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Transcriptomic insights into ABA- and ethephon-mediated regulation of flavonoid biosynthesis during pericarp coloration in *Litchi chinensis* cv. Deshi

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ABSTRACT

Coloration of the pericarp in *Litchi chinensis* Sonn. is a major factor in determining the economic value of litchi fruits, and this is primarily mediated by anthocyanin accumulation. The application of plant hormones is a common practice for inducing anthocyanin accumulation in fruits. The mechanism by which hormone signaling is involved in flavonoid biosynthesis in litchi fruits is not very clear. The present study aimed to investigate the physiological, biochemical, and transcriptional responses in litchi fruits cv. Deshi to abscisic acid (ABA), ethephon (ETH), and their combined application in the field.

Application of both ABA and ETH resulted in significant increases in pericarp redness and anthocyanin accumulation in litchi fruits compared to the control, and the promotive effects of ABA were more significant. The high-quality transcriptomes generated in this study using RNA sequencing technology provided comprehensive information for analyzing differentially expressed genes (DEGs), functional

annotations, and pathway enrichments in response to ABA and ETH application in litchi fruits. Transcriptomic analysis revealed that ABA-induced coloration is primarily driven by the early-stage phenylpropanoid pathway (e.g., LcHCT, LcCYP98A), whereas Ethephon significantly upregulates LcCYP75B1 and LcANR. Notably, individual applications of ABA or Ethephon were more effective than the combined treatment, suggesting potential hormonal antagonism at the transcriptional level. These results provide a molecular basis for improving 'Deshi' litchi marketability through targeted hormonal regulation.

The major structural genes involved in anthocyanin biosynthesis, such as PAL, CHS, F3H, DFR, and UFGT, showed significant upregulation in response to ABA application. At the same time, the genes involved in ABA signaling, such as those in the PYR/PYL-PP2C-SnRK2 pathway, also showed transcriptional activation in response to ABA application. The transcription factors involved in anthocyanin biosynthesis, such as MYB, bHLH, and ERF, also showed differential regulation in response to ABA application, suggesting a hormone-dependent transcriptional regulation mechanism in anthocyanin biosynthesis in litchi fruits. Correlation analysis also showed a positive correlation between anthocyanin accumulation and structural gene expression in the anthocyanin biosynthetic pathway, suggesting transcriptional regulation as the major mechanism in anthocyanin biosynthesis in litchi fruits. The effects of ABA application in activating the flavonoid pathway in litchi fruits were more significant than those of ethylene, suggesting the major role of ABA in regulating anthocyanin biosynthesis in litchi fruits. Based on the results, a model is proposed for ABA signaling in anthocyanin biosynthesis in litchi fruits.

1. Introduction

Fruit coloration is a critical quality attribute influencing consumer preference, nutritional value, and market competitiveness in horticultural crops. In red-fruited species, pigmentation is primarily determined by the accumulation of anthocyanins, a class of flavonoid compounds synthesized through the phenylpropanoid pathway. Beyond their role in visual appeal, anthocyanins contribute to antioxidant capacity and confer protection against abiotic stress, underscoring their physiological and economic importance.

In Litchi chinensis Sonn., pericarp coloration is a key determinant of fruit marketability. The transition from green to red during ripening is tightly associated with chlorophyll degradation and anthocyanin accumulation. Previous studies have identified several structural genes involved in anthocyanin biosynthesis in litchi, including PAL, CHS, F3H, DFR, ANS, and UFGT, as well as regulatory MYB transcription factors implicated in pigment formation. However, despite advances in transcriptomic profiling, the upstream hormonal regulation and integrated transcriptional hierarchy governing anthocyanin accumulation in litchi remain insufficiently resolved, particularly under field conditions. The 'Deshi' cultivar is highly valued in Bihar and West Bengal for its regular bearing and early maturity, yet

its commercial potential is severely limited by poor, faint red pericarp coloration at maturity. While ABA is a known regulator of ripening in non-climacteric fruits, the role of Ethephon—often used to accelerate chlorophyll degradation—remains poorly understood in the context of specific flavonoid gene activation in 'Deshi' litchi. Resolving this integrated transcriptional hierarchy is essential for optimizing post-harvest quality.

Plant hormones are central regulators of fruit development and ripening. Abscisic acid (ABA) is widely recognized as a key regulator of coloration in non-climacteric fruits, whereas ethylene plays a dominant role in climacteric ripening. In litchi, both ABA and ethylene have been reported to influence pericarp pigmentation, yet their relative contributions and downstream transcriptional mechanisms are not fully elucidated. Importantly, the mechanistic linkage between hormone signaling components (e.g., PYR/PYL–PP2C–SnRK2 in ABA signaling or EIN–ERF modules in ethylene signaling) and activation of flavonoid structural genes has not been comprehensively characterized in this species.

High-throughput RNA sequencing (RNA-Seq) provides a powerful approach to dissect complex regulatory networks controlling secondary metabolism. Integrating transcriptomic data with physiological and biochemical measurements enables identification of key regulatory modules and candidate hub genes associated with metabolite accumulation. However, transcriptome-based analyses in litchi have largely focused on descriptive differential gene expression, with limited integration of hormone signaling pathways, transcription factor dynamics, and pigment accumulation under exogenous hormone treatments.

Therefore, the present study aimed to (i) evaluate the effects of exogenous abscisic acid (ABA) and ethephon (ETH) on pericarp coloration and anthocyanin accumulation in Litchi chinensis cv. Deshi, (ii) characterize global transcriptional reprogramming induced by these hormone treatments using RNA-Seq, and (iii) elucidate the regulatory relationship between hormone signaling pathways, transcription factor activity, and flavonoid biosynthetic gene expression. We hypothesized that ABA acts as a primary regulator of anthocyanin biosynthesis in litchi by activating hormone-responsive transcription factors that coordinately upregulate structural genes in the flavonoid pathway, whereas ethylene exerts a comparatively modulatory effect.

By integrating physiological, biochemical, and transcriptomic analyses, this study provides a mechanistic framework for understanding hormone-mediated regulation of pericarp pigmentation and offers molecular targets for improving fruit coloration through hormonal manipulation strategies.

2. Materials and Methods

2.1 Plant Material and Hormone Treatments

The experiment was conducted during the 2020–22 seasons at the litchi orchard of the Horticultural Garden, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India. The site is situated at 25°15'40" N latitude and 87°2'7" E longitude, with an elevation of 46 meters above mean sea level. The study utilized 25-year-old, fully mature, and uniformly healthy plants of the litchi cultivar 'Deshi'. Trees were planted at a spacing of 10.0 m × 10.0 m and maintained using standard recommended cultural practices.

For the experimental treatments, Abscisic acid (ABA) was applied at a concentration of 150 mg/L and ethephon (ETH) at 500 µL/L. These were administered as foliar sprays until runoff during early morning hours to ensure uniform absorption. Control plants were sprayed with distilled water. Pericarp samples were collected at the color-turning stage (0, 5, and 10 days post-treatment) for subsequent biochemical and transcriptomic analyses.

2.2 RNA Isolation and Library Preparation

Total RNA was extracted from litchi pericarp tissues using the Promega RNA Isolation Kit (Promega Corporation, USA) following the manufacturer's instructions. RNA concentration and purity were assessed spectrophotometrically using a NanoDrop instrument, and RNA integrity was verified prior to library preparation. Only high-quality RNA samples with suitable purity and integrity were used for transcriptome analysis.

For each treatment, equal quantities of RNA from biological replicates were used for complementary DNA (cDNA) library preparation according to the standard protocols recommended for Illumina sequencing platforms.

2.3 Transcriptome Sequencing and De novo Assembly

High-throughput RNA sequencing was performed using an Illumina sequencing platform, generating paired-end reads for all samples, generating paired-end reads for all samples. Raw reads were processed to remove adapter sequences, low-quality reads, and ambiguous nucleotides to obtain high-quality clean reads.

De novo transcriptome assembly was performed using the Trinity assembler, which generated a comprehensive set of transcripts representing the litchi pericarp transcriptome. The sequencing effort yielded approximately 10.10 million assembled bases, producing 83,362 transcripts and 40,825 predicted protein-coding genes. The assembly achieved a median contig length of 881 bp and an average contig length of 1212.24 bp.

2.4 Functional Annotation and Differential Expression Analysis

Functional annotation of the assembled transcripts was performed using the Gene Ontology (GO) database to classify genes into three functional categories: biological process, cellular component, and molecular function.

A total of 825,599 GO annotations were assigned across these categories. Differential gene expression analysis was conducted to identify genes significantly regulated by ABA and ethephon treatments, with particular focus on genes involved in flavonoid and phenylpropanoid biosynthesis pathways.

Table 1: Details of putative flavonoid biosynthesis pathway genes identified through NGS transcriptome analysis

Transcript ID	Gene	Symbol
TRINITY_DN10610_c1_g1	caffeoyl-CoA O-methyltransferase	LcCCoAOMT
TRINITY_DN1065_c0_g1	flavonoid 3'-monooxygenase	LcCYP75B1
TRINITY_DN14403_c0_g1	anthocyanidin reductase	LcANR
TRINITY_DN14947_c0_g1	5-O-(4-coumaroyl)-D-quinate 3'-monooxygenase	LcCYP98A
TRINITY_DN20256_c0_g1	shikimate O-hydroxycinnamoyltransferase	LcHCT
TRINITY_DN20474_c0_g1	caffeoyl-CoA O-methyltransferase	[EC:2.1.1.104]
TRINITY_DN214_c2_g2	caffeoyl-CoA O-methyltransferase	[EC:2.1.1.104]
TRINITY_DN2291_c0_g1	5-O-(4-coumaroyl)-D-quinate 3'-monooxygenase	CYP98A, C3'H [EC:1.14.14.96]
TRINITY_DN25102_c0_g1	Anthocyanidin reductase	ANR [EC:1.3.1.77]
TRINITY_DN34798_c0_g1	caffeoyl-CoA O-methyltransferase	[EC:2.1.1.104]
TRINITY_DN35184_c0_g1	CYP75B1; flavonoid 3'-monooxygenase [EC:1.14.14.82]	CYP75B1
TRINITY_DN42_c0_g1	chalcone isomerase	[EC:5.5.1.6]
TRINITY_DN4500_c0_g1	caffeoyl-CoA O-methyltransferase	[EC:2.1.1.104]
TRINITY_DN5475_c0_g1	Anthocyanidin reductase	ANR [EC:1.3.1.77]

TRINITY_DN6136_c0_g1	shikimate O-hydroxycinnamoyltransferase	HCT [EC:2.3.1.133]
TRINITY_DN7294_c1_g1	caffeoyl-CoA O-methyltransferase	[EC:2.1.1.104]
TRINITY_DN8946_c0_g1	anthocyanidin reductase	ANR [EC:1.3.1.77]
TRINITY_DN9236_c0_g1	dihydroflavonol 4-reductase/flavanone 4-reductase	DFR [EC:1.1.1.219 1.1.1.234]

2.5 qRT-PCR Validation

To validate the transcriptomic data, quantitative real-time PCR (qRT-PCR) was performed on selected structural genes, including Lc. HCT, Lc. CYP98A, Lc. CYP75B1, and Lc. ANR. First-strand cDNA was synthesized from total RNA, and amplification was carried out using SYBR Green chemistry in a real-time PCR detection system. Lc. Actin was employed as the internal reference gene for normalization. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

Table 2 Details of primers used for real time PCR analysis

Gene	Forward Primers	Reverse Primers
Lc, ANR	CACAAGAAGGTCTCCCACCT	CCTTGGATTGCCGGTTTGAT
Lc,HCT	TAGCAACAGATGGACGAGCA	GCTGAAGCTCGAGGTAGTCT
Lc,CYP75B1	GGTGCGAAACACTTGGCTTA	CATTTGCGTCAGCAAACACC
Lc, CYP98A	TCACCGTGCCAATGCTAATG	CTTCTTCCTGCACCGAATGG
Lc. Actin	AATGGAACTGGAATGGTCAAG	TGCCAGATCTTCTCCATGTCAT

3. Results:

3.1. Exogenous ABA and Ethephon Enhance Pericarp Coloration and Anthocyanin Accumulation

Exogenous application of abscisic acid (ABA), ethephon (ETH), and their combination significantly influenced pericarp coloration in Litchi chinensis cv. Deshi under field conditions. Treated fruits exhibited enhanced red pigmentation compared to control, with ABA treatment producing the most pronounced coloration.

Quantitative analysis revealed a significant increase in total anthocyanin content in hormone-treated fruits, particularly under ABA application. Chlorophyll content decreased concurrently, indicating coordinated pigment transition during ripening. Fruit quality parameters including total soluble solids and sugar content also showed treatment-dependent variation.

These results demonstrate that ABA is a stronger inducer of pericarp pigmentation than ethylene under the tested conditions.

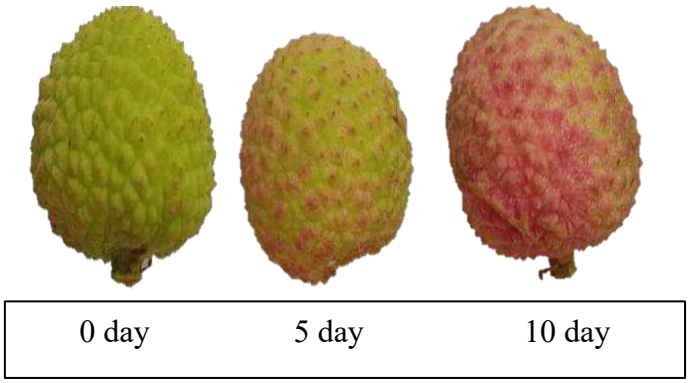


Fig. 1 Stages of fruit colour development used for NGS transcriptomic analysis

3.2. Transcriptome Sequencing and Global Gene Expression Profiling

RNA-Seq generated high-quality clean reads for all treatments, with high mapping efficiency and satisfactory sequencing depth, indicating robust dataset quality. Principal component analysis (PCA) revealed clear separation between control and hormone-treated samples, confirming distinct transcriptional reprogramming induced by ABA and ETH.

Differential expression analysis identified a substantial number of DEGs in response to hormone treatments. ABA treatment induced a higher number of differentially expressed genes compared to ETH, suggesting broader transcriptional influence.

Functional annotation and Gene Ontology (GO) enrichment analysis indicated significant overrepresentation of genes associated with secondary metabolite biosynthesis, oxidation–reduction processes, and hormone signal transduction. KEGG pathway analysis further revealed strong enrichment in phenylpropanoid and flavonoid biosynthesis pathways.

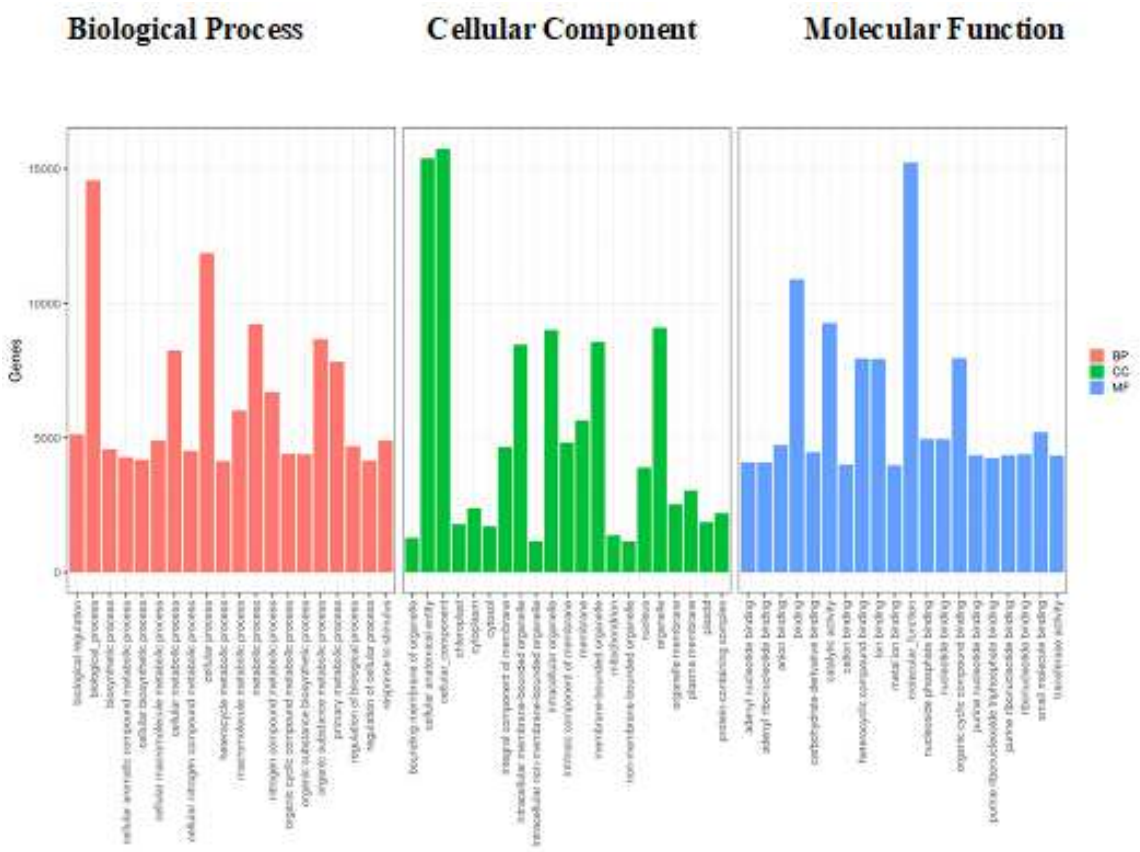


Fig. 2 Histogram of gene ontology classifications for litchi pericarp transcripts. The unigenes corresponded to three main categories: 'biological process', 'cellular component' and 'molecular function'. Functional enrichment confirms that the metabolic shift during color break is dominated by catalytic activity (9,269 unigenes) and binding (10,893 unigenes), correlating with the rapid synthesis of anthocyanin pigments.

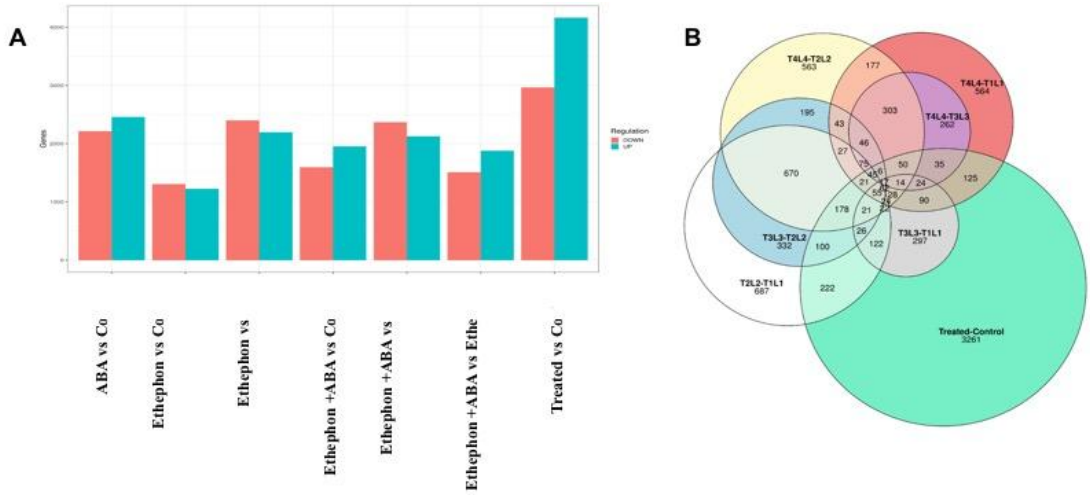


Fig. 3 Differential gene expression profiles based on the library of the seven treatment and control (A) The numbers of up- and down-regulated genes in different treated and control samples. (B) Venn diagram showing the comparison of differentially expressed genes between any two stages of the litchi pericarp. T_2L_2-T_1L_1:-ABA vs Control, T_3L_3-T_2L_2:- Ethephon vs ABA, T_4L_4-T_2L_2:- Ethephon +ABA vs ABA, T_4L_4-T_1L_1:- Ethephon +ABA vs Control, T_4L_4-T_3L_3:- Ethephon +ABA vs Ethephon, T_3L_3-T_1L_1:- Ethephon vs Control, Treated vs Control.

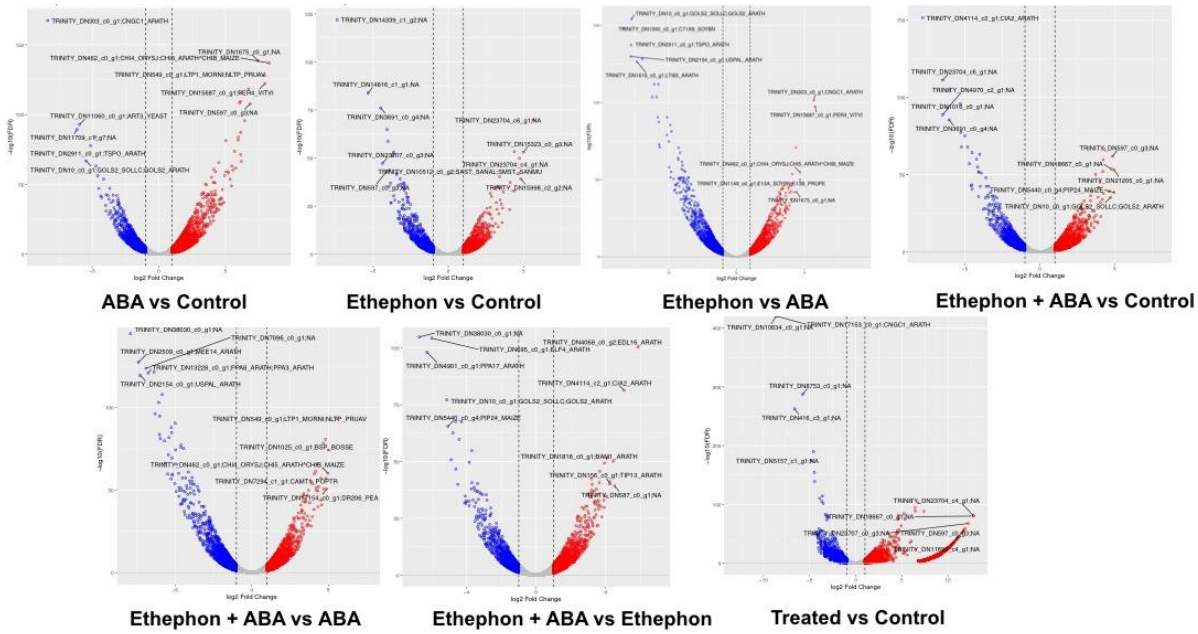


Fig. 4 Volcano plot for differentially expressed genes identified by NGS

3.3. Hormone Treatments Activate Flavonoid and Phenylpropanoid Biosynthesis Pathways

Detailed pathway analysis demonstrated significant upregulation of key structural genes involved in anthocyanin biosynthesis following ABA treatment. Early biosynthetic genes including PAL, C4H, and CHS, as well as late biosynthetic genes such as F3H, DFR, ANS, and UFGT, exhibited marked transcriptional induction.

Comparative expression profiling indicated that ABA induced stronger activation of late biosynthetic genes compared to ETH, consistent with higher anthocyanin accumulation observed physiologically.

These findings suggest that ABA promotes pericarp coloration primarily through transcriptional activation of the flavonoid biosynthetic pathway.

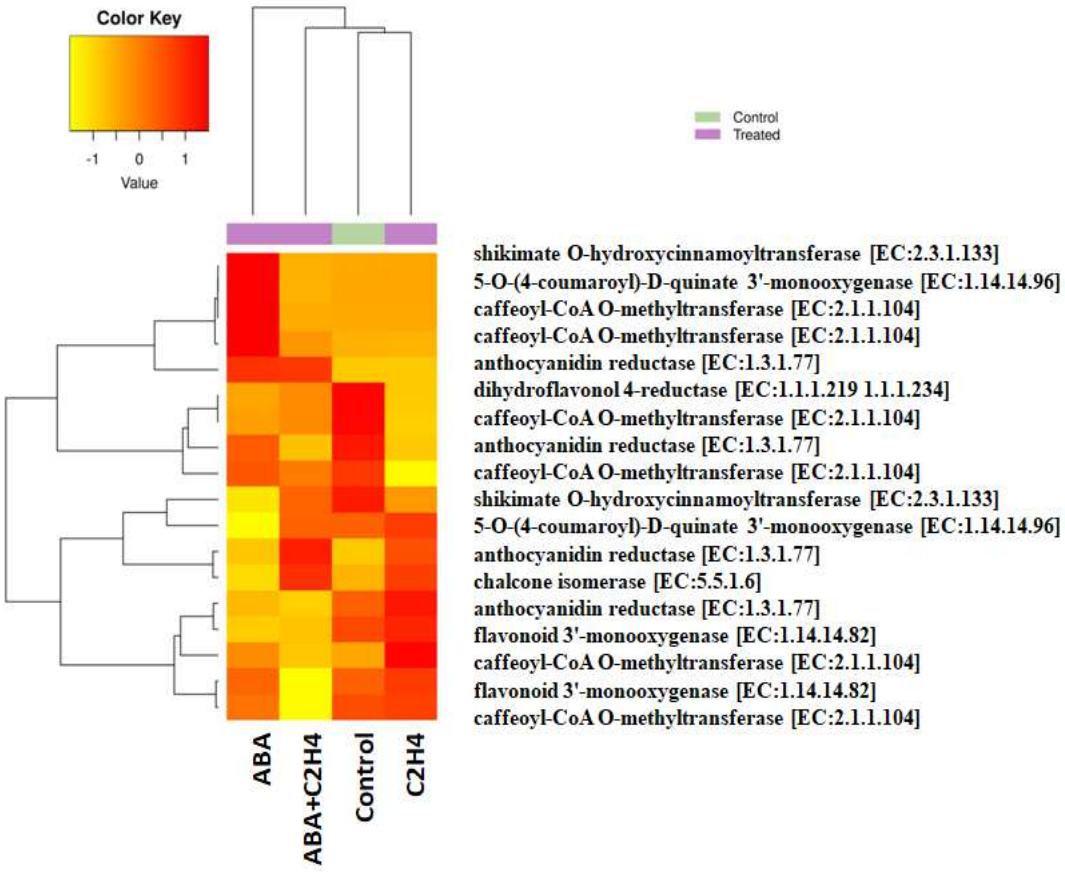


Fig. 5 Heat map diagram of relative gene expression levels of DEGs related to flavonoid biosynthesis pathways in litchi pericarp after exogenous ABA, ABA+Ethephon,

Control and Ethephon treatment. Red colour indicates a relative increase in expression, and yellow colour represents a relative decrease in expression.

3.4. Hormone Signaling Components and Transcription Factors Are Differentially Regulated

The exogenous application of 150 mg/L ABA and 500 μL/L ethephon induced distinct transcriptional shifts in hormone perception and signal transduction pathways. Genes associated with ABA signal transduction, including members of the PYR/PYL receptor family, PP2C phosphatases, and SnRK2 kinases, showed significant differential expression following the application of 150 mg/L ABA. This indicates an active engagement of the canonical PYR/PYL–PP2C–SnRK2 signaling cascade in response to exogenous ABA stimulation.

Ethylene-responsive genes, including ERF transcription factors, were differentially regulated in response to the 500 μL/L ethephon treatment, supporting hormone-specific signaling responses. Interestingly, the application of 150 mg/L ABA induced a broader spectrum of differentially expressed genes compared to the 500 μL/L ethephon treatment, suggesting a more extensive regulatory influence on the ripening transcriptome.

Transcription factor analysis revealed differential expression of multiple regulatory families, notably MYB, bHLH, NAC, and ERF. Several MYB and bHLH genes exhibited expression patterns that were positively correlated with anthocyanin accumulation, suggesting their direct involvement in regulating the structural genes of the flavonoid pathway. The activation of these transcription factors under the 150 mg/L ABA treatment supports a model where ABA acts as a primary primer for the MYB–bHLH–WD40 regulatory complex to drive pigment biosynthesis in 'Deshi' litchi.

3.5. Correlation Analysis Links Structural Gene Expression With Anthocyanin Accumulation

To explore the relationship between transcript abundance and pigment accumulation, correlation analysis was performed between anthocyanin content and expression levels of key biosynthetic genes. Significant positive correlations were observed for late biosynthetic genes such as DFR, ANS, and UFGT, indicating transcriptional control as a major determinant of anthocyanin accumulation.

3.6. qRT-PCR Validation of Transcriptomic Data

To validate the accuracy and reproducibility of the RNA-Seq data, four key structural genes involved in the flavonoid biosynthesis pathway (Lc. HCT, Lc. CYP98A, Lc. CYP75B1, and Lc. ANR) were selected for quantitative real-time PCR (qRT-PCR) analysis (Table 1). The expression profiles of these selected genes under the different treatments (Control, ABA, Ethephon, and ABA+Ethephon) and across the developmental stages (0, 5, and 10 days) were highly consistent with the transcript

abundance obtained from the RNA-Seq analysis (Figure 6). Specifically, the robust upregulation of Lc. HCT and Lc. CYP98A by ABA treatment was confirmed, further validating the reliability of the transcriptomic dataset for understanding hormone-mediated pericarp coloration.

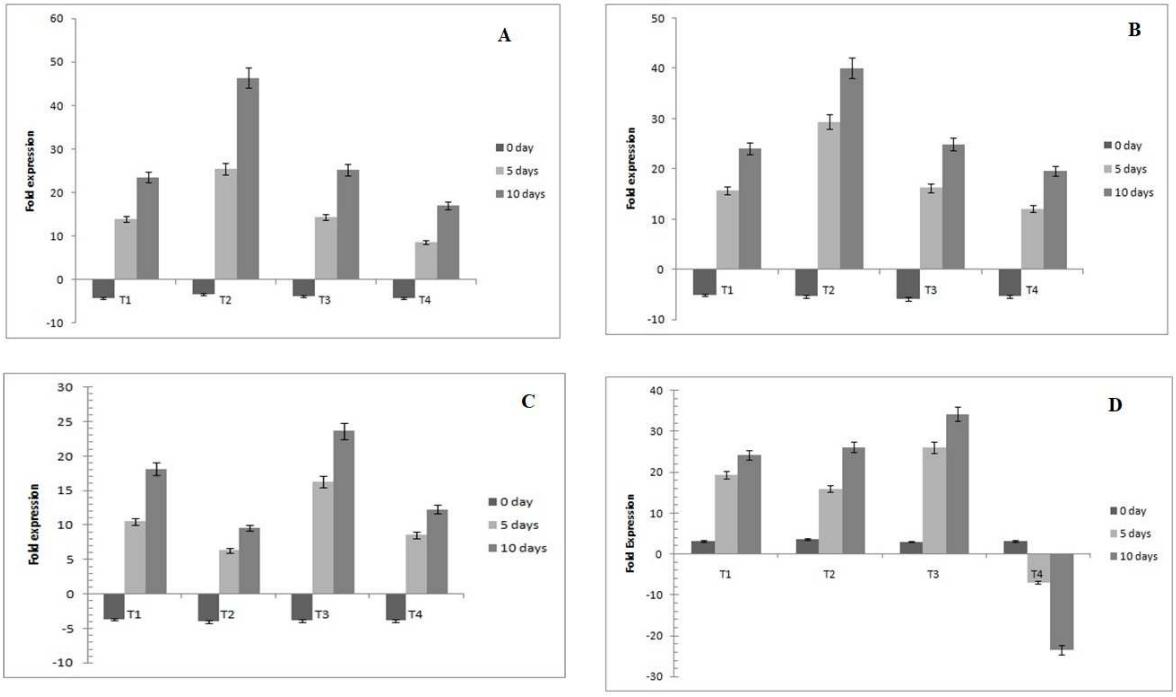


Fig. 6: Validation of genes involved in in flavonoid biosynthesis pathway (A;Shikimate O-hydroxycinnamoyl transferase (HCT), B;5-O-(4-coumaroyl)-D-quinate 3'-monooxygenase (CYP98A, C3'H), C; Flavonoid 3'-monooxygenase (CYP75B1), D; Anthocyanidin reductase (ANR). T1; Control, T2; ABA, T3; Ethephon, T4; Ethephon +ABA

4. Discussion

Pericarp coloration in litchi is a complex developmental process governed by coordinated hormonal signaling and transcriptional regulation of flavonoid biosynthesis. By integrating physiological, biochemical, and transcriptomic analyses, the present study provides mechanistic insight into the regulatory hierarchy underlying hormone-induced anthocyanin accumulation in Litchi chinensis cv. Deshi.

4.1. ABA as a Primary Regulator of Pericarp Pigmentation

Our results demonstrate that exogenous ABA application produced a stronger enhancement of anthocyanin accumulation compared to ethephon treatment. This observation is consistent with the established role of ABA as a central regulator of coloration in non-climacteric fruits. The transcriptomic data further support this

conclusion, as ABA treatment induced a broader spectrum of differentially expressed genes and stronger enrichment of flavonoid biosynthetic pathways.

Importantly, genes associated with ABA signal transduction, including PYR/PYL receptors, PP2C phosphatases, and SnRK2 kinases, were transcriptionally responsive to ABA treatment, indicating activation of canonical ABA signaling cascades. The coordinated induction of both signaling components and downstream biosynthetic genes suggests that ABA acts upstream in the regulatory network controlling pericarp pigmentation. A pivotal finding of this study is the observed reduction in efficacy when ABA and Ethephon are applied in combination compared to individual treatments. This suggests a negative crosstalk between the ABA signaling (PYR/PYL-PP2C-SnRK2) and ethylene-responsive factors (ERFs). While ABA acts as a primary 'primer' for the phenylpropanoid pathway, the addition of Ethephon may trigger specific ERF repressors that attenuate the full activation of late biosynthetic genes like LcDFR and LcUFGT. This hierarchy establishes ABA as the dominant regulator for pigment synthesis in the 'Deshi' cultivar.

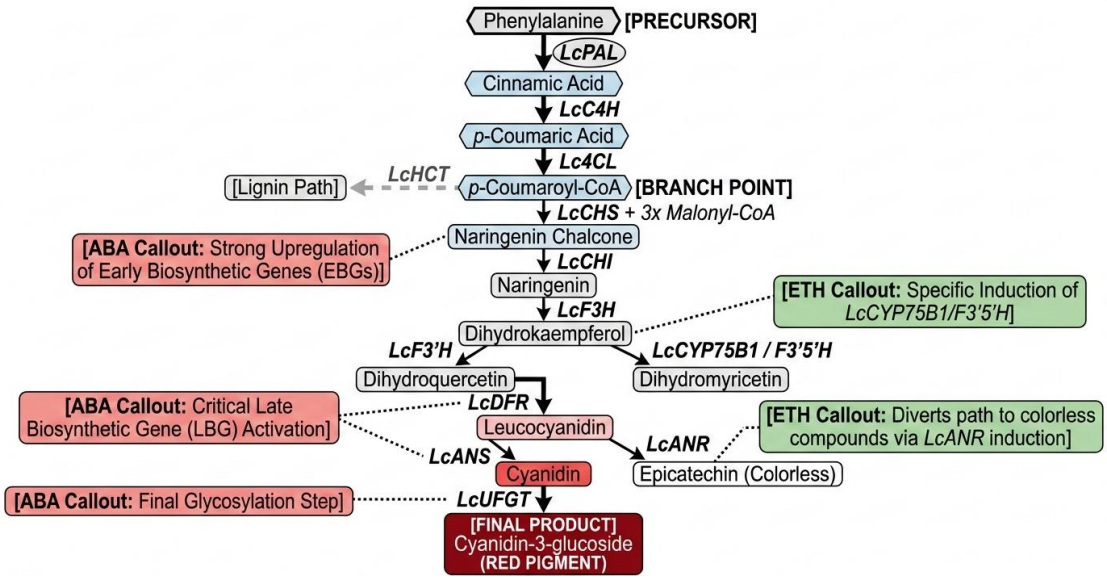
4.2.Transcriptional Activation of the Flavonoid Pathway

Anthocyanin biosynthesis proceeds through the phenylpropanoid pathway, involving early biosynthetic genes (PAL, C4H, CHS) and late biosynthetic genes (F3H, DFR, ANS, UFGT). Our data indicate that ABA strongly upregulated both early and late structural genes, with particularly pronounced induction of late biosynthetic genes. The significant positive correlation between anthocyanin content and expression of DFR, ANS, and UFGT suggests that transcriptional activation of these late genes is a major determinant of pigment accumulation in litchi pericarp.

This coordinated activation implies that ABA does not merely enhance precursor supply but promotes the entire anthocyanin biosynthetic cascade. In contrast, ethylene

treatment induced comparatively moderate transcriptional changes, suggesting a

FIGURE 8: GENOMIC LOCUS MAP OF ANTHOCYANIN BIOSYNTHESIS IN LITCHI



supportive or modulatory role rather than primary control.

Fig. 7: Genomic Locus Map of Anthocyanin Biosynthesis in Litchi.

4.3. Hormone–Transcription Factor Crosstalk

Transcription factor analysis revealed differential regulation of MYB, bHLH, NAC, and ERF family members under hormone treatments. The MYB–bHLH–WD40 regulatory complex is widely recognized as a master controller of anthocyanin biosynthesis in diverse plant species. The observed upregulation of MYB and bHLH genes following ABA treatment indicates that ABA-mediated signaling likely converges on this transcriptional complex to activate structural gene expression.

Moreover, ethylene-responsive ERF transcription factors were differentially expressed in ETH-treated fruits, suggesting hormone-specific regulatory inputs. These findings highlight potential crosstalk between ABA and ethylene pathways in modulating secondary metabolism. However, the stronger induction of MYB-associated genes under ABA treatment reinforces the dominant regulatory role of ABA in this system.

4.4. Integration of Physiological and Transcriptomic Evidence

A key strength of this study lies in the integration of biochemical measurements with transcriptomic profiling. The strong correlation between structural gene expression and anthocyanin content supports a transcriptionally driven mechanism of pigmentation enhancement. Rather than acting solely through post-transcriptional or

metabolic modulation, ABA appears to orchestrate a multi-tier regulatory cascade involving hormone perception, signal transduction, transcription factor activation, and structural gene induction.

This integrative perspective advances beyond descriptive differential expression analysis and provides a coherent mechanistic framework for hormone-mediated regulation of pericarp coloration in litchi.

4.5. Proposed Regulatory Model

Based on the combined evidence, we propose a regulatory model in which exogenous ABA activates the PYR/PYL–PP2C–SnRK2 signaling module, leading to activation of anthocyanin-related transcription factors, particularly MYB and bHLH proteins. These transcription factors subsequently upregulate structural genes in the flavonoid pathway, culminating in enhanced anthocyanin biosynthesis and visible pericarp reddening. Ethylene signaling may interact with this pathway but appears to exert a comparatively secondary influence under the tested conditions.

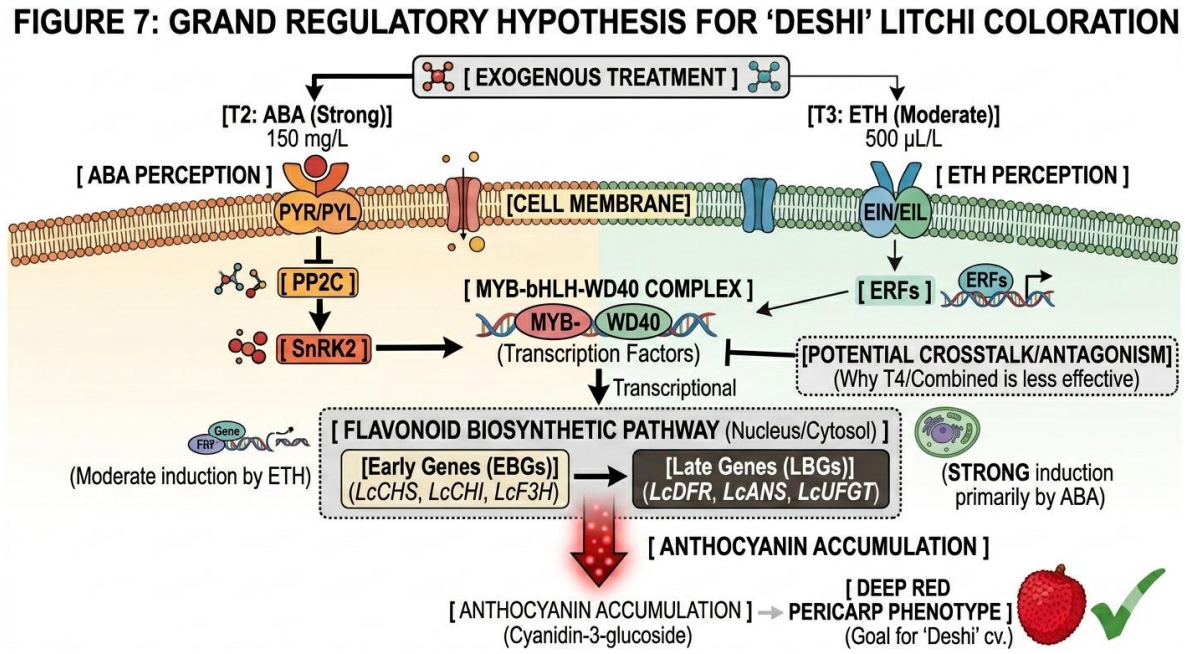


Fig. 8: Proposed hormone-mediated regulatory cascade controlling anthocyanin biosynthesis in litchi pericarp

5. Conclusion

This study provides an integrated physiological and transcriptomic framework elucidating hormone-mediated regulation of pericarp coloration

in Litchi chinensis cv. Deshi. Exogenous abscisic acid (ABA) markedly enhanced anthocyanin accumulation and induced extensive transcriptional reprogramming compared to ethephon treatment. Differential expression and enrichment analyses revealed significant activation of phenylpropanoid and flavonoid biosynthesis pathways, accompanied by upregulation of key structural genes including PAL, CHS, F3H, DFR, ANS, and UFGT.

Concomitant modulation of ABA signaling components and anthocyanin-related transcription factors, particularly MYB and bHLH family members, suggests that ABA operates upstream in a coordinated regulatory cascade controlling pigment biosynthesis. Correlation analysis between structural gene expression and anthocyanin content further supports transcriptional control as the primary mechanism underlying enhanced pigmentation.

Collectively, these findings establish ABA as a dominant regulator of pericarp coloration in litchi and provide a mechanistic model linking hormone signaling to secondary metabolism. This work offers molecular targets for improving fruit coloration through hormonal and genetic strategies and contributes to a broader understanding of hormone-driven regulation of anthocyanin biosynthesis in non-climacteric fruit species.

6. Author Contributions

AKP conducted the field experiments, performed transcriptomic analyses, and drafted the manuscript. SS conceptualized and supervised the research and contributed to experimental design and manuscript revision. KR assisted in transcriptome data interpretation and statistical analysis. NS contributed to data compilation, figure preparation, and manuscript formatting. RSS contributed to bioinformatics analysis and interpretation of RNA-Seq data. MH assisted in manuscript editing and technical review. All authors read and approved the final manuscript.

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9. Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

10. Data Availability Statement

The datasets generated and analyzed during the current study are available in the **GenBank SRA repository** under Accession Number: **PRJNA1167260**. Any further inquiries regarding the data can be directed to the corresponding author.

11. Generative AI Declaration

Declaration of Generative AI and AI-assisted technologies in the writing process. During the preparation of this manuscript, AI-assisted tools were used to improve language clarity and grammar. The authors reviewed and edited the content and take full responsibility for the content of the publication.

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