by conducting a comprehensive genome-wide analysis of the PAL gene family in strawberries. Specifically, it seeks to identify and characterize PAL genes in the strawberry genome, analyze their promoter regions for stress-responsive elements, profile their expression patterns under abiotic stress, explore their roles in phenylpropanoid metabolism, and investigate their evolutionary relationships with PAL genes from other plant species. Overall, this research deepens our understanding of PAL's functional diversity in strawberries and provides a foundation for future studies aimed at enhancing strawberry stress resilience through targeted genetic and molecular strategies.

Materials and Methods

Retrieval of protein sequences containing the PAL genes in *Fragaria × ananassa*

The amino acid sequences of *Fragaria × ananassa* were retrieved from the Phytozome v13 database (https://phytozome-next.jgi.doe.gov/) (Goodstein et al. 2012). To identify *Fragaria × ananassa* PAL gene family members, the protein sequences of four PAL genes from Arabidopsis and nine PAL genes from rice were downloaded from the TAIR database (https://www.arabidopsis.org/) (Lamesch et al. 2012) and the Rice Genome Annotation Project (RGAP) database (https://rice.uga.edu/) (Hamilton et al. 2025), respectively. Different subfamilies of the PAL gene from Arabidopsis and rice were used as queries to search against the whole *Fragaria × ananassa* genome by the BLASTP (http://blast.ncbi.nlm.nih.gov) (Mahram and Herbordt 2015) program with an E-value<1e-6. Further, the Pfam (Bateman 2004) and SMART (http://smart.embl-heidelberg.de/) databases (Schultz 2000) were used for the identification and confirmation of PAL-conserved domains.

Physicochemical properties, subcellular localization, and cis elements

We gathered information on eight FaPAL proteins from two sources: Phytozome and Protparam (https://web.expasy.org/protparam). Phytozome provided information on the number and position of chromosomes as well as the gene's direction (sense or antisense) in that particular region. Peptide size and mRNA length (CDS) were also provided. Protparam provided these proteins' theoretical pI, molecular weight, GRAVY

(Grand Average of Hydropathy), and stability index (Bjellqvist et al. 1993 & 1994, Wilkins et al. 1998). To determine the location, the WoLF PSORT database (https://wolfpsort.hgc.jp/) (Horton et al. 2007) was utilized. Upstream promoter regions of 2000 base pairs were taken from phytozomeV3 (https://phytozome-next.jgi.doe.gov). In each case, a prediction was made using the web tool PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) (Lescot 2002) to survey the potential stress response and hormone-related cis-regulatory elements in the promoter region based on 5 to 20 base pairs of upstream sequence from the first nucleotide. These outputs were shown as a heat map, using TBtools for visualization (Bülow and Hehl 2016, Chen et al. 2020).

Analysis of conserved motif domain and exon-intron arrangement

The MEME program, which can be found online at (http://meme.sdsc.edu/meme/website/intro.html) (Bailey et al. 2009) was used to find motifs (motif-10 default setting) in the amino acid sequences. Then, the amino acid sequences were added to the CDD (https://www.ncbi.nlm.nih.gov/ Structure/cdd/wrpsb.cgi), which uses domains identified by NCBI as a database. To analyze the exon and intron distribution, the GSDS web tool at (http://gsds.cbi. pku.edu.cn/) (Hu et al. 2015) was used to process the genomic and CDS sequences of the FaPAL gene family.

Comparative phylogenetic analysis

The amino acid sequences of FaPAL proteins were aligned with those from *Fragaria × ananassa*, *Oryza sativa*, *Arabidopsis thaliana*, *Cucumis sativus,* and *Triticum aestivum* to make a phylogenetic tree. The software MEGA 11 (Tamura et al. 2021) was used with the NJ (neighbor-joining) method (Gascuel 2006) aligned by MUSCLE, to build the tree and bootstrapping was done with 1000 replications (Edgar 2021). The tree was visually adjusted using the iTOL program (https://itol.embl.de/), which allows users to examine and annotate the phylogenetic relationships (Letunic and Bork 2024).

miRNA analysis

The target sites of all FaPAL gene family were identified using the PmiREN website (https://www.pmiren.com/) (Guo et al. 2020). There were nine main groups of miRNAs (Fan-miR482, Fan-miR11293, Fan-miR2118, Fan-miR396, Fan-miR11293, Fan-miR477, Fan-miR827, Fan-miR397, and Fan-miRN1080) that targeted the FaPAL genes. When considering their subgroups, there were 47 miRNAs in total that targeted the FaPAL genes. The CDS sequences of the genes and mature miRNA sequences were compared using the PsRNA online server tool (https://www.zhaolab.org/psRNATarget/) with default settings (Dai and Zhao 2011). Then, the Cytoscape program was used to show the interactions between target genes and the predicted miRNA (Shannon et al. 2003) and identified the degree by the CytoHubba plugin that shows the highest interaction among genes and microRNAs (miRNAs). The normalized degree was calculated by the following equation:

$$\text{Normalized degree (\%)} = \frac{ki}{N-1} \times 100$$

Here,
ki = Degree of node i (number of edges connected to that gene)
N = Total number of nodes (genes) in the network
N-1 = The maximum possible number of connections a node could have

Results

Identifying PAL genes in *Fragaria × ananassa* and their localization

The molecular features of the FaPAL genes were extensively analyzed to uncover their specific characteristics. FaPAL8 had the lowest molecular weight of 77910.01 Da, while FaPAL3 had the highest of 79043.13 Da. The pI values varied from a low of 6.0 in FaPAL7 to a high of 6.29 in FaPAL2.

All genes had negative Gravy scores, indicating that they were hydrophilic. Moreover, peptide length ranged from a low of 718 in FaPAL4, FaPAL5, FaPAL7, and FaPAL8 to a high of 724 in FaPAL1, FaPAL2, and FAPAL3. Upon further examination of gene orientations, FaPAL2, FaPAL3, FaPAL4, FaPAL5, and FaPAL7 oriented in the forward (F) direction, while FaPAL1, FaPAL6, and FaPAL8 oriented in the reverse (R) direction. In terms of subcellular localization, notable variations existed among the FaPAL genes. FaPAL6, FaPAL7, and FaPAL8 exhibited the highest localization in the cytoplasm; FaPAL5 shows significant localization in the chloroplast (Table 1, Fig. 1).

Table 1: Details on the FaPAL gene family include information on eight genes identified in the strawberry genome.

Gene name	Transcript ID	PAC ID	Start	End	Strand	AA	Weight (Da)	PI	GRAVY
FaPAL1	maker-Fvb6-2-augustus-gene-174.36-mRNA-1	50471421	17475966	17479498	-	724	78844.82	6.13	-0.201
FaPAL2	maker-Fvb6-4-augustus-gene-113.28-mRNA-1	50519463	11331124	11334816	+	724	78885.96	6.29	-0.198
FaPAL3	maker-Fvb6-1-augustus-gene-255.38-mRNA-1	50524180	25529383	25532885	+	724	79043.13	6.1	-0.191
FaPAL4	maker-Fvb7-3-augustus-gene-83.24-mRNA-1	50540694	8289114	8292620	+	718	77036.83	6.06	-0.181
FaPAL5	maker-Fvb7-3-augustus-gene-86.41-mRNA-1	50478551	8658484	8662340	+	718	77079.9	6.06	-0.176
FaPAL6	maker-Fvb7-1-augustus-gene-214.51-mRNA-1	50487771	21468170	21471563	-	720	78081.13	6.21	-0.184
FaPAL7	maker-Fvb7-2-augustus-gene-144.48-mRNA-1	50492072	14469059	14471953	+	718	78022.99	6	-0.198
FaPAL8	maker-Fvb7-1-augustus-gene-236.35-mRNA-1	50488933	23663916	23667335	-	718	77910.01	6.21	-0.183

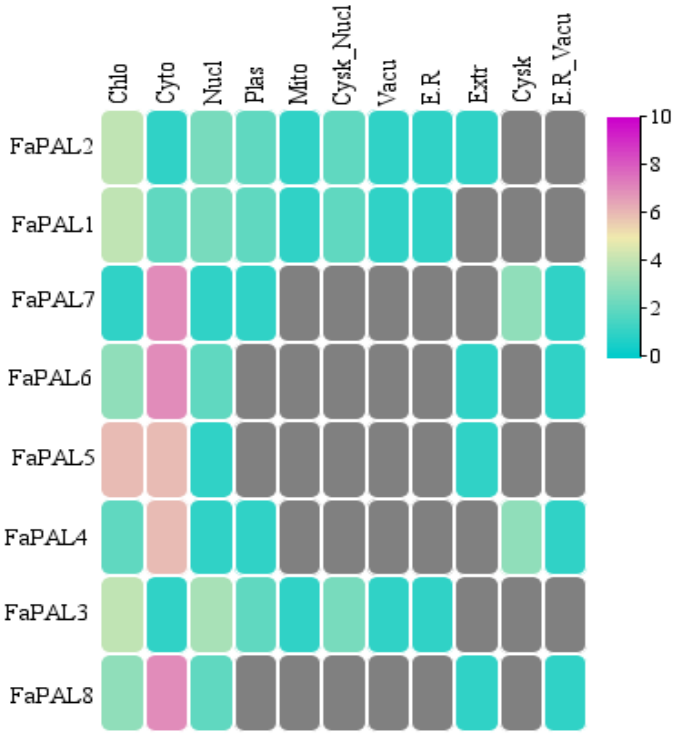

Fig. 1: Heat map displays 8 FaPAL genes distribution in plant cell components; violet indicates higher functional significance, revealing specific gene presence in cytoplasm, nucleus, chloroplast, mitochondria, endoplasmic reticulum, Extracellular, plasmid, cytoskeleton_nucleus, vacuole, cytoskeleton and endoplasmic reticulum_vacuole.

Phylogenetic analyses of FaPALs

To understand the evolutionary relationships of these 8 FaPAL proteins, a maximum likelihood phylogenetic tree was built based on all PAL proteins in strawberries, wheat, rice, Arabidopsis, and cucumber. The phylogenetic tree showed that all PAL proteins were divided into four Groups (Group I, II, III, and IV) (Fig. 2), where FaPALs belonged to the group II having significant similarities with PAL of Arabidopsis and cucumber. Overall, Group I contained the largest number of PALs (42), and Group IV had the lowest number of PALs (1).

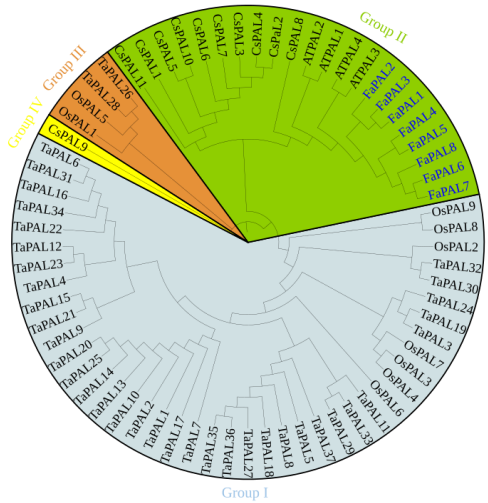

Fig. 2: The phylogenetic analysis of 69 PAL genes from five plant species, *F. ananassa*, *O. sativa*, *C. sativus*, *A. thaliana*, and *T. aestivum,* was systematically grouped into three distinct clades: clade I (blue), clade II (green), clade III (orange), and clade IV (yellow).

Chromosomal distribution and syntenic relationships of PAL genes in strawberry

The identified PAL genes in *Fragaria × ananassa* were mapped onto multiple chromosomes, revealing their specific chromosomal locations and syntenic relationships. As shown in Fig. 3, the PAL genes (designated as FaPAL1, FaPAL2, FaPAL3, FaPAL4, FaPAL5, FaPAL6, FaPAL7 & FaPAL8) are distributed across seven distinct chromosomes (Fvb6-1, Fvb6-2, Fvb6-4, Fvb7-1, Fvb7-2, Fvb7-3, and Fvb7-4). Chromosomes are represented as vertical green bars, while blue lines indicate syntenic relationships between the PAL genes. Notably, significant gene duplication events are observed, particularly among the PAL genes located on Fvb6-1, Fvb6-2, and Fvb6-4, as multiple interconnections are evident between these loci. These tandem and segmental duplications may have contributed to the expansion of the PAL gene family in strawberry. In addition, synteny analysis revealed cross-chromosomal relationships, where PAL genes on Fvb7 chromosomes (Fvb7-1, Fvb7-2, Fvb7-3, and Fvb7-4) share conserved regions with those on Fvb6. This complex network of syntenic connections highlights the evolutionary conservation and potential functional redundancy of PAL genes in *Fragaria × ananassa*.

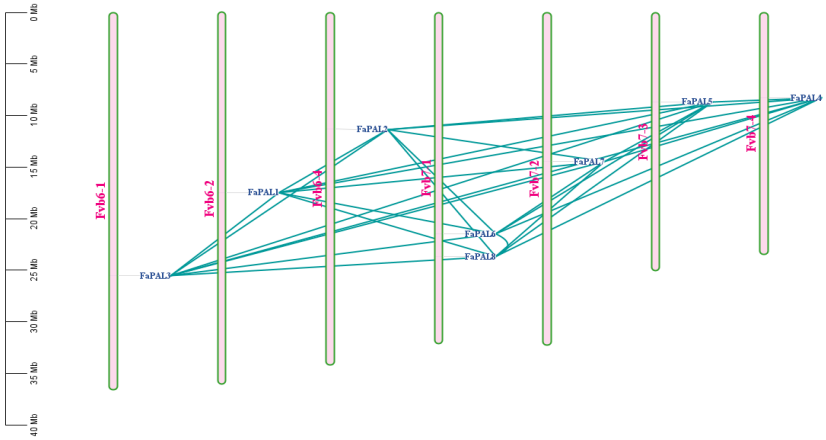

Fig. 3: Synteny and chromosomal localization of FaPAL genes across multiple chromosomes. Green vertical bars represent chromosomes, and the blue lines indicate syntenic relationships between FaPAL genes. Genes are labeled in blue (FaPAL) and chromosome names or markers in pink (e.g., Fvb6-1, Fvb6-2, etc.). The scale on the left indicates chromosomal length in megabases (Mb).

Analysis of gene structure, protein motifs, and sequence conservation for FaPALs

The gene structure and conserved motif distribution of the PAL gene family in *Fragaria × ananassa* were analyzed to understand their diversity and functional evolution. A total of eight PAL genes (FaPAL1 to FaPAL8) were identified, and their structural organization and conserved domains were visualized (Fig. 4). Ten conserved motifs were identified across the FaPAL proteins (Motif 1–10), indicating their functional conservation. The arrangement and composition of these motifs were highly conserved among the FaPALs, with all eight genes sharing motifs 1, 4, 6, and 8. This indicates their involvement in core enzymatic activities. Motifs 2, 3, 5, 7, 9, and 10 were consistently observed in most FaPALs, highlighting their importance in maintaining structural stability and facilitating catalytic function. FaPAL genes revealed relatively simple structures, with all genes possessing uninterrupted coding sequences (CDS) flanked by untranslated regions (UTRs). FaPAL2 and FaPAL5 exhibited slightly extended UTRs compared to other FaPALs. These differences in UTR length may play a role in gene expression regulation. The phylogenetic relationships among the FaPAL genes clustered them into closely related subgroups, with FaPAL6 and FaPAL8 forming a distinct clade. This suggests potential functional divergence or neofunctionalization events among the PAL family members during evolution. All FaPAL genes contained canonical PAL domains, further confirming their identity as bona fide members of the phenylalanine ammonia-lyase family.

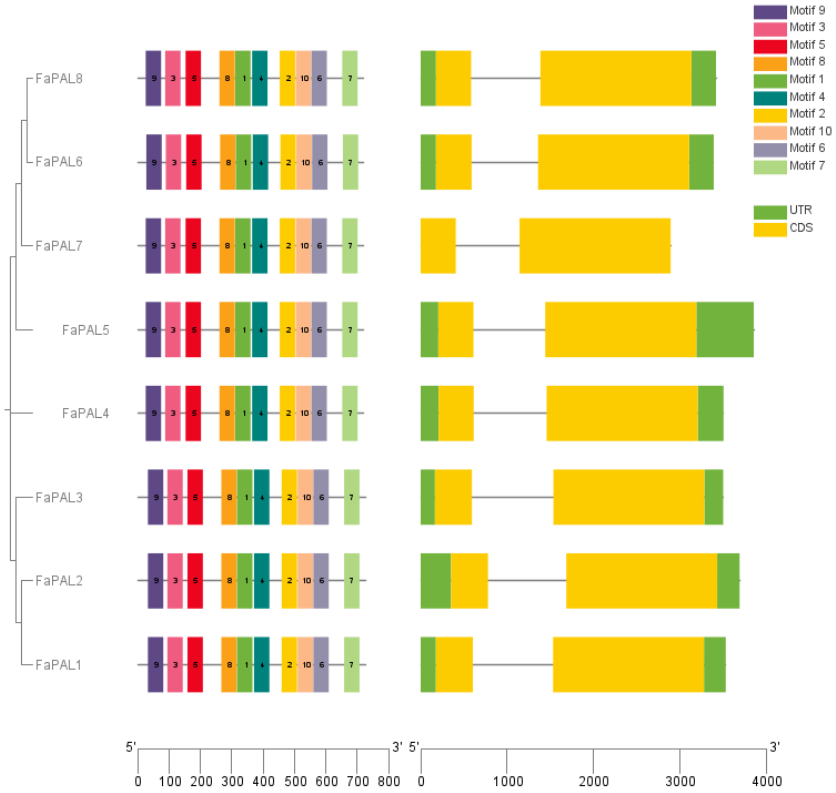

Fig. 4: The motifs' distribution across the 8 FaPAL protein family members. The FaPAL proteins reveal consistent presence of specific motifs across the protein set, highlighting distinct patterns in domain and superfamily structures.

Cis elements analysis

The genome-wide analysis of PAL genes in *Fragaria × ananassa* identified eight members (*FaPAL1–FaPAL8*), which clustered into distinct phylogenetic groups, suggesting evolutionary diversification. Cis-regulatory element analysis revealed the presence of multiple elements associated with abiotic stress responses, including light responsiveness, anaerobic induction, abscisic acid responsiveness, and defense/stress signals. Notably, light-responsive elements were the most abundant across all PAL gene promoters, emphasizing their role in light-mediated regulation. Elements linked to hormonal regulation, such as MeJA-responsiveness, auxin-responsive, and gibberellin-responsiveness, were also detected, highlighting the involvement of *FaPAL* genes in hormone signaling pathways. Moreover, the presence of drought-inducibility, low-temperature responsiveness, and salicylic acid-

responsive elements underscores the potential roles of PAL genes in abiotic stress adaptation. Seed-specific regulation and meristem expression elements suggest additional developmental functions of PAL genes (Fig. 5).

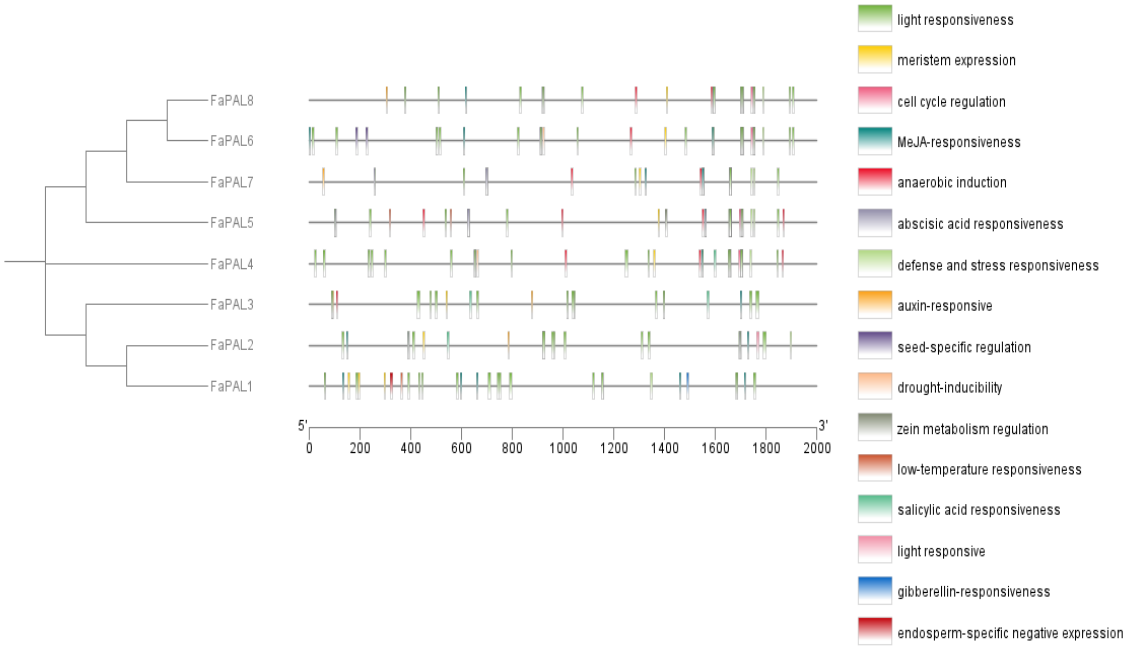

Fig. 5: Cis-element analysis on the promoter region of the FaPAL genes. The potential cis-regulatory elements in the 2000 bp promoter regions were predicted. Different colors indicated the elements related to different functional categories.

MicroRNA analysis

We identified eleven putative miRNAs targeting eight *FaPAL* genes to construct an interaction network using Cytoscape software, aiming to better understand the regulatory mechanisms of miRNAs involved in *PAL* regulation. In the connection distribution and regulatory network, *FaPAL3*, *FaPAL5*, *Fan-miR482h*, *Fan-miR482e*, and *Fan-miR482d* exhibited the highest degree, with a score of seven and a degree percentage of 38.9%, which was statistically significant.

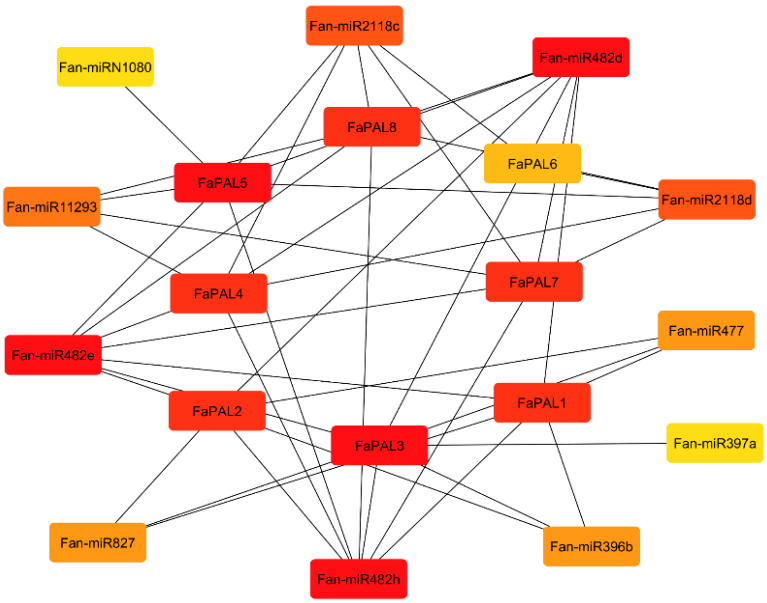

Fig. 6: Regulatory network relationship between the miRNA and their targeted FaPAL genes. Dark red color indicates the highest interconnection among genes and miRNAs.

Discussion

Phenylalanine ammonia-lyase (PAL) plays a crucial role in secondary metabolism in plants, especially in strawberries (*Fragaria × ananassa*), as demonstrated by its functional characterization and genome-wide research. PAL catalyzes the first step of the phenylpropanoid pathway, producing metabolites such as lignins, flavonoids, and phenolics, which play crucial roles in structural integrity, antioxidative defense, and pathogen resistance. These metabolites are essential for plant growth, development, and stress tolerance (Siebeneichler et al. 2024, Xu et al. 2024). The study identified eight PAL genes (FaPAL1–FaPAL8) in strawberries, highlighting their chromosomal distribution, gene duplication events, and evolutionary relationships with PAL genes in other plant species like *Arabidopsis thaliana, Triticum aestivum, Oryza sativa,* and *Cucucis sativus* (Rawal et al. 2013, Amjad et al. 2024). These duplications, both tandem and segmental, have contributed to the diversification of PAL functions, enabling their adaptation to different environmental stressors (Zhan et al. 2022). Phylogenetic analysis positioned the strawberry PAL genes into evolutionary clusters alongside those from other plant species, indicating both functional conservation and divergence. The conservation of key motifs in the PAL proteins, such as motifs 1, 4, 6, and 8, highlights their central enzymatic roles in phenylpropanoid metabolism, while structural variations, including differences in untranslated regions (UTRs), suggest potential regulatory diversity and functional specialization.

Promoter analysis of FaPAL genes revealed various cis-regulatory elements associated with abiotic stress responses, including light responsiveness, hormone regulation, and drought and temperature stress. This emphasizes PAL's crucial role in environmental adaptation through the phenylpropanoid pathway. Light-responsive elements were especially abundant, indicating a key role in light-mediated regulation of secondary metabolism. Hormone-related elements, such as those responsive to abscisic acid, salicylic acid, and jasmonic acid, further highlight PAL's involvement in hormone signaling networks during stress responses. These findings support previous studies that showed PAL's role in structural reinforcement and antioxidative defense under stress conditions (Hong et al. 2024). PAL's ability to mediate the synthesis of phenolic compounds, including anthocyanins, is particularly important in strawberries, as these compounds enhance fruit quality by enhancing attributes like color, flavor, and nutritional value. This not only increases the economic value of strawberries but also underscores the significance of PAL in strengthening crop resilience to environmental stressors.

MicroRNA analysis added a layer of regulatory complexity to the understanding of PAL gene expression. Eleven miRNAs targeting FaPAL genes were found in the study; the highest degree of interaction (38.9%) was found for FaPAL3, FaPAL5, Fan-miR482h, Fan-miR482e, and Fan-miR482d, with a score of 7. This suggests that these genes are important regulatory hubs in the network of miRNA–mRNA interactions. This implies that PAL activity is significantly modulated by miRNA-mediated post-transcriptional regulation, particularly in the presence of abiotic stress. The regulatory interactions may provide fine-tuned control over phenylpropanoid metabolism, allowing the plant to dynamically respond to environmental cues. This is consistent with findings in other plants, such as rice and cucumber, where miRNA regulation of PAL genes has been implicated in stress tolerance and metabolic adjustments (Yu et al. 2018, Amjad et al. 2024).

The chromosomal mapping of FaPAL genes revealed their distribution across multiple chromosomes, specifically on Fvb6 and Fvb7. Significant syntenic relationships and duplication events were observed among these loci, highlighting the evolutionary conservation and expansion of the PAL gene family in strawberries. These duplication events may contribute to functional redundancy and specialization, allowing different PAL genes to perform distinct roles under varying conditions. For instance, specific PAL genes might be more active in structural reinforcement during stress, while others focus on flavonoid production for antioxidative defense. The synteny analysis also identified cross-chromosomal relationships, indicating a complex evolutionary history and potential functional interplay between different PAL genes.

The study's integrative approach, combining structural analysis, phylogenetics, cis-regulatory elements, and miRNA interactions, provides a comprehensive understanding of PAL's roles in strawberry biology. While PAL genes have been extensively studied in model plants like *Arabidopsis*, their characterization and identification in strawberries fills a critical research gap. Strawberries, being economically significant and highly sensitive to abiotic stresses like drought, salinity, and temperature extremes, present a compelling case for studying PAL-mediated stress responses. By regulating phenylpropanoid metabolism, PAL genes enhance the plant's structural integrity, antioxidative defenses, and overall resilience, ensuring better growth, development, and fruit quality under adverse

conditions. Moreover, the phenolic compounds produced through PAL activity, such as anthocyanins and lignins, not only protect the plant but also contribute to the nutritional and medicinal value of strawberries (Gho et al. 2020, Chen et al. 2022, Ullah et al. 2024).

Despite its contributions, the study leaves several avenues for future research. The regulatory pathways influencing PAL expression under stress, particularly the roles of cis-elements and miRNAs, require further exploration. Additionally, the functional characterization of individual PAL genes in specific stress scenarios would provide deeper insights into their roles. For instance, understanding how PAL genes interact with hormonal pathways during drought or salinity stress could aid in developing targeted genetic interventions. Integrating these findings into breeding programs or genetic engineering strategies could improve strawberry resilience and productivity, aligning with global agricultural priorities to enhance crop performance in the face of climate change. This research thus lays a strong foundation for future efforts to harness the potential of PAL genes in improving stress tolerance and fruit quality in strawberries, contributing to sustainable agriculture and food security.

By building on previous research on PAL in plants (Dong and Shang 2013, Cass et al. 2015) and leveraging advanced genomic tools, this study advances our understanding of how PAL genes function in a crop of significant economic and nutritional importance. The findings not only offer insights into the genetic and functional dynamics of the PAL gene family but also provide practical implications for improving strawberry cultivation through molecular breeding and biotechnological approaches.

Our study provides a comprehensive genome-wide analysis of the PAL genes found in strawberries. We found eight PAL genes (FaPAL1-FaPAL8) and characterized them, allowing us to demonstrate their evolutionary relationship, conserved catalytic properties, and regulatory function in the metabolism of phenylpropanoid in strawberry. The presence of cis-regulatory elements that are influenced by stress and hormones. FaPAL3 and FaPAL5 genes show the highest degree of interaction with microRNAs, with a statistically significant percentage of 38.9%, highlighting their role in stress adaptation and improving fruit quality. The findings provide vital molecular information for creating better, stress-resistant strawberry cultivars in the future through the use of genetic improvement techniques.

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Author contributions: MAH conceived and designed the study, supervised the research, and finalized the manuscript. MRP conducted all bioinformatics analyses and prepared the initial manuscript draft. MFA, SA, and SRM contributed to data analysis and interpretation of the results. All authors reviewed, revised, and approved the final version of the manuscript.

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Data availability: All data generated in the study are reported in the manuscript, and unprocessed data will be available to the corresponding author upon request.

References

Achnine L, Blancaflor EB, Rasmussen S and Dixon RA (2004). Colocalization of L-phenylalanine ammonia-lyase and cinnamate 4-hydroxylase for metabolic channeling in phenylpropanoid biosynthesis. The Plant Cell 16(11): 3098–3109. https://doi.org/10.1105/tpc.104.024406

Amjad M, Wang Y, Han S, Haider MZ, Sami A, Batool A, Shafiq M, Ali Q, Dong J, Sabir IA and Manzoor MA (2024). Genome wide identification of phenylalanine ammonia-lyase (PAL) gene family in *Cucumis sativus* (cucumber) against abiotic stress. BMC Genomic Data 25(1): 76. https://doi.org/10.1186/s12863-024-01259-1

Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, Ren J, Li WW and Noble WS (2009). MEME SUITE: Tools for motif discovery and searching. Nucleic Acids Research 37(Web Server): W202–W208. https://doi.org/10.1093/nar/gkp335

Bateman A (2004). The Pfam protein families database. Nucleic Acids Research 32(Database issue): D138–D141. https://doi.org/10.1093/nar/gkh121

Bjellqvist B, Basse B, Olsen E and Celis JE (1994). Reference points for comparisons of two-dimensional maps of proteins from different human cell types defined in a pH scale where isoelectric points correlate with polypeptide compositions. Electrophoresis 15(1): 529–539. https://doi.org/10.1002/elps.1150150171

Bjellqvist B, Hughes GJ, Pasquali C, Paquet N, Ravier F, Sanchez JC, Frutiger S and Hochstrasser D (1993). The focusing positions of polypeptides in immobilized pH gradients can be predicted from their amino acid sequences. Electrophoresis 14(1): 1023–1031. https://doi.org/10.1002/elps.11501401163

Bülow L and Hehl R (2016). Bioinformatic identification of conserved cis-sequences in coregulated genes. Methods in Molecular Biology 1482: 233-245

Cass CL, Peraldi A, Dowd PF, Mottiar Y, Santoro N, Karlen SD, Bukhman YV, Foster CE, Thrower N, Bruno LC, Moskvin OV, Johnson ET, Willhoit ME, Phutane M, Ralph J, Mansfield SD, Nicholson P and Sedbrook JC (2015). Effects of phenylalanine ammonia lyase (PAL) knockdown on cell wall composition, biomass digestibility, and biotic and abiotic stress responses in Brachypodium. Journal of Experimental Botany 66(14): 4317–4335. https://doi.org/10.1093/jxb/erv269

Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He Y and Xia R (2020). TBtools: An integrative toolkit developed for interactive analyses of big biological data. Molecular Plant 13(8): 1194–1202. https://doi.org/10.1016/j.molp.2020.06.009

Chen X, Wang P, Gu M, Hou B, Zhang C, Zheng Y, Sun Y, Jin S and Ye N (2022). Identification of PAL genes related to anthocyanin synthesis in tea plants and its correlation with anthocyanin content. Horticultural Plant Journal 8(3): 381–394. https://doi.org/10.1016/j.hpj.2021.12.005

Dai X and Zhao PX (2011). psRNATarget: A plant small RNA target analysis server. Nucleic Acids Research 46 (W1): W49–W54. https://doi.org/10.1093/nar/gky316

Dixon RA, Achnine L, Kota P, Liu CJ, Reddy MSS and Wang L (2002). The phenylpropanoid pathway and plant defense a genomics perspective. Molecular Plant Pathology 3(5): 371–390. https://doi.org/10.1046/j.1364-3703.2002.00131.x

Dong CJ and Shang QM (2013). Genome-wide characterization of phenylalanine ammonia-lyase gene family in watermelon (*Citrullus lanatus*). Planta 238(1): 35–49. https://doi.org/10.1007/s00425-013-1869-1

Edgar RC (2021). High-accuracy alignment ensembles enable unbiased assessments of sequence homology and phylogeny. https://doi.org/10.1101/2021.06.20.449169

Gascuel O (2006). Neighbor-joining revealed. Molecular Biology and Evolution 23(11): 1997–2000. https://doi.org/10.1093/molbev/msl072

Gho YS, Kim SJ and Jung KH (2020). Phenylalanine ammonia-lyase family is closely associated with response to phosphate deficiency in rice. Genes and Genomics 42(1): 67–76. https://doi.org/10.1007/s13258-019-00879-7

Goodstein DM, Shu S, Howson R, Neupane R, Hayes RD, Fazo J, Mitros T, Dirks W, Hellsten U, Putnam N and Rokhsar DS (2012). Phytozome: A comparative platform for green plant genomics. Nucleic Acids Research 40(D1): D1178–D1186. https://doi.org/10.1093/nar/gkr944

Guo Z, Kuang Z, Wang Y, Zhao Y, Tao Y, Cheng C, Yang J, Lu X, Hao C, Wang T, Cao X, Wei J, Li L and Yang X (2020). PmiREN: A comprehensive encyclopedia of plant miRNAs. Nucleic Acids Research 48(D1): D1114–D1121. https://doi.org/10.1093/nar/gkz894

Hamilton JP, Li C and Buell CR (2025). The rice genome annotation project: An updated database for mining the rice genome. Nucleic Acids Research 53(D1): D1614–D1622. https://doi.org/10.1093/nar/gkae1061.

Hong J, Meng K, Thomas HR, Yang Y, Williams B, Kang H and Zhou Y (2024). Reframing agriculture by light: The role of light-mediated jasmonates/salicylic acid regulation in plant defense, development and beyond. Vegetable Research 4(1). https://doi.org/10.48130/vegres-0024-0026

Horton P, Park KJ, Obayashi T, Fujita N, Harada H, Adams-Collier CJ and Nakai K (2007). WoLF PSORT: Protein localization predictor. Nucleic Acids Research 35(Web Server): W585–W587. https://doi.org/10.1093/nar/gkm259

Hu B, Jin J, Guo AY, Zhang H, Luo J and Gao G (2015). GSDS 2.0: An upgraded gene feature visualization server. Bioinformatics 31(8): 1296–1297. https://doi.org/10.1093/bioinformatics/btu817

Lamesch P, Berardini TZ, Li D, Swarbreck D, Wilks C, Sasidharan R, Muller R, Dreher K, Alexander DL, Garcia-Hernandez M, Karthikeyan AS, Lee CH, Nelson WD, Ploetz L, Singh S, Wensel A and Huala E (2012). The

Arabidopsis information resource (TAIR): Improved gene annotation and new tools. Nucleic Acids Research 40(D1): D1202–D1210. https://doi.org/10.1093/nar/gkr1090

Lescot M (2002). PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for *in silico* analysis of promoter sequences. Nucleic Acids Research 30(1): 325–327. https://doi.org/10.1093/nar/30.1.325

Letunic I and Bork P (2024). Interactive Tree of Life (iTOL) v6: Recent updates to the phylogenetic tree display and annotation tool. Nucleic Acids Research 52(W1): W78–W82. https://doi.org/10.1093/nar/gkae268

Mahram A and Herbordt MC (2015). NCBI BLASTP on high-performance reconfigurable computing systems. ACM Transactions on Reconfigurable Technology and Systems 7(4): 1–20. https://doi.org/10.1145/2629691

Ninkuu V, Aluko OO, Yan J, Zeng H, Liu G, Zhao J, Li H, Chen S and Dakora FD (2025). Phenylpropanoids metabolism: Recent insight into stress tolerance and plant development cues. Frontiers in Plant Science 16: 1571825. https://doi.org/10.3389/fpls.2025.1571825

Olsen KM, Lea US, Slimestad R, Verheul M and Lillo C (2008). Differential expression of four *Arabidopsis* PAL genes; PAL1 and PAL2 have functional specialization in abiotic environmental-triggered flavonoid synthesis. Journal of Plant Physiology 165(14): 1491–1499. https://doi.org/10.1016/j.jplph.2007.11.005

Rasmussen S, Barah P, Suarez-Rodriguez MC, Bressendorff S, Friis P, Costantino P, Bones AM, Nielsen HB and Mundy J (2013). Transcriptome responses to combinations of stresses in *Arabidopsis*. Plant Physiology 161(4): 1783–1794. https://doi.org/10.1104/pp.112.210773

Rawal HC, Singh NK and Sharma TR (2013). Conservation, divergence, and genome-wide distribution of PAL and POX A gene families in plants. International Journal of Genomics 2013: 1–10. https://doi.org/10.1155/2013/678969

Schultz J (2000). SMART: A web-based tool for the study of genetically mobile domains. Nucleic Acids Research 28(1): 231–234. https://doi.org/10.1093/nar/28.1.231

Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B and Ideker T (2003). Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Research 13(11): 2498–2504. https://doi.org/10.1101/gr.1239303

Siebeneichler TJ, Crizel RL, Rombaldi CV and Galli V (2024). Regulation of phenylpropanoid biosynthesis in strawberry ripening: Molecular and hormonal mechanisms. Phytochemistry Reviews 23(3): 923–941. https://doi.org/10.1007/s11101-023-09907-7

Tamura K, Stecher G and Kumar S (2021). MEGA11: Molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution 38(7): 3022–3027. https://doi.org/10.1093/molbev/msab120

Ullah I, Toor MD, Yerlikaya BA, Mohamed HI, Yerlikaya S, Basit A and Rehman AU (2024). High-temperature stress in strawberry: Understanding physiological, biochemical and molecular responses. Planta 260(5): 118. https://doi.org/10.1007/s00425-024-04544-6

Vogt T (2010). Phenylpropanoid biosynthesis. Molecular Plant 3(1): 2–20. https://doi.org/10.1093/mp/ssp106

Weisshaar B and Jenkins GI (1998). Phenylpropanoid biosynthesis and its regulation. Current Opinion in Plant Biology 1(3): 251–257. https://doi.org/10.1016/S1369-5266(98)80113-1

Wilkins MR, Gasteiger E, Bairoch A, Sanchez JC, Williams KL, Appel RD and Hochstrasser DF (1998). Protein identification and analysis tools in the ExPASy server. 2-D Proteome Analysis Protocols. 531–552.

Xu C, Fan X, Shen G and Guo B (2024). Genome-wide identification of the phenylalanine ammonia-lyase gene from *Epimedium pubescens* Maxim. (Berberidaceae): Novel insight into the evolution of the PAL gene family. BMC Plant Biology 24(1): 831. https://doi.org/10.1186/s12870-024-05480-z

Yu XZ, Fan WJ, Lin YJ, Zhang FF and Gupta DK (2018). Differential expression of the PAL gene family in rice seedlings exposed to chromium by microarray analysis. Ecotoxicology 27(3): 325-335. https://doi.org/10.1007/s10646-018-1897-5

Zhan C, Li Y, Li H, Wang M, Gong S, Ma D and Li Y (2022). Phylogenomic analysis of phenylalanine ammonia-lyase (PAL) multigene family and their differential expression analysis in wheat (*Triticum aestivum* L.) suggested their roles during different stress responses. Frontiers in Plant Science 13: 982457. https://doi.org/10.3389/fpls.2022.982457.

Zhang C, Guo H, Li Z and Yue S (2025). Evolution of phenylalanine ammonia-lyase protein family from algae to angiosperm. Functional and Integrative Genomics 25(1): 40. https://doi.org/10.1007/s10142-025-01548-7.