



Integrated physiological and multi-omics analysis identifies *MdMAPKKK17*-mediated anthocyanin suppression in apple red-skin patterning

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ABSTRACT

Apple (*Malus domestica* Borkh.) red-skin coloration, primarily determined by anthocyanin accumulation, is a critical trait affecting consumer preference and market value. However, the molecular mechanisms underlying distinct red-skin patterns (striped vs. blushed) remain unclear. Here, we performed an integrated multi-omics analysis comparing the red-striped 'Fuji' apple, which exhibits alternating light-red stripes (LRS) and dark-red stripes (DRS), with its red-blushed mutant (FBM). Physiological and microstructural analyses showed that FBM and DRS had higher anthocyanin content, thicker cuticles, and denser epidermal cells than LRS. Metabolomic profiling identified key differential metabolites positively associated with fruit blush intensity, which mainly consisted of six anthocyanins, fourteen flavonols, and twelve flavones within flavonoids, alongside fifteen phenolic acids, four amino acids and derivatives, one alkaloid, three lignans and coumarins, as well as two tannins. Transcriptomic analysis uncovered *MdMAPKKK17*, a mitogen-activated protein kinase kinase kinase gene, whose expression was negatively associated with anthocyanin accumulation across red-skin patterns. Functional validation based on transient and stable overexpression demonstrated that *MdMAPKKK17* negatively regulates anthocyanin biosynthesis by interacting with *MdMYB10* and suppressing its transcriptional activity. Our findings clarify the metabolic and transcriptional networks governing apple red-skin patterning, identify *MdMAPKKK17* as a key repressor of anthocyanin deposition and pattern formation, and provide potential targets for molecular breeding to tailor apple skin coloration to meet market demands and enhance nutritional value.

1. Introduction

Apple (*Malus domestica* Borkh.) is one of the most economically important temperate fruit crops globally, valued not only for its extensive cultivation and commercial significance but also for its rich content of health-promoting constituents, including vitamins, minerals, dietary fiber, and various polyphenols (Du Toit et al., 2024; Zheng et al., 2025). Skin coloration, particularly the development of red pigmentation, plays a critical role as a quality trait in influencing consumer preference and marketability (Cliff et al., 2002; Shi et al., 2022). In red apple varieties,

visual appeal is largely determined by the accumulation and distribution of anthocyanins, a major class of water-soluble flavonoids that impart red, purple, and blue hues in plant tissues (Duan et al., 2025; Bu et al., 2025). Beyond their aesthetic roles, anthocyanins possess notable antioxidant, anti-inflammatory, and cardioprotective properties, contributing to the nutritional and pharmacological value of apples (Grosso, 2025; Zhang, Jing, 2023). Therefore, elucidating the regulatory mechanisms underlying anthocyanin biosynthesis and red-skin patterning is essential for breeding strategies aimed at enhancing both the visual appeal and health benefits of apple fruits.

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The red-skin coloration of apple fruits shows diverse polymorphic patterns, which are predominantly classified into two phenotypes: red-striped (characterized by alternating red and green/yellow stripes) and red-blushed (uniform solid red) (Jia et al., 2020). Consumer preferences vary significantly; for instance, red-striped apples are favored in New Zealand, whereas red-blushed types are more popular in North America (Jaeger et al., 2018). This red-skin phenotypic diversity suggests distinct genetic and/or epigenetic controls underlying color patterning. Anthocyanin biosynthesis proceeds through the flavonoid pathway and involves several key structural genes such as *PAL*, *CHS*, *CHI*, *DFR*, *ANS*, and *UFGT*, whose transcription is coordinately regulated by the MYB-BHLH-WD40 (MBW) ternary complex (Chen et al., 2019; Jia et al., 2020; Xu et al., 2015; Zhang et al., 2019). Environmental cues (e.g., light and temperature) and internal signals (e.g., hormones and sugars) modulate the activity of this complex, thereby influencing anthocyanin production and distribution (Alabd et al., 2022; Li et al., 2024; Wang et al., 2024a,2024b). Thus, further elucidation of the physiological, biochemical, and molecular bases of red-skin pattern formation is essential for a comprehensive understanding of the anthocyanin regulatory network and its role in fruit quality.

In addition to transcriptional regulation, anthocyanin biosynthesis is modulated epigenetically through DNA methylation and phosphorylation. For instance, promoter hypermethylation of *PcMYB10* leads to green-skinned sports in red pears (Wang et al., 2013), while increased methylation in the *MdMYB10* promoter is linked to yellow-skinned apple mutants (El-Sharkawy et al., 2015). Conversely, demethylation of the *MdMYB10* promoter enhances red pigmentation in apple (Jia et al., 2020). Phosphorylation also plays a critical role in various processes: MPK4 stabilizes MYB75 to promote light-induced anthocyanin synthesis (Li et al., 2016), whereas YDA negatively regulates anthocyanin accumulation (Meng et al., 2016) in *Arabidopsis*. In apple, MPK4-mediated phosphorylation of MYB1 (Yang et al., 2021), MPK6-mediated phosphorylation of HY5 (Xing et al., 2023), and overexpression of *MdMCK9* (Sun et al., 2022) collectively modulate anthocyanin biosynthesis. Nevertheless, the involvement of mitogen-activated protein kinase kinase kinase (MAPKKK)-mediated signaling pathways in apple coloration remains largely unexplored.

While significant progress has been made in understanding anthocyanin biosynthesis and regulation, the specific mechanisms governing the patterning of red color in apple skin—the distinction between red-striped and red-blushed phenotypes—remain less clear. *MdMYB10* and *MdMYB90*-like promoter methylations have also been implicated, with higher methylation levels often associated with red-striped patterns and reduced anthocyanin accumulation (Jia et al., 2020; Sun et al., 2021). Comparison of methylation levels of the *MdMYB10* promoter in ‘Honeycrisp’ red and green stripes indicated that an enrichment of methylation was observed in the green stripes (Telias et al., 2011). In our preliminary research, although treatment of ‘CF-2’ with 5-aza-dC increased anthocyanin accumulation, the fruit remained red-striped, suggesting that *MdMYB10* methylation is not the sole determinant of red-skin patterning and this red pattern needs further study (Jia et al., 2020). Furthermore, recent work shows that MdMPK4-mediated phosphorylation of MdERF17 influences skin degreening, further implicating post-translational regulation in apple coloration (Wang et al., 2022). Unraveling the genetic and molecular mechanisms controlling these pigmentation patterns is essential for targeted breeding strategies aimed at improving fruit quality and market appeal.

To address this knowledge gap, we conducted an integrative study employing the red-striped ‘Fuji’ parent (with alternating light-red stripes, LRS, and dark-red stripes, DRS) and a red-blushed mutant (FBM) derived from it on the same tree. By integrating physiological, microstructural, metabolomic and transcriptomic analyses, we systematically compared external characteristics, internal quality, and key physiological attributes among these phenotypes. The objectives of this research were to: (1) characterize the microstructural and physiological differences underlying variations in red skin patterns; (2) identify

differentially accumulated metabolites associated with red-skin patterns; (3) elucidate the transcriptional regulatory network governing red-skin pattern formation; and (4) identify and validate the function of candidate genes. This study provides new, multi-faceted insights into the physiological and molecular foundations of apple red-skin patterning.

2. Materials and methods

2.1. Fruit materials

FBM was identified as a bud sport derived from a lateral branch of a red-striped ‘Fuji’ apple tree, which typically displays alternating light-red stripes (LRS) and dark-red stripes (DRS). The parental tree was grown in Penglai District, Yantai City, Shandong Province (37°47′N, 120°50′E) under standard orchard management practices. Fruit bag removal was conducted on October 5, 2022, and fruit tissues were sampled at 0, 2, 4, 6, 8, 10, and 14 days after bag removal (DABR) during the red coloration stage. At each sampling time point corresponding to the DABR coloring period, 45 red-striped ‘Fuji’ apples and 15 FBM fruits were collected, with 15 red-striped ‘Fuji’ and 5 FBM fruits defined as one biological replicate. Fruit skin and flesh tissues were dissected separately. For red-striped ‘Fuji’ apples, LRS and DRS skin regions were sampled independently starting at 2 DABR. All samples were immediately frozen in liquid nitrogen and stored at −80 °C until further analysis.

2.2. Measurement of apple skin color traits

At each coloration stage, 15 ‘Fuji’ and 15 FBM apples were randomly selected. Fruit skin color was evaluated using a CR-400 colorimeter (Konica Minolta, Japan). For each fruit, three randomly chosen points on the equatorial region (widest part) were measured as technical replicates and CIE L*, a*, and b* values were recorded. The L* value denotes lightness (0 for black, 100 for white), a* represents red-green chromaticity (positive values indicate red, negative values indicate green), and b* represents yellow-blue chromaticity (positive values indicate yellow, negative values indicate blue).

2.3. Determination of anthocyanin content

Anthocyanins were extracted according to the method of Jia et al. (2020) with minor modifications. Briefly, 1.0 g of apple skin powder was homogenized in 10 mL of methanol–acetic acid (99:1, v/v), followed by incubation at 4°C in the dark for 24 h. After centrifugation at 10,000 × g for 15 min, and the supernatant was collected. The extraction procedure was repeated twice. Subsequently, 1 mL of the combined supernatant was mixed with 4 mL of KCl buffer (pH 1.0) or sodium acetate buffer (pH 4.5), respectively, and incubated for 15 min. Absorbance values at 510 nm and 700 nm were measured using a UV-Vis spectrophotometer. The anthocyanin content was calculated based on the absorbance differential between the two pH conditions.

2.4. Carotenoid extraction and quantification

Carotenoids were extracted and quantified using established methodologies (Dang et al., 2021; Jia et al., 2019). Briefly, 0.5 g of skin or 1 g of flesh tissue was homogenized in 5 mL of 80% acetone, followed by extraction in darkness for 2 h with intermittent mixing. The supernatant was collected after centrifugation, and the remaining pellet was re-extracted twice under identical conditions. The combined supernatants were diluted to a final volume of 10 mL with 80% acetone. The absorbance was measured at 440 nm for carotenoid quantification.

2.5. Determination of total flavonoid content

Total flavonoid content was extracted and quantified according to

the method described by Alizadeh and Fattahi (2021). Briefly, 0.5 g of skin or 1 g of flesh was homogenized with 4 mL of 80% ethanol and extracted overnight in the dark. The mixture was subsequently shaken for 30 min and sonicated for an additional 30 min. The supernatant was collected after centrifugation, and the residual pellet was re-extracted twice with 80% ethanol (without overnight incubation). The combined supernatants were adjusted to a final volume of 10 mL with 80% ethanol.

For quantification, 0.5 mL of the extract was sequentially mixed with 2.5 mL of distilled water, 0.15 mL of 5% sodium nitrite, and 0.15 mL of 10% aluminum nitrate. Following vortex mixing, the mixture was incubated in the dark for 6 min. Subsequently, 1 mL of 1 M sodium hydroxide was added and the total volume was adjusted to 5 mL with distilled water. Flavonoid content was estimated by measuring absorbance at 500 nm.

2.6. Determination of ascorbic acid content

According to the method (Felicetti and Mattheis, 2010), ascorbic acid was extracted from 0.5 g of apple skin or flesh powder with 4 mL of oxalic acid–EDTA solution under dark conditions for 2 h. The extraction procedure was repeated twice, and the combined supernatants were collected after centrifugation and adjusted to a final volume of 10 mL. For colorimetric analysis, 1 mL of the extract was sequentially mixed with 0.1 mL of metaphosphoric acid–glacial acetic acid, 0.2 mL of 5% sulfuric acid, and 0.4 mL of 5% ammonium molybdate. The reaction mixture was diluted to 5 mL, incubated for 40 min for color development, and absorbance was measured at 760 nm using a UV-Vis spectrophotometer.

2.7. Determination of protopectin content

Protopectin was extracted and quantified using a commercial assay kit (G0703F, Suzhou Grace Biotechnology Co., Ltd., Suzhou, China). Briefly, 0.1 g of skin was desugared by heating at 85°C for 10 min in 1.5 mL of 80% ethanol. After cooling and centrifugation, the supernatant was discarded, and the washing procedure was repeated twice. The resulting precipitate was hydrolyzed by adding 1 mL of extraction solution, followed by incubation at 95°C for 60 min. The mixture was cooled to room temperature, centrifuged, and the supernatant was collected for subsequent analysis. For colorimetric determination, 105 µL of supernatant (with distilled water as the blank control) was mixed with 630 µL of concentrated sulfuric acid and 21 µL of chromogenic reagent. The absorbance was measured at 530 nm against the blank control.

2.8. Analysis of soluble sugars

Soluble sugars were extracted from 2.5 g of skin powder or 5 g of flesh powder with 10 mL of Watson's distilled water. The mixture was incubated at 75°C for 30 min with intermittent mixing, followed by centrifugation and supernatant collection. This extraction procedure was repeated twice and the combined supernatants were diluted to a final volume of 25 mL with Watson's distilled water. After filtration through a 0.45 µm aqueous membrane, the extract was analyzed by ultra-performance liquid chromatography coupled with evaporative light scattering detection (UPLC-ELSD). Chromatographic separation was performed on a Waters ACQUITY H-Class UPLC system equipped with an ELSD detector, according to the method described by Zhao et al. (2016). The mobile phase consisted of deionized water and acetonitrile containing 0.2% triethylamine (28:72, v/v) at a flow rate of 0.15 mL min⁻¹. Other chromatographic parameters were set as follows: column temperature 35°C, injection volume 5 µL, ELSD gain 1, nebulization temperature 60°C, drift tube temperature 80°C, and gas pressure 40 psi. Authentic standards of fructose, glucose, and sucrose (Solarbio) were used for quantification.

2.9. Analysis of organic acids

Organic acids were extracted following the same procedure as that for soluble sugars (Jia et al., 2021). The extract was filtered through a 0.45 µm organic membrane, and the filtrate was used for subsequent analysis. Chromatographic analysis was conducted using a Waters ACQUITY H-Class UPLC system equipped with a photodiode array (PDA) detector, according to the method described by Kong et al. (2024). For sample purification, 4 mL of supernatant was passed through a strong anion exchange (SAX) cartridge, and the filtrate was collected. A 2 mL aliquot of the filtrate was then transferred to a 50 mL vial, vortexed, and evaporated at 37°C. The resulting residue was reconstituted in 2 mL of deionized water and filtered through a 0.22 µm membrane. This purification process was repeated three times per sample.

Chromatographic separation was achieved on an ACQUITY UPLC HSS T3 column (2.1 × 100 mm, 1.8 µm) with a mobile phase of 0.01 mol L⁻¹ potassium dihydrogen phosphate-methanol (99:1, v/v, pH 2.38) at a flow rate of 0.13 mL min⁻¹. The chromatographic conditions were as follows: column temperature, 33°C; detection wavelength, 210 nm; and injection volume, 5 µL. Quantification analysis was performed using authentic standards purchased from Solarbio, including an aqueous mixed standard (BYO8010, containing L-malic, L-lactic, citric, and succinic acids) and tartaric acid (ST9760).

2.10. Metabolite extraction and analysis

Metabolomic profiling was performed by MetWare Biotechnology Co., Ltd. (Wuhan, China). Fruit skin samples were freeze-dried using a Scientz-100F freeze dryer and pulverized with a Mixer Mill 400 grinder (Retsch, Germany) at 30 Hz for 1.5 min. Subsequently, 50 mg of the resulting powder was extracted with 1200 µL of pre-chilled (−20 °C) 70% aqueous methanol. The mixture was vortexed six times at 30-min intervals (30 s each) and centrifuged at 12,000 rpm for 3 min. The supernatant was filtered through a 0.22 µm membrane prior to ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) analysis.

Chromatographic separation was performed on an ExionLC™ AD UPLC system (Sciex, USA) equipped with an Agilent ZORBAX StableBond C18 column (2.1 × 100 mm, 1.8 µm), which was maintained at 40°C. The mobile phase comprised (A) water containing 0.1% formic acid and (B) acetonitrile containing 0.1% formic acid, delivered at a flow rate of 0.35 mL min⁻¹ with the following gradient program: 5% B (0 min), linear increase to 95% B over 9 min, maintaining at 95% B from 9 to 10 min, returning to 5% B from 10 to 11.1 min, and re-equilibration from 11.1 to 14 min. MS/MS detection was conducted using an Applied Biosystems 6500 QTRAP mass spectrometer (Sciex, USA) equipped with an electrospray ionization (ESI) source.

The mass spectrometry conditions were set as follows: the ESI source was operated at 500 °C, with ion spray voltages of 5500 V in positive mode and −4500 V in negative mode. Ion source gases (GSI and GSII) and curtain gas (CUR) were maintained at 50, 60, and 25 psi, respectively, while collision-induced dissociation (CID) parameters were set to a high level. Multiple reaction monitoring (MRM) mode was employed on a triple quadrupole (QQQ) instrument, with nitrogen used as the collision gas at a medium setting. Since MRM mode was utilized, a global scan range was not defined; instead, collision energy (CE) and declustering potential (DP) were individually optimized for each MRM ion transition. Additionally, specific MRM transitions were monitored within scheduled time windows based on the elution profiles of the target metabolites.

2.11. Preparation and observation of paraffin sections

Based on a modified protocol reported by Li et al. (2022), paraffin sectioning was conducted using fruit samples at different coloration

stages. Tissue blocks ($3 \times 3 \times 3$ mm) comprising both skin and adjacent flesh were excised from the equatorial zone of each fruit. For red-striped 'Fuji' fruits, samples included both LRS and DRS skin regions. All tissues were fixed in FAA (50% ethanol: formalin: glacial acetic acid = 90: 5: 5, v/v), subjected to vacuum infiltration for 20 min, and subsequently processed through dehydration, clearing, infiltration, embedding, sectioning, and staining. The sections were then observed under a fluorescence microscope for histological evaluation.

2.12. Scanning electron microscopy (SEM)

For SEM observation, peel segments ($5 \times 5 \times 1$ mm) were excised from the equatorial region using a sharp blade. The samples were fixed in 2.5% glutaraldehyde at 4°C overnight and then processed through graded acetone dehydration and CO₂ critical point drying according to Lin et al. (2022). After sputter-coating with gold, observations were conducted using a JSM-7500F SEM (JEOL Ltd., Tokyo, Japan).

2.13. RNA extraction and quantitative real-time PCR (RT-qPCR) analysis

Total RNA was extracted using the TIANGEN Plant Polysaccharide & Polyphenol RNA Kit (DP419) according to the manufacturer's protocol, as described previously by Jia et al. (2020). Complementary DNA (cDNA) was synthesized using a commercial cDNA synthesis kit (Takara Bio Inc., Kyoto, Japan). Quantitative real-time PCR (RT-qPCR) was conducted using the Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific, USA) with SYBR Green reagents (Takara) and *EF1 α* and *Actin* as reference genes (Ampomah-Dwamena et al., 2022; Jia et al., 2021). Gene expression levels were calculated using the 2^{- $\Delta\Delta C_t$} method, with three biological replicates (each comprising three technical replicates) included for each sample. The primers used for RT-qPCR are listed in Table S1.

2.14. Transient transformation of apple fruit

The full-length coding sequence (CDS) of *MdMAPKKK17* was cloned from 'Fuji' skin cDNA and subsequently inserted into the pRI101-FLAG overexpression vector to generate *MdMAPKKK17*-OE (primers used in Table S1), as described by Dang et al. (2021). The constructed pRI101-FLAG-*MdMAPKKK17* recombinant vector was transformed into *Agrobacterium tumefaciens* strain EHA105. The bacterial culture was expanded, and bacterial cells were collected by centrifugation. After discarding the supernatant, the bacterial pellet was resuspended in infiltration buffer (10 mM MES, 10 mM MgCl₂, 200 μ M acetosyringone-AS) and adjusted to an OD₆₀₀ of 0.5–0.6.

Red-striped 'Fuji' fruits at 170 days after full bloom (without bag removal) were used as experimental materials. Following the protocol described by Dang et al. (2021), the bacterial suspension was injected into the fruit epidermis using a 1 mL sterile syringe. After injection, the fruits were incubated in a light incubator (24°C with 18 h light/19°C with 6 h dark) for 7–14 days. Fruit coloration was observed, and skin tissues from the injection sites were collected. Three biological replicates were performed for each treatment. All samples were rapidly frozen in liquid nitrogen and stored at –80°C for subsequent analysis.

2.15. Stable transformation of apple callus

The engineered strain EHA105/pRI101-FLAG-*MdMAPKKK17* was cultured for expansion, and bacterial cells were collected by centrifugation. After discarding the supernatant, the bacterial pellet was resuspended in MS liquid medium (containing 1 mol L⁻¹ betaine and 0.5 mol L⁻¹ AS) and adjusted to an OD₆₀₀ of 0.5–0.6. The suspension was then used to infect 'Orin' apple callus. Stably transformed positive calli was obtained through antibiotic selection, with calli transformed with the empty pRI101-FLAG vector (P101F) serving as controls. The

MdMAPKKK17-OE and P101F calli were cultured in a light incubator for 7–14 days under the following conditions: 23°C with 20 h light/19°C in darkness for 4 h. Callus coloration was observed, and samples were collected. Three biological replicates were performed for each treatment. All samples were rapidly frozen in liquid nitrogen and stored at –80°C for subsequent analysis.

2.16. Yeast two-hybrid (Y2H) assay

The CDSs of *MdMYB10* and *MdMYB114* were separately inserted into the pGBKT7 vector and the CDS of *MdMAPKKK17* was inserted into the pGADT7 vector. Different plasmid combinations (experimental groups: pGADT7-*MdMAPKKK17* + pGBKT7-*MdMYB10*/pGBKT7-*MdMYB114*; control groups: pGADT7-*MdMAPKKK17* + empty pGBKT7, empty pGADT7 + pGBKT7-*MdMYB10*/ pGBKT7-*MdMYB114*) were co-transformed into Y2H Gold yeast competent cells. The transformed yeast cells were first cultured on SD/-Trp/-Leu medium. Subsequently, serial dilutions of each transformant were spotted onto SD/-Trp/-Leu/-His/-Ade medium and the protein-protein interactions were determined based on the growth status of the yeast cells (Jia et al., 2021).

2.17. Dual-luciferase (LUC) transactivation assay

The promoter region of *MdDFR* was cloned into the pGreenII 0800-LUC reporter vector. The CDSs of *MdMAPKKK17* and *MdMYB10* were inserted into the effector vector pGreenII 62-SK, respectively (primers used in Table S1). Corresponding effector and reporter plasmids were co-infiltrated into *Nicotiana benthamiana* leaves. At 72 h post-infiltration, firefly and renilla luciferase activities (expressed as LUC/REN ratios) were measured using Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) with a GloMax® 20/20 Luminometer (Promega). Relative LUC activity was normalized to REN activity as described by Jia et al. (2021), with six biological replicates per treatment.

2.18. Statistical analysis

Statistical analysis was conducted using SPSS 22.0 software. Multiple comparisons versus the control group were conducted with one-way ANOVA followed by Dunnett's test, and two-group comparisons were performed using an independent samples *t*-test. Significance levels are denoted as * *P* < 0.05, ** *P* < 0.01, and *** *P* < 0.001. Graphs were generated using GraphPad Prism 8.0.2 and Origin 2024 software. Electron micrographs and paraffin sections were processed and analyzed with ImageJ and Saiviewer scanning software, respectively.

3. Results and discussion

3.1. Phenotypic characterization and quality trait factor analysis of red-striped 'Fuji' and its red-blushed mutant FBM

3.1.1. Phenotype and pigment accumulation differences define red-skin pattern traits

The red-striped 'Fuji' displayed distinct light-red stripe (LRS) and dark-red stripe (DRS) regions, while its red-blushed mutant (FBM) showed a uniformly solid red color throughout the fruit coloring period (days after bag removal, DABR) (Fig. 1A, E). Phenotypic observation of red-striped 'Fuji' indicated that the red-striped pattern was established at or after 6 DABR (Fig. 1A). Colorimetric analysis confirmed consistent differences in CIELAB parameters: across all coloring stages, 'Fuji' exhibited significantly higher lightness (L*) than FBM, with differences ranging from 7.9 to 18.0, and significantly lower redness (a*) than FBM, with differences spanning from 11.1 to 22.5. Yellowness (b*) was higher in 'Fuji' during the early coloring stages (2, 4, and 6 DABR) by 3.3–9.5 units; however, this difference faded at 14 DABR (Fig. 1B–D). These values reflect the visual distinction between red-striped and red-blushed

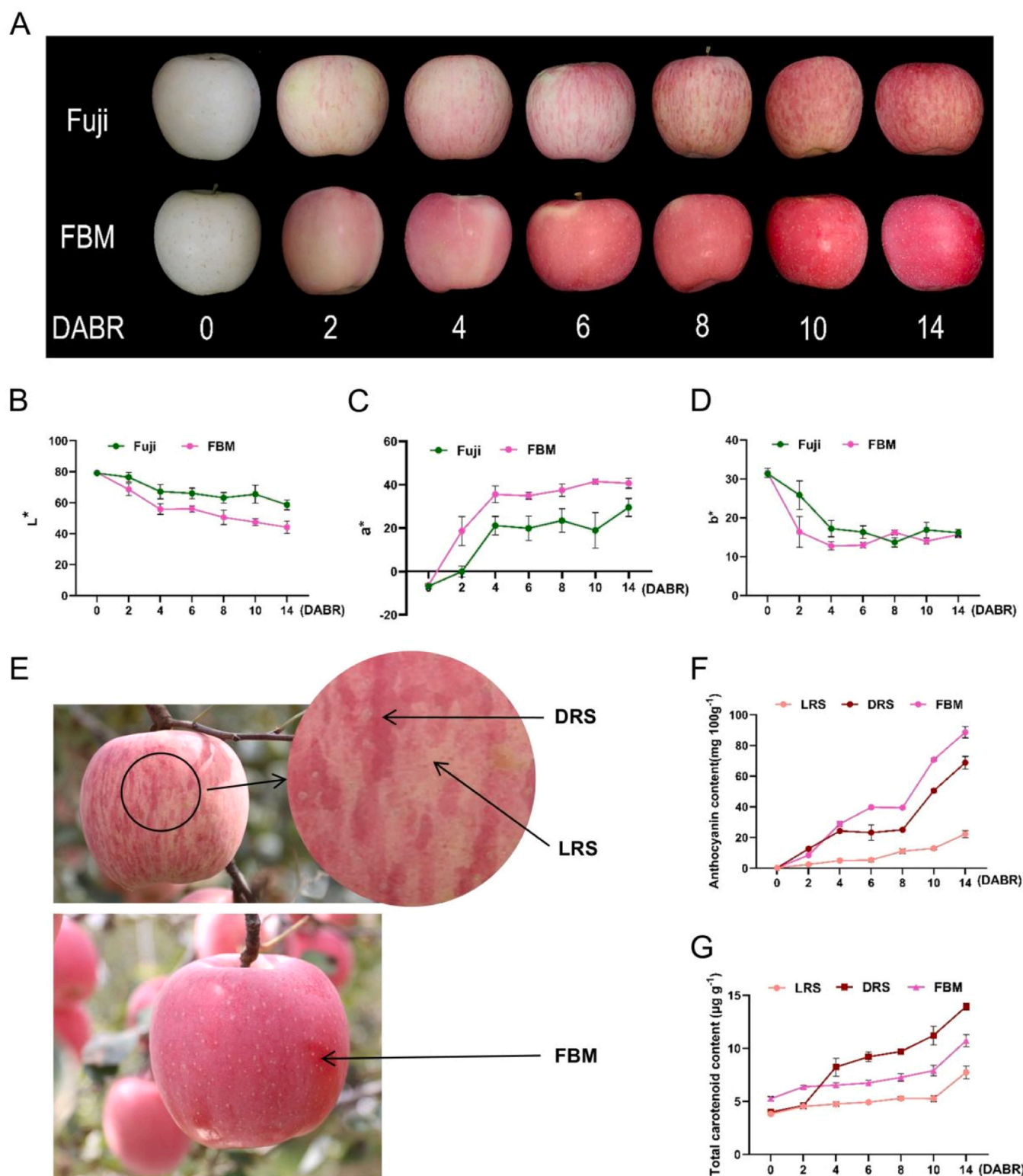


Fig. 1. Changes of the apple red-skin pattern traits in 'Fuji' and FBM during the coloring period at 0, 2, 4, 6, 8, 10, and 14 DABR. A. Phenotypes of 'Fuji' and FBM fruits during the coloring period. B–D. L*, a*, and b* in 'Fuji' and FBM fruits during the coloring period. E. Picture for distinguishing LRS, DRS, and FBM. F. Total anthocyanin content. G. Total carotenoid content. Data represent mean $\pm$ SE. FBM, red-blushed mutant; DABR, days after bag removal; LRS, light-red stripes; DRS, dark-red stripes.

phenotypes.

Anthocyanins and carotenoids are critical pigments that influence the coloration of fruits (Hu et al., 2024). Time-course pigment analysis revealed a graded anthocyanin accumulation pattern across the three

phenotypes: LRS < DRS < FBM (Fig. 1F). Specifically, DRS anthocyanin content was approximately fivefold higher than that of LRS during early coloring (2–6 DABR) and decreased to about threefold at the mature stage (14 DABR); FBM anthocyanin content reached five–seven times

that of LRS during mid-coloring (6–10 DABR). This trend aligns with the findings of Jia et al. (2020), who reported elevated anthocyanin levels in a red-blushed mutant compared to a striped variety, suggesting that differential anthocyanin accumulation plays a crucial role in red-skin patterning.

Carotenoid levels exhibited distinct temporal dynamics: FBM showed higher concentrations during early coloration (0–2 DABR), approximately 1.37–1.41 times that of LRS and 1.34–1.38 times that of DRS; DRS surpassed FBM after 4 DABR, reaching 1.73–2.13 times that of LRS and approximately 1.26–1.42 times that of FBM; LRS consistently displayed the lowest levels (Fig. 1G). Carotenoids impart yellow hues and interact with anthocyanins to modulate overall fruit color (Li et al., 2025a, 2025b). These results indicate that both the abundance and temporal dynamics of anthocyanins and carotenoids in the skin jointly regulate color pattern formation. This aligns with findings in blood orange (*Citrus sinensis*), where carotenoids and anthocyanins collectively promote pigment accumulation and skin coloration during fruit development (Fu et al., 2025), implying interplay between their biosynthetic pathways, potentially mediated by shared regulators or metabolic precursors.

3.1.2. Fruit quality trait profiling reveals distinct red-skin patterns

Apple skin accumulates various quality-related metabolites that affect color, flavor, and antioxidant capacity (Das et al., 2022; Kuang et al., 2024; Li et al., 2025a, 2025b). Comparative analysis of LRS, DRS, and FBM identified distinct profiles of flavonoids, ascorbic acid, protopectin, soluble sugars, and organic acids.

Total flavonoids and ascorbic acid, key antioxidants co-regulated with anthocyanins (Ibrahim et al., 2012), showed distinct temporal profiles among the three phenotypes. DRS accumulated the highest concentrations of both compounds during the late coloring stage, while LRS had the lowest flavonoid content and FBM had the lowest ascorbic acid levels (Fig. 2A, B). Protopectin content was significantly higher in DRS and FBM than in LRS (Fig. 2C). As a key cell wall component, protopectin affects tissue firmness and may indirectly influence pigment accumulation by altering the cellular microenvironments (Zhang et al., 2021). These variations support the association between red pigmentation and enhanced antioxidant capacity (Yang et al., 2023; Zou et al., 2025), indicating that coordinated regulation of antioxidant metabolism mediates differences in red skin patterning.

Soluble sugars and organic acids are essential for plant growth and fruit flavor. Soluble sugar analysis showed that FBM exhibited the

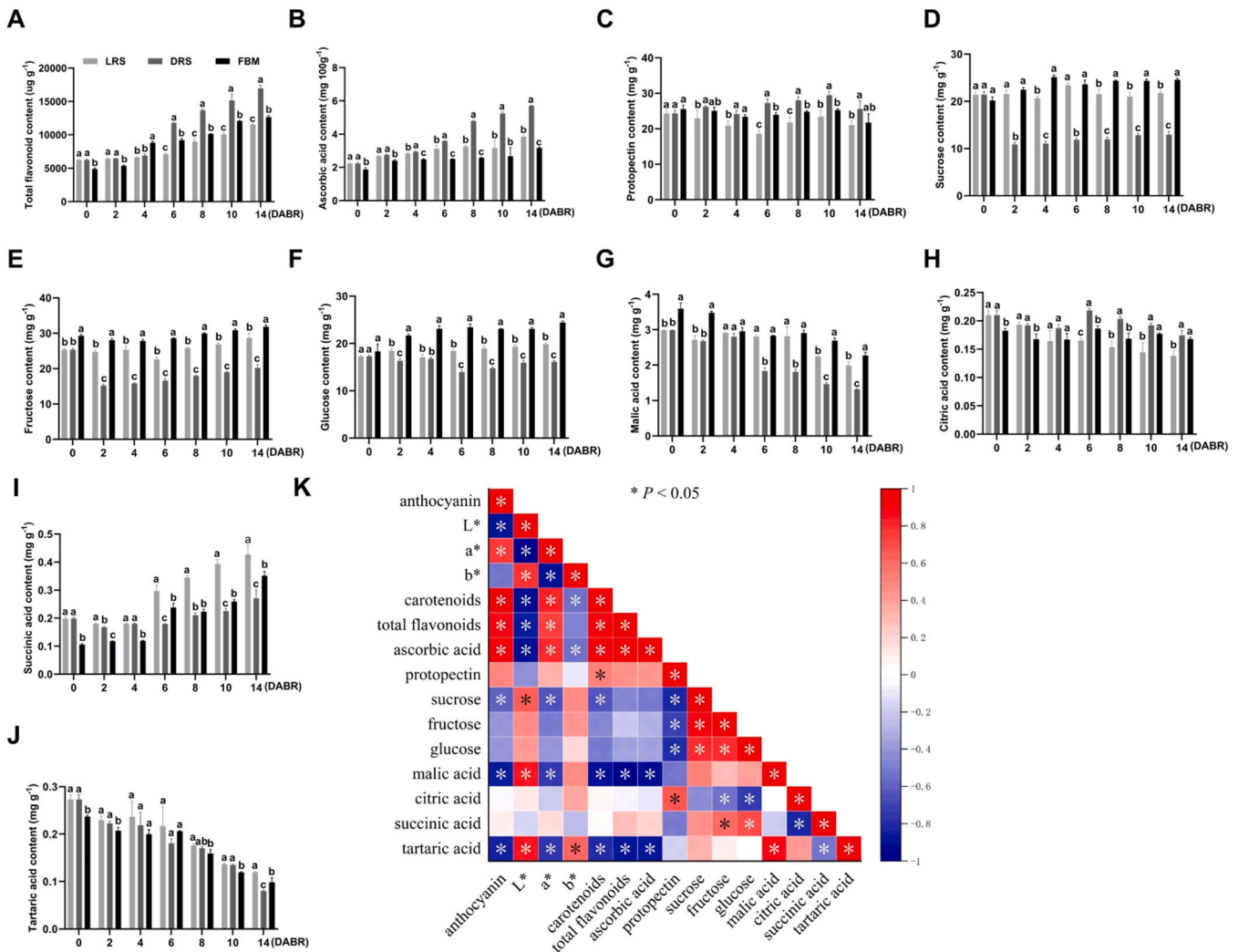


Fig. 2. Dynamic changes and correlation analysis of fruit quality traits in LRS, DRS and FBM. A–J. Analysis of the levels of fruit quality traits in LRS, DRS, and FBM. Total flavonoid (A), ascorbic acid (B), protopectin (C), sucrose (D), fructose (E), glucose (F), malic acid (G), citric acid (H), succinic acid (I), tartaric acid (J). K. Correlation analysis between color difference and the contents of carotenoid, total flavonoid, ascorbic acid, protopectin, soluble sugars, organic acids, and anthocyanin in ‘Fuji’ and FBM at 0 DABR and in LRS, DRS, and FBM at 2, 4, 6, 8, 10, and 14 DABR. Data represent mean ± SE. Statistical analysis was performed using one-way ANOVA followed by Dunnett’s test. Different letters indicate significant differences ($P < 0.05$). Asterisks indicate significant differences ($P < 0.05$). LRS, light red stripes; DRS, dark red stripes; FBM, red-blushed mutant; DABR, days after bag removal.

highest levels of sucrose, fructose and glucose, followed by LRS, and then DRS (Fig. 2D–F). As sugars serve as both signaling molecules and metabolic precursors in anthocyanin biosynthesis, their elevated accumulation in FBM likely contributes to its intensified pigmentation. Organic acid profiles showed distinct red pattern-specific variations during the 6–14 DABR: FBM and DRS showed significantly higher citric acid levels but lower succinic acid and tartaric acid compared to LRS (Fig. 2H–J); malic acid was higher in LRS and FBM than in DRS (Fig. 2G). Since malate transporters modulate both fruit acidity and anthocyanin synthesis (Zhang et al., 2025), this finding suggests that organic acid metabolism is integrated into the regulatory network controlling red-skin patterning. Collectively, apples with different red-skin patterns exhibit distinct sugar-acid accumulation profiles, indicating that red-pattern differentiation is closely associated with spatiotemporal reorganization of internal sugar-acid metabolism.

Flesh tissue analysis revealed additional compositional differences: compared to ‘Fuji’, FBM generally had higher concentrations of carotenoids (Fig. S1A), total flavonoids (Fig. S1B), sucrose (Fig. S1D), citric acid (Fig. S1H), and tartaric acid (Fig. S1I), but lower levels of fructose (Fig. S1E), glucose (Fig. S1F), and malic acid (Fig. S1G) during the late fruit coloring period. ‘Fuji’ and FBM showed no significant difference in ascorbic acid content (Fig. S1C).

3.1.3. Analysis of the correlation among factors affecting red-skin pattern

To clarify the associations between red-skin pattern differentiation and quality-related metabolites, correlation analyses were performed across the three color types. Overall correlation analysis showed that anthocyanin content was positively correlated with a^* , carotenoids, total flavonoids, ascorbic acid, protopectin, and succinic acid, but negatively correlated with L^* and b^* , sucrose, fructose, glucose, malic acid, and tartaric acid. Notably, a^* , carotenoids, total flavonoids, and ascorbic acid showed significantly positive correlations with anthocyanins, while L^* , sucrose, malic acid, and tartaric acid exhibited significantly negative correlations (Fig. 2K).

Type-specific correlation analyses revealed distinct metabolite association patterns with anthocyanins across different red-skin patterns. In LRS, anthocyanins were significantly positively correlated with a^* , carotenoids, total flavonoids, ascorbic acid, fructose, glucose, and succinic acid, but significantly negatively correlated with L^* , malic acid, citric acid, and tartaric acid (Fig. S2A). In DRS, anthocyanins showed significantly positive correlations with a^* , carotenoids, total flavonoids, ascorbic acid, and succinic acid, but significantly negative correlations with L^* , malic acid, and tartaric acid (Fig. S2B). In FBM, anthocyanins were significantly positively associated with a^* , carotenoids, total flavonoids, ascorbic acid, fructose, glucose, and succinic acid, and significantly negatively associated with L^* , malic acid, and tartaric acid (Fig. S2C).

Across all red-skin patterns, a^* , carotenoids, total flavonoids, ascorbic acid, and succinic acid consistently showed significant positive correlations with anthocyanin accumulation, while L^* , tartaric acid, and malic acid displayed significant negative correlations (Fig. S2D, E). Notably, sucrose has been reported as the sugar most strongly linked with anthocyanin accumulation in blood oranges (Giménez-Sanchis et al., 2024), while high ascorbic acid levels may reduce anthocyanin extract stability (De Rosso and Mercadante, 2007). These results suggest that the development of distinct red-skin patterns involves type-specific co-regulation of multiple quality-related metabolites.

3.2. Metabolomic profiling of LRS, DRS, and FBM

Metabolomic profiling by UPLC-MS/MS was performed on the skins of three red-skin apple types (LRS, DRS, and FBM) at 0 and 8 DABR, identifying 1660 metabolites categorized into 12 classes, including alkaloids, flavonoids, organic acids, and phenolic acids. (Fig. S3A). This finding confirms the metabolic diversity among apple cultivars, consistent with previous reports (Chen et al., 2025). Cluster analysis revealed

distinct metabolic profiles across the three red-skin types (Fig. S3B), while principal component analysis (PCA) of the 15 samples provided insights into skin color variation among these types (Fig. S3C).

The classification of differential metabolites by red-skin type showed that flavonoids were the primary differential metabolites (Fig. S4), including flavonols, flavones, flavanones, anthocyanins, other flavonoids, flavanols, chalcones, flavanols, auronones, and isoflavones. At 8 DABR, flavonols and flavones contents were significantly higher in DRS than in LRS but lower than those in FBM (Fig. S5), indicating that darker red skins (e.g. DRS and FBM) accumulate higher levels of anthocyanins, flavonols, and flavones, which jointly contribute to red color variation. This observation is consistent with findings in bilberry, where anthocyanins and flavonols co-regulate ripening pigmentation (Jaakola et al., 2002), and supports reports of competitive biosynthetic relationships between these flavonoid classes in other species (Cheng et al., 2022). Beyond flavonoids, other secondary metabolite classes—phenolic acids, amino acids and derivatives, alkaloids, lignans, coumarins, and tannins—were also differentially accumulated among red-skin patterning types, a phenomenon not previously reported in apple skin.

3.2.1. Comparative analysis of flavonoid metabolites among LRS, DRS, and FBM

Comparative flavonoid metabolomic analysis revealed distinct profiles among the three red-skin patterning types. At 8 DABR, venn analysis identified six differentially accumulated anthocyanins (Fig. 3A): cyanidin–3-O-galactoside, cyanidin–3-O-glucoside (Kuromanin), cyanidin–3-O-(2''O-xylosyl) gentiobioside, cyanidin 3-xyloside, procyanidin B1, and procyanidin B5. The accumulation patterns of these anthocyanins correlated with visible coloration and total anthocyanin content, indicating that both anthocyanin levels and spatial distribution determine red-skin patterning. Cyanidin–3-O-galactoside and cyanidin–3-O-glucoside, the most abundant anthocyanins in apple, are catalyzed by UFGaT and UFGT, respectively, representing key terminal steps in red coloration (Bu et al., 2025). As minor components, cyanidin–3-O-gentiobioside and cyanidin–3-xyloside may be generated from differential glycosyltransferase activities among skin types (Vimolmangkang et al., 2013; Zhang et al., 2025). Procyanidin B1 is a key fruit coloration-related metabolite; research on plum shows that its levels at the young fruit stage determine mature fruit color, with cyanidin derived from procyanidins maintaining red coloration at maturity (Chen et al., 2022a, 2022b). Similarly, procyanidin B5 variation may reflect differences in carbon flux allocation between anthocyanin synthesis and precursor metabolism (Mao et al., 2025).

In addition to anthocyanins, 14 flavonols and 12 flavones were differentially accumulated among the different skin types at 8 DABR (Fig. 3B, C). Flavonols and flavones share the upstream phenylpropanoid pathway with anthocyanins, their biosynthesis is catalyzed by F3H and FLS, respectively. Flavonols (e.g., quercetin and kaempferol derivatives) act as photoprotectants and copigments that enhance and stabilize red coloration via intermolecular interactions with anthocyanins (Rezende et al., 2025; Trouillas et al., 2016; Wang et al., 2024a, 2024b), while flavonoids may indirectly regulate anthocyanin accumulation through cofactor availability or signaling (Duan et al., 2026). A heatmap visually illustrates the differential accumulation of these anthocyanins, flavonols, and flavones among LRS, DRS, and FBM (Fig. 3I). Additionally, metabolomic analysis of ‘Fuji’ and FBM at 0 DABR detected anthocyanins, flavonols, and flavones prior to coloration onset, suggesting their involvement in preparatory regulation (Fig. S6).

In summary, comparative analysis of LRS, DRS, and FBM revealed significant differences in flavonoid metabolite distribution among red-skin patterning types. Consistent with Mao et al. (2025), variations in upstream carbon flux and downstream modification within the flavonoid pathway drive differential metabolite accumulation in apple skin. This fine-tuned regulation likely modulates anthocyanin color intensity and stability by altering accessory pigment composition. These findings support previous reports identifying flavonols and flavones as key

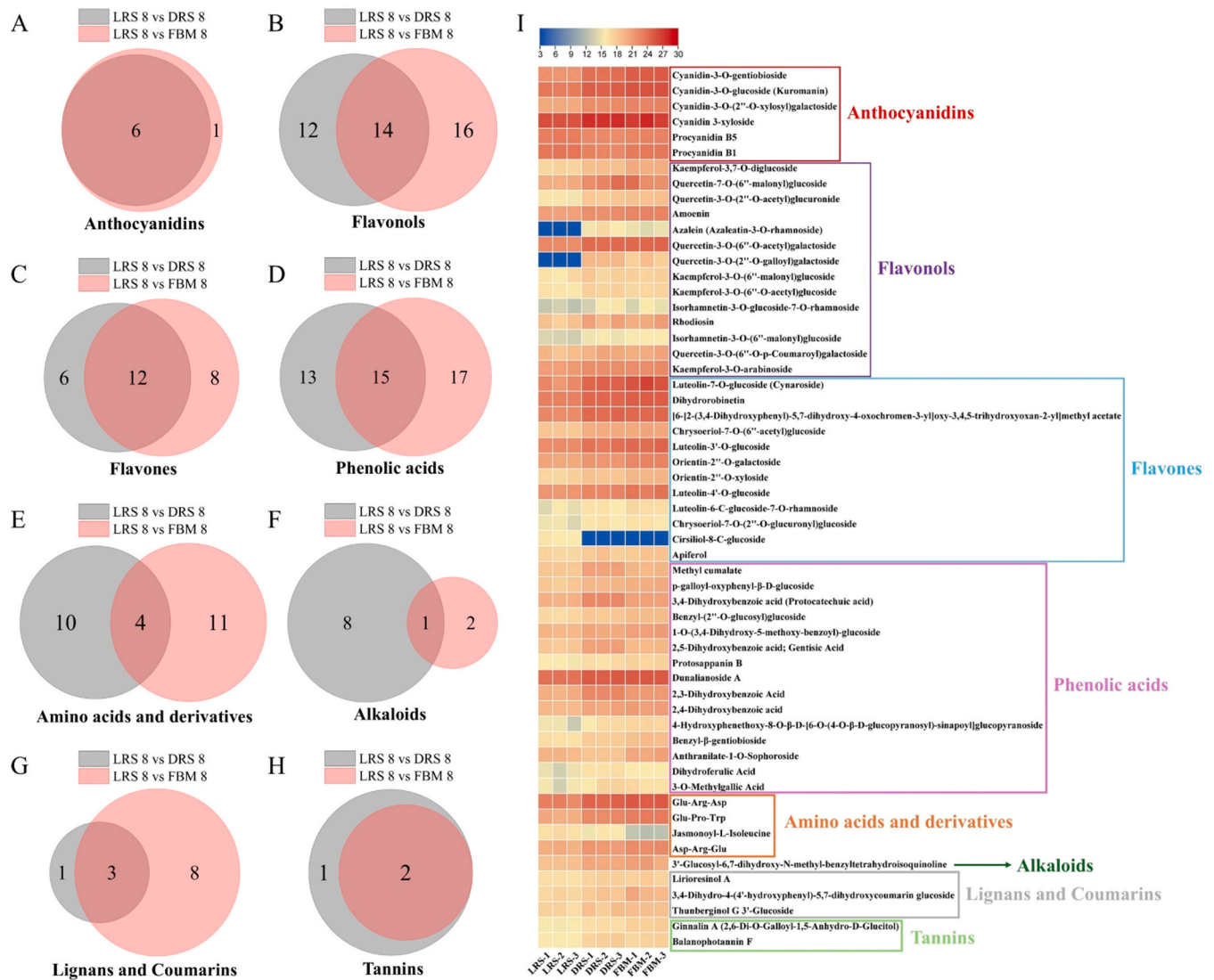


Fig. 3. Comparative metabolomic analysis of fruit skins from LRS, DRS, and FBM at 8 DABR. A–H. Venn diagrams of differential metabolites classified into eight classes: anthocyanins (A), flavonols (B), flavones (C), phenolic acids (D), amino acids and derivatives (E), alkaloids (F), lignans and coumarins (G), and tannins (H). I. Heatmap profiling of differential metabolites in LRS, DRS, and FBM fruit skins at 8 DABR; deeper red denotes higher metabolite abundance. LRS, light-red stripes; DRS, dark-red stripes; FBM, red-blushed mutant; DABR, days after bag removal.

regulators of light-induced apple skin coloration (Xue et al., 2022) and further demonstrate their synergistic role in forming distinct red-skin patterning types.

3.2.2. Profiling of other secondary metabolites among LRS, DRS, and FBM

Venn analysis of differentially accumulated metabolites between LRS 8 vs DRS 8 and LRS 8 vs FBM 8 identified common secondary metabolites across LRS, DRS, and FBM, including 15 phenolic acids, 4 amino acids and derivatives, 1 alkaloid, 3 lignans and coumarins, and 2 tannins (Fig. 3D–H). Heatmap analysis revealed that at 8 DABR, these metabolites were generally more abundant in darker red skins (DRS and FBM) than in lighter red skin (LRS) (Fig. 3I); this trend was also observed at 0 DABR (Fig. S6). This differential accumulation has important biological implications: phenolic acids contribute to plant defense and enhance anthocyanin coloration via copigmentation (Chen et al., 2022a,2022b); amino acids and derivatives act as precursors or signaling molecules in secondary metabolism (Wu et al., 2024); and alkaloids, lignans, coumarins, and tannins are associated with antioxidant activity and tissue browning, with higher accumulation in darker skins indicating enhanced antioxidant capacity and metabolic activity (Duan et al.,

2021). Collectively, these results demonstrate that red-skin patterning differences extend beyond anthocyanins and flavonoids to involve broad secondary metabolic network reprogramming. Darker red skins exhibit a more active secondary metabolic state, consistent with the view that multiple metabolites synergistically regulate fruit coloration (Gong et al., 2021).

3.3. Microstructural characterization of LRS, DRS, and FBM

Distinct red-skin patterns in apple fruits are closely associated with differences in microscopic cellular and cuticular structures. Paraffin sectioning showed no significant structural variations among the three red-skin types during the initial coloring stage (0–6 DABR). However, starting from 8 DABR, the cell density of LRS was significantly lower than that of DRS and FBM, accompanied by a noticeably thinner cuticle and epidermal cell wall. Specifically, the cuticle thickness of DRS and FBM was approximately 1.05 and 1.35 times that of LRS, respectively, while the epidermal cell wall thickness was approximately 1.05 and 1.17 times that of LRS (Fig. 4A–C).

SEM further revealed that ‘Fuji’ had smaller cells and a thinner

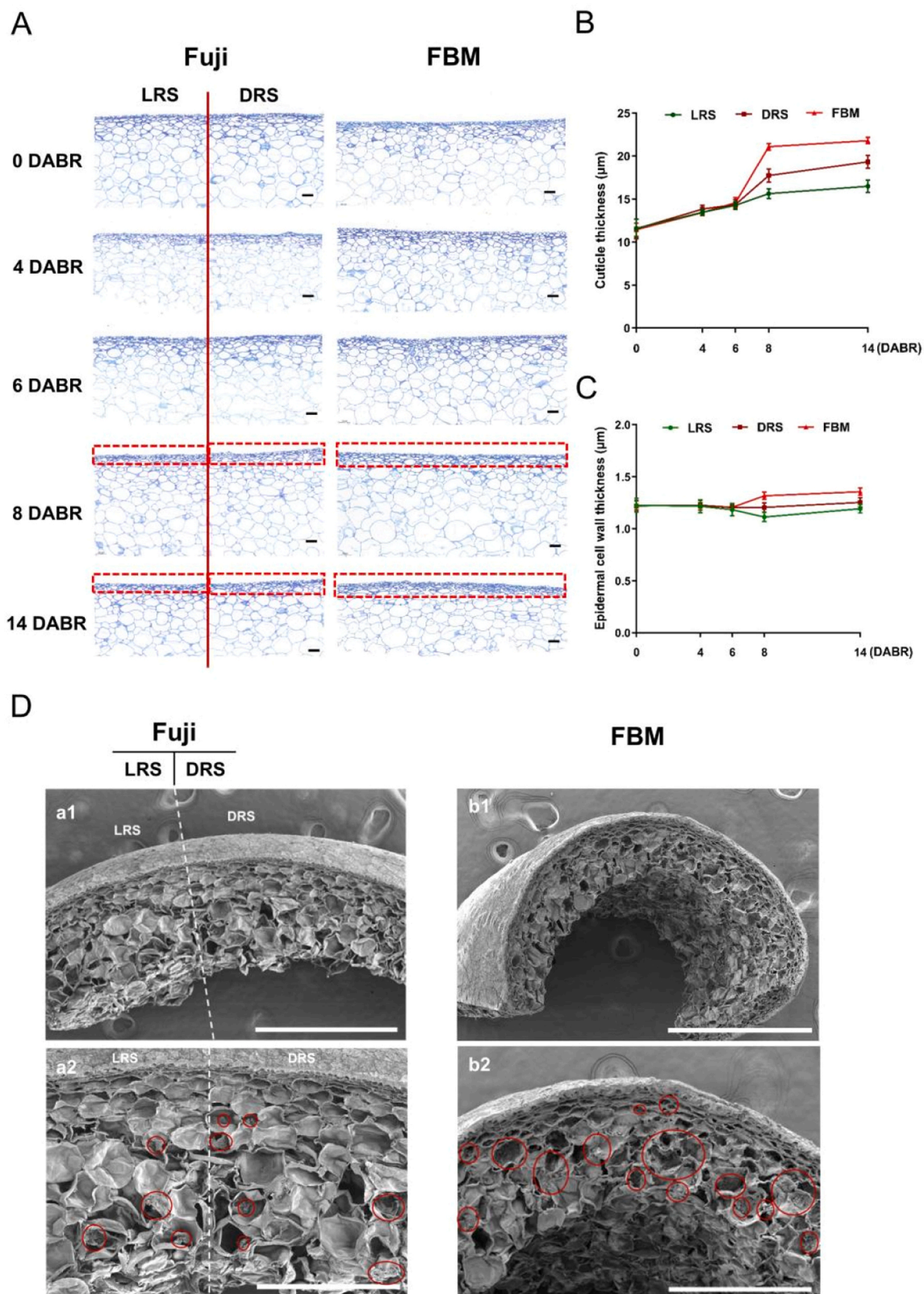


Fig. 4. Observation of microstructures in 'Fuji' and FBM apples through paraffin sections and scanning electron microscopy. A. Paraffin section observation images of LRS, DRS, and FBM at 0, 4, 6, 8, and 14 DABR; scale bar = 100 μm . B. Skin cuticle thickness. C. Skin epidermal cell wall thickness. D. Scanning electron microscopy (SEM) images of 'Fuji' and FBM at 8 DABR, images of 'Fuji' at 50 $\times$ (a1) and 100 $\times$ (a2) under a scanning electron magnification, with the area on the left side of the dotted line representing LRS and the area on the right side representing DRS; images of FBM at 50 $\times$ (b1) and 100 $\times$ (b2) under SEM. In panels a1 and b1, the scale bar = 1 mm and the magnification = 50 $\times$; in panels a2 and b2, the scale bar = 500 μm and the magnification = 100 $\times$. Data represent mean $\pm$ SE. LRS, light-red stripes; DRS, dark-red stripes; FBM, red-blushed mutant; DABR, days after bag removal.

cuticle compared to FBM; FBM exhibited more tightly arranged epidermal cells and more pronounced cuticular deposition in adjacent flesh tissues, with its cuticle thickness approximately 2–4 times that of ‘Fuji’ (Fig. 4D). These findings suggest that cell morphology and arrangement serve as important structural factors influencing fruit coloration performance.

Previous studies have established that the cuticle—a protective layer composed of cutin and wax—contributes not only to fruit gloss and

crack resistance (Lara et al., 2019; Yang et al., 2024) but also to limiting water loss and enhancing resistance against biotic and abiotic stresses (Anton et al., 2010). Accumulating evidence further indicates a direct link between cuticle properties and anthocyanin accumulation. Regression analysis has shown a positive association between cuticular thickness and anthocyanin accumulation in apple skin (Li et al., 2004). Similarly, tomato studies have demonstrated that mutants with impaired cuticle biosynthesis exhibit reduced anthocyanin

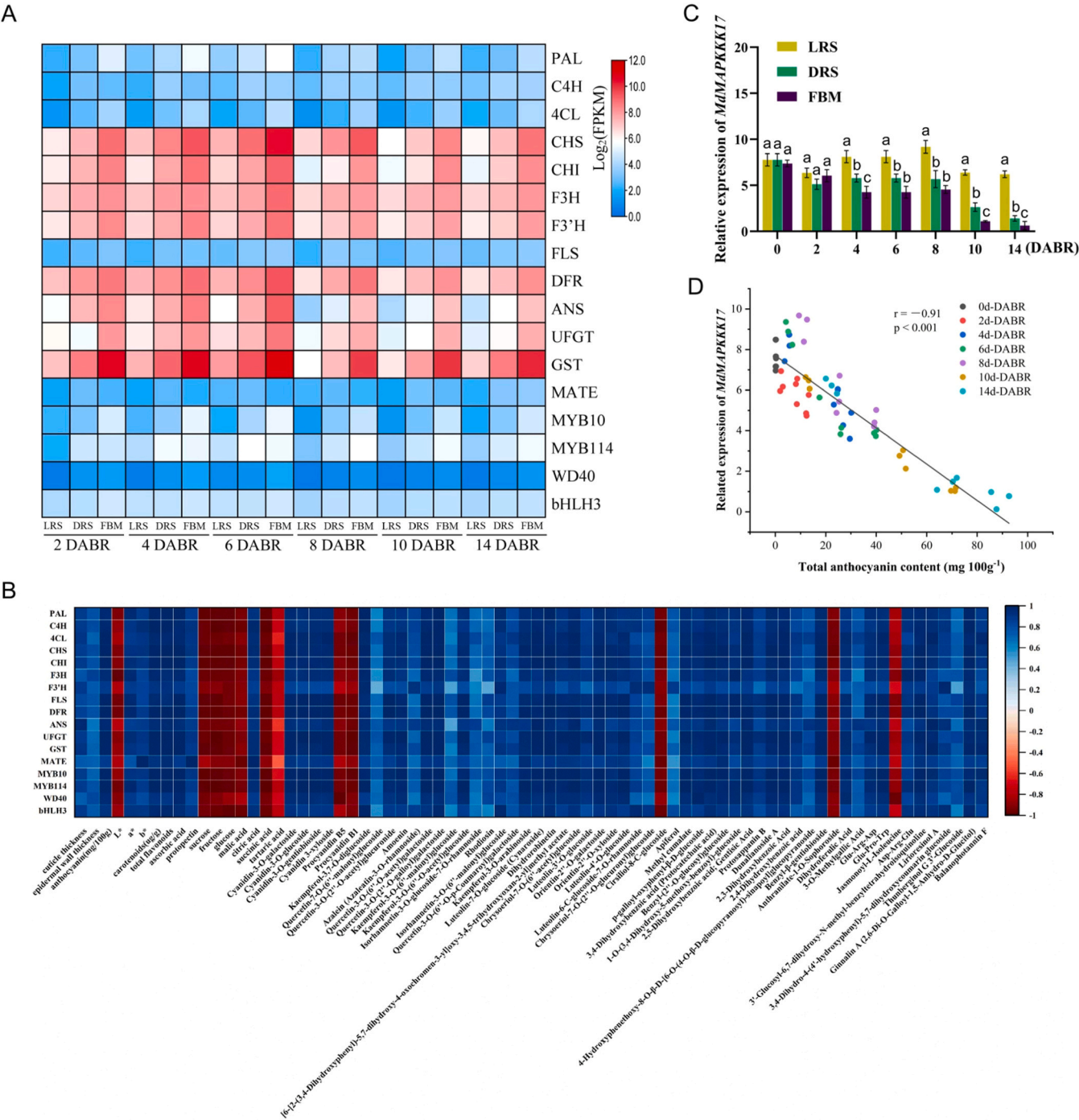


Fig. 5. Transcriptional profiling of anthocyanin biosynthesis and identification of a negative regulator *MdMAPKKK17*. A. Heatmap showing the expression patterns of 13 anthocyanin biosynthetic genes and four regulatory genes in LRS, DRS, and FBM at 0, 2, 4, 6, 8, 10, and 14 DABR. B. Heatmap of correlation analysis between expression levels of anthocyanin-related genes and fruit quality metabolites in LRS, DRS, and FBM during the coloring period. C. Expression level of *MdMAPKKK17* in LRS, DRS, and FBM skins at 0, 2, 4, 6, 8, 10, and 14 DABR. D. Correlation between the *MdMAPKKK17* expression level and anthocyanin content. Data represent mean $\pm$ SE. Statistical analysis was performed using one-way ANOVA followed by Dunnett's test. Different letters indicate significant differences ($P < 0.05$). LRS, light-red stripes; DRS, dark-red stripes; FBM, red-blushed mutant; DABR, days after bag removal.

accumulation, suggesting that cuticle development and anthocyanin synthesis may share common regulatory pathways or that cuticle integrity provides a favorable microenvironment for pigment production (Nadakuduti et al., 2012). These relationships require further investigation.

In this study, the thicker cuticle and denser cell arrangement observed in FBM likely contribute to a more stable cellular microenvironment, thereby facilitating energy-intensive secondary metabolic processes such as anthocyanin biosynthesis. Furthermore, previous research has shown that MdPRE6-like promotes anthocyanin accumulation by regulating morphological features such as cell size (Huang et al., 2024). Our results align with this regulatory mechanism and further reveal that cellular structural differentiation during the critical coloring period may cooperate with the genetic regulatory network of pigment synthesis to jointly determine the final red-skin phenotype.

3.4. Transcriptional dynamics of anthocyanin pathway and identification of a novel repressor for red-skin pattern

To identify the key regulators of red-skin patterns, RNA sequencing (RNA-seq) was conducted on skin tissues from LRS, DRS, and FBM at 4, 8, and 14 DABR. Analysis of the data (NCBI: PRJNA1379484) revealed strong associations of anthocyanin biosynthetic genes (e.g., *MdPAL*, *MdC4H*, *Md4CL*, *MdCHS*, *MdCHI*, *MdF3H*, *MdF3'H*, *MdFLS*, *MdDFR*, *MdANS*, *MdUGT*, *MdGST*, and *MdMATE*) and transcription factors (e.g., *MdMYB10*, *MdMYB114*, *MdbHLH3*, and *MdWD40*) with these red-skin patterns (Jia et al., 2020). RT-qPCR validation confirmed consistent expression gradients, with the lowest levels in LRS, intermediate in DRS, and highest in FBM across multiple time points (Fig. 5A). Diverging from prior studies focused on overall coloration (Yang et al., 2019), we identified distinct expression differences directly correlating with specific red-skin patterns. In summary, differential expression of core MBW complex members serves as a key upstream determinant influencing the phenotypic divergence between red-striped and red-blushed apples, with expression gradients being coordinated with structural gene transcription and reinforcing the MBW complex's central role (Zhao et al., 2025). Notably, 5-azacytidine treatment enhanced anthocyanin content but did not convert striped to blushed patterns (Jia et al., 2020; Telias et al., 2011), confirming that anthocyanin abundance alone is insufficient to determine color patterns and implying additional regulatory mechanisms.

Correlation analysis between gene expression and metabolite levels indicated widespread positive associations between most structural genes/transcription factors and anthocyanin/flavonoid components. Specifically, several key biosynthetic genes (e.g., *MdCHS*, *MdCHI*, *MdF3'H*, *MdANS*, *MdUGT*, *MdGST*, and *MdMATE*) and transcription factors (e.g., *MdMYB10*, *MdMYB114* and *MdWD40*) exhibited positive correlations with anthocyanin, flavonols, and flavones levels and secondary metabolites (Fig. 5B), highlighting the essential role of anthocyanin pathway genes in shaping striped and blushed apple phenotypes (Jia et al., 2020).

Based on the RNA-seq data and metabolomic profiling, further analysis identified *MdMAPKKK17*, a mitogen-activated protein kinase kinase kinase (MAPKKK-class) gene, as strongly linked to red-skin patterns. Its expression was highest in LRS, intermediate in DRS, and lowest in FBM—opposite to the trend of anthocyanin biosynthesis-related genes (Fig. 5C). A significant negative correlation was observed between *MdMAPKKK17* expression and anthocyanin content (Fig. 5D), suggesting a distinct regulatory function in anthocyanin biosynthesis. Furthermore, correlation analysis was performed between the expression levels of *MdMAPKKK17* and those of anthocyanin biosynthetic genes and related regulatory factors in LRS, DRS and FBM throughout the entire coloring period. The heatmap results showed that *MdMAPKKK17* was negatively correlated with all these anthocyanin biosynthesis-related genes, among which significant negative correlations were observed with *C4H*, *4CL*, *CHI*, *F3H*, *FLS*, *GST*, *MATE*, *MYB10*, *MYB114*, *WD40* and

bHLH3 (Fig. S7), suggesting that *MdMAPKKK17* may act as a broad-spectrum negative regulator of anthocyanin biosynthesis, cooperatively inhibiting anthocyanin accumulation by broadly suppressing the expression of multiple structural genes and key transcriptional regulators. This aligns with previous reports indicating that phosphorylation is involved in regulating anthocyanin production (Meng et al., 2016; Sun et al., 2022; Xing et al., 2023; Yang et al., 2021).

3.5. Functional validation of *MdMAPKKK17* as a negative regulator of anthocyanin biosynthesis

Previous studies have reported that ZmMAPKKK45, a Raf-subfamily MAPKKK protein containing an STKc_MAP3K-like domain, confers quantitative resistance to multiple foliar fungal diseases in maize by regulating ROS accumulation, and its mechanism of action is independent of the canonical MAPK cascade (Zhong et al., 2026). In the present study, *MdMAPKKK17* contains a conserved kinase catalytic domain (Fig. 6A). To elucidate the functional role of *MdMAPKKK17* in anthocyanin synthesis, we overexpressed it in 'Fuji' apple fruits via transient assays (Fig. 6B, C) and in 'Orin' calli via stable transformation assays (Fig. 6F, G). *MdMAPKKK17* overexpression reduced red-pigmentation in both tissues (Fig. 6B, F), with significantly reduced anthocyanin content (79.9% and 89.9% decrease in fruits and calli, respectively) compared to the P101F control (Fig. 6D, H). These results confirm *MdMAPKKK17* as a negative regulator of anthocyanin accumulation. This aligns with the conserved role of MAPK signaling in fruit coloration. FvMAPK3 (Strawberry, Mao et al., 2022) and YDA (*Arabidopsis*, Meng et al., 2016) similarly repress anthocyanin accumulation, supporting evolutionary conservation of MAPK-mediated regulation of anthocyanin biosynthesis. Additionally, the expression of anthocyanin biosynthetic genes was significantly downregulated in *MdMAPKKK17*-overexpressing tissues, whereas the transcript levels of the key regulators *MdMYB10* and *MdMYB114* remained unchanged (Fig. 6E, I). This suggests *MdMAPKKK17* inhibits anthocyanin accumulation by modulating the transcriptional activity of these or other anthocyanin-related transcription factors, not their transcriptional expression.

3.6. *MdMAPKKK17* interacts with *MdMYB10* and suppresses its transcriptional activity

Previous studies have demonstrated that the *Arabidopsis* mitogen-activated protein kinase kinase kinase MEKK1 directly interacts with the senescence-associated transcription factor WRKY53 (Miao et al., 2007), and that the rice Group C Raf-like MAPKKK ILA1 interacts with IIP to modulate leaf angle (Ning et al., 2011). Collectively, these findings support the notion that MAPKKK family members can directly interact with transcription factors. In the present study, Y2H assays were performed to examine potential protein–protein interactions between *MdMAPKKK17* and the key anthocyanin-related transcription factors *MdMYB10* and *MdMYB114*. Yeast cells co-transformed with AD-*MdMAPKKK17* and BK-*MdMYB10* grew vigorously on quadruple dropout medium (SD/-Trp/-Leu/-His/-Ade) at all dilution, whereas no growth was observed in the corresponding negative controls (Fig. 7A), indicating a direct physical interaction between *MdMAPKKK17* and *MdMYB10*. By contrast, no interaction was detected between *MdMAPKKK17* and *MdMYB114* (Fig. 7B). Moreover, co-transformation of 35S:*MdMYB10* and 35S:*MdMAPKKK17* with ProMdDFR:LUC resulted in significantly lower relative LUC/REN activity than that of 35S:*MdMYB10* alone (Fig. 7C), collectively demonstrating that *MdMAPKKK17* interacts with *MdMYB10* and suppresses its transcriptional activation. Nonetheless, the detailed molecular mechanism by which *MdMAPKKK17* represses anthocyanin accumulation through phosphorylation regulation of *MdMYB10* or other proteins remains to be fully elucidated in future investigations.

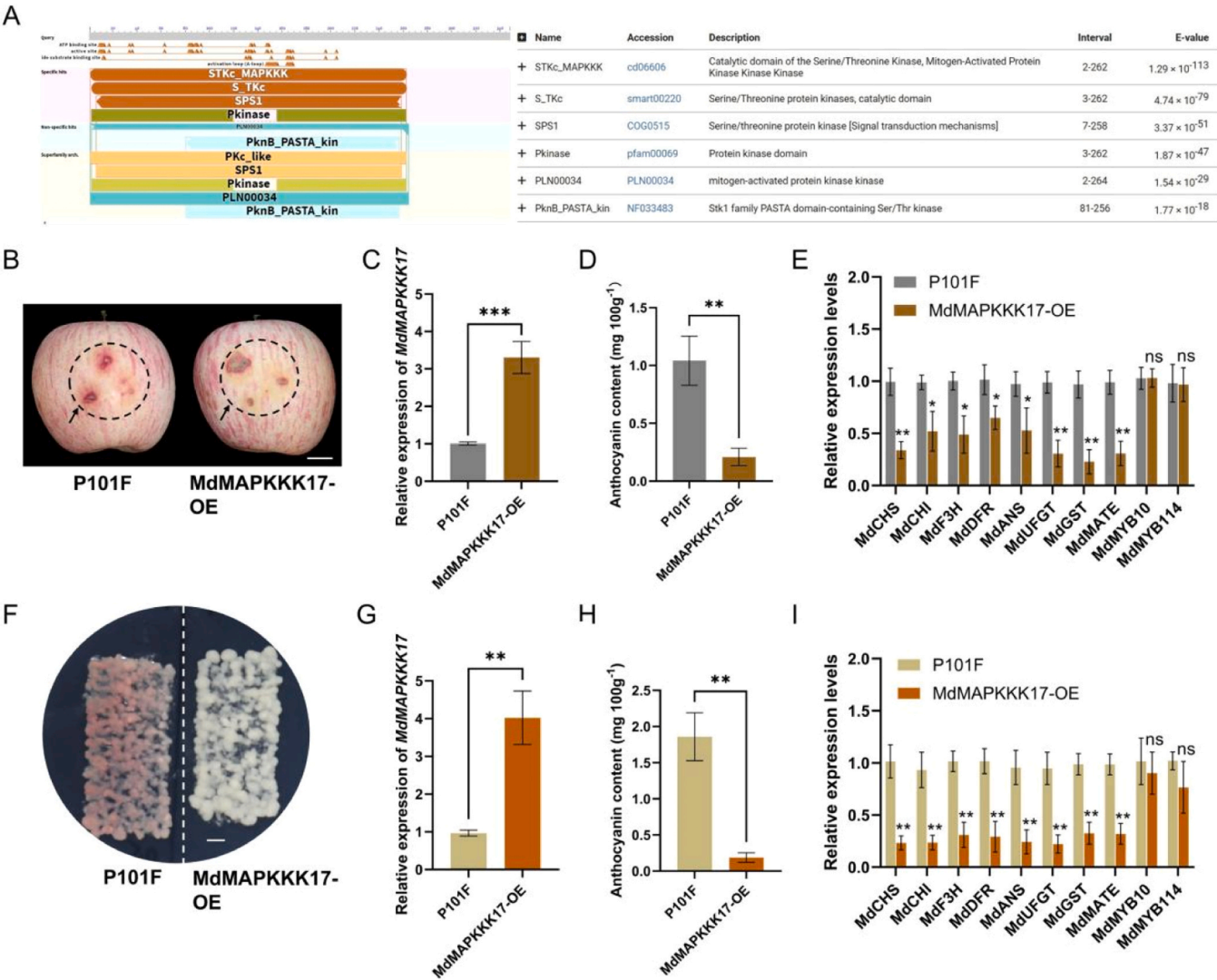


Fig. 6. Functional validation of *MdMAPKKK17*. **A.** Schematic diagram of the amino acid core domain of *MdMAPKKK17*. **B.** Phenotype of *MdMAPKKK17*-over-expressing (*MdMAPKKK17*-OE) and empty P101F 'Fuji' fruit. Scale bar = 2 cm. **C–D.** Levels of *MdMAPKKK17* expression (**C**) and anthocyanin (**D**) in *MdMAPKKK17*-OE and P101F 'Fuji' skin. **E.** Relative expression levels of anthocyanin-related genes in *MdMAPKKK17*-OE and P101F 'Fuji' skin. **F.** Phenotype of *MdMAPKKK17*-OE and P101F 'Orin' calli. Scale bars = 1 cm. **G–H.** Levels of *MdMAPKKK17* expression (**G**) and anthocyanin (**H**) in *MdMAPKKK17*-OE and P101F 'Orin' calli. **I.** Relative expression levels of anthocyanin-related genes in *MdMAPKKK17*-OE and P101F 'Orin' calli. Data represent mean $\pm$ SE. Asterisks indicate significant differences as determined by a Student's *t*-test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$); ns indicates no significant difference.

4. Conclusion

This multi-omics study systematically deciphers the physiological and molecular mechanisms underlying distinct red-skin patterns in apple. Physiological, metabolomic, and microstructural analyses confirmed that anthocyanins, carotenoids, flavonoids (six anthocyanins, fourteen flavonols, twelve flavones), fifteen phenolic acids, four amino acids, one alkaloid, three lignans and coumarins, two tannins, thicker cuticles, and denser epidermal cells are positively correlated with red-skin patterning. Notably, we identified *MdMAPKKK17* as a novel negative regulator of anthocyanin biosynthesis linked to red-skin patterns, which functions by interacting with *MdMYB10* and suppressing its transcriptional activity (Fig. 8). Collectively, these findings demonstrate that apple red-skin patterning is orchestrated by a coordinated network involving specific metabolic fluxes, transcriptional regulation and supportive anatomical features. The study provides concrete molecular targets, particularly *MdMAPKKK17*, for future breeding strategies aimed at precisely tailoring fruit color patterns to enhance both visual appeal and nutritional quality.

CRedit authorship contribution statement

Xiuqi Sui: Conceptualization. **Xinyuan Wang:** Investigation. **Dongjie Jia:** Writing – review & editing, Visualization, Validation, Project administration, Funding acquisition, Conceptualization. **Yongbing Yuan:** Writing – review & editing, Validation, Funding acquisition, Conceptualization. **Xudong Guo:** Software, Methodology, Investigation. **Meihua Mo:** Software, Methodology, Investigation. **Huimin Wang:** Investigation. **Xiaoya Han:** Methodology, Investigation. **Yishu Xu:** Writing – original draft, Software, Methodology, Investigation, Formal analysis. **Chunyu Li:** Writing – original draft, Software, Methodology, Investigation, Formal analysis. **Yuchen Li:** Writing – original draft, Software, Methodology, Investigation, Formal analysis.

Declaration of Competing Interest

In the submission, no conflict of interest exists in the submission of this manuscript, and manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work

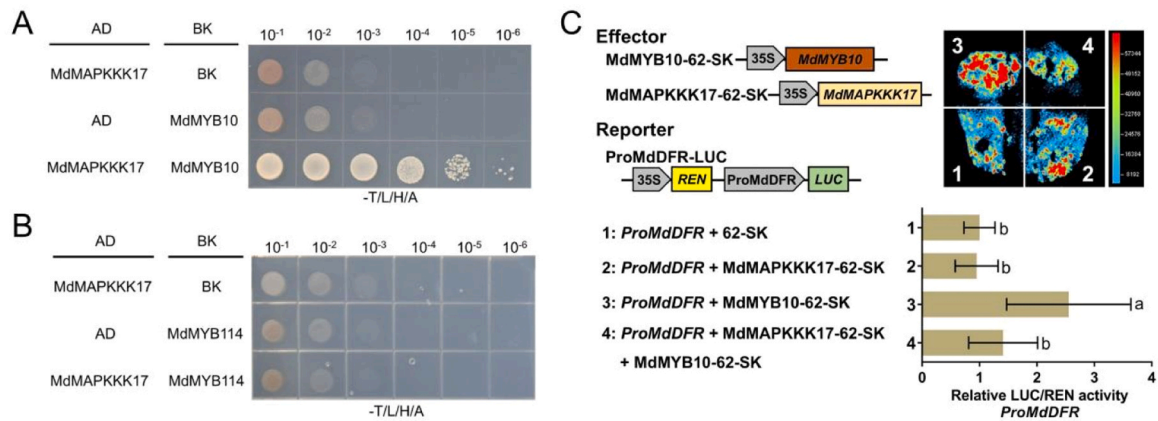


Fig. 7. MdMAPKKK17 interacts with MdMYB10 and enhances its transcriptional activity. A. Y2H assay was performed to detect the protein interaction between MdMAPKKK17 and MdMYB10. B. Y2H assay revealed that MdMAPKKK17 did not interact with MdMYB114. C. Dual LUC assays confirmed that MdMAPKKK17 represses the transcriptional activity of MdMYB10. Data represent mean $\pm$ SE. Statistical analysis was performed using one-way ANOVA followed by Dunnett's test. Different letters indicate significant differences ($P < 0.05$). Y2H, yeast two-hybrid; LUC, firefly luciferase.

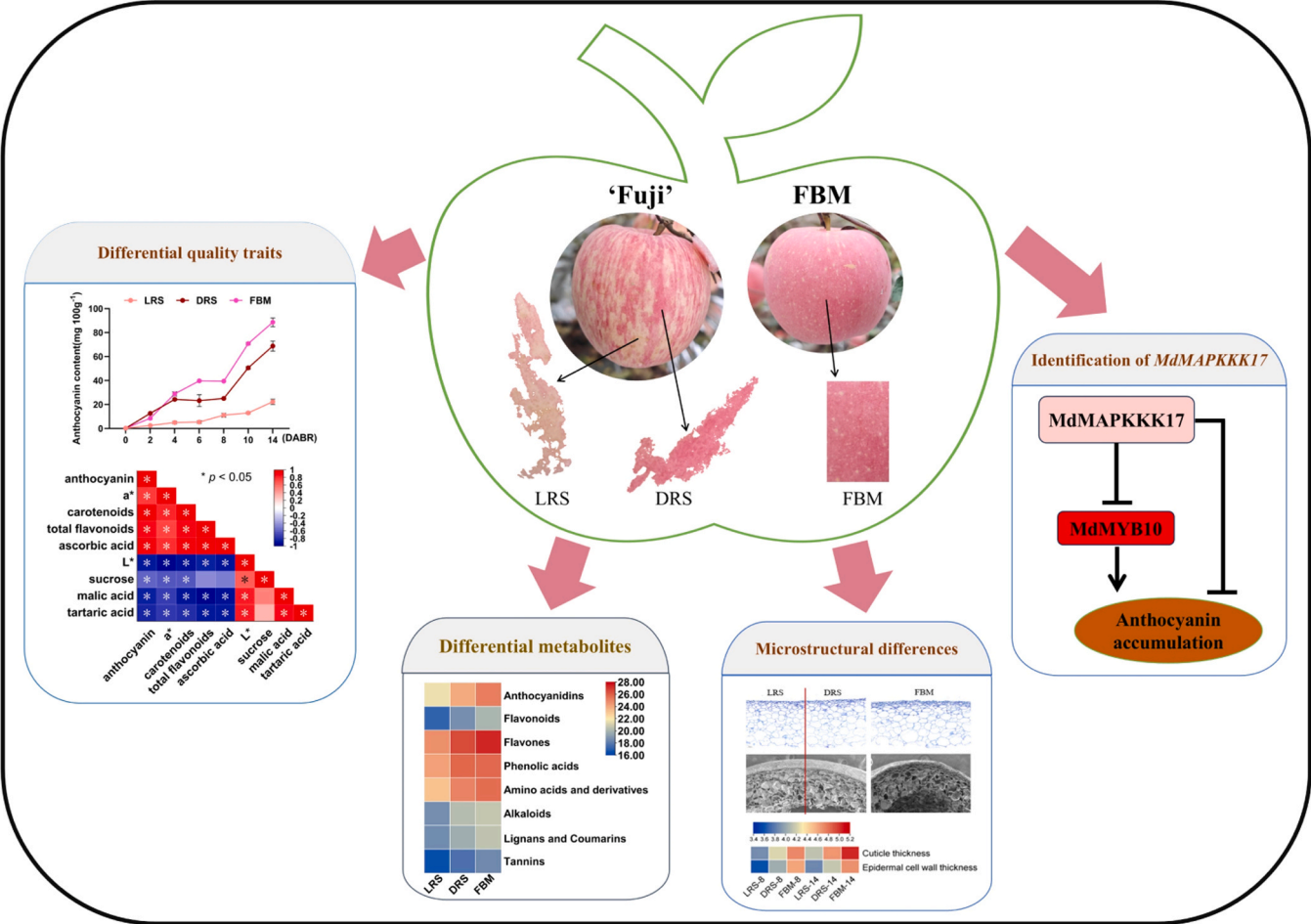


Fig. 8. A multi-omics model systematically deciphers the physiological and molecular mechanisms underlying distinct red-skin patterns in apple. Physiological, metabolomic, and microstructural analyses revealed that total anthocyanin, carotenoid, flavonoids (including six anthocyanins, fourteen flavonols, twelve flavones), fifteen phenolic acids, four amino acids and derivatives, one alkaloid, three lignans and coumarins, two tannins, thicker cuticles, and denser epidermal cells are positively correlated with red-skin patterning. Importantly, MdMAPKKK17 was identified as a novel negative regulator of anthocyanin biosynthesis linked to red-skin patterns, acting through interaction with MdMYB10 and suppression of its transcriptional activity.

described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part. All the authors listed have approved the manuscript that is enclosed.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.postharvbio.2026.114531](https://doi.org/10.1016/j.postharvbio.2026.114531).

Data availability

Data will be made available on request.

References

- Alabd, A., Ahmad, M., Zhang, X., Gao, Y., Peng, L., Zhang, L., Ni, J., Bai, S., Teng, Y., 2022. Light-responsive transcription factor PpWRKY44 induces anthocyanin accumulation by regulating PpMYB10 expression in pear. *Hortic. Res.* 9, 199. <https://doi.org/10.1093/hr/uhac199>.
- Alizadeh, Z., Fattahi, M., 2021. Essential oil, total phenolic, flavonoids, anthocyanins, carotenoids and antioxidant activity of cultivated Damask Rose (*Rosa damascena*) from Iran: with chemotyping approach concerning morphology and composition. *Sci. Hortic.* 290, 110341. <https://doi.org/10.1016/j.scienta.2021.110341>.
- Ampomah-Dwamena, C., Tomes, S., Thirumawithana, A.H., Elborough, C., Bhargava, N., Rebstock, R., Sutherland, P., Ireland, H., Allan, A.C., Espley, R.V., 2022. Overexpression of *PSY1* increases fruit skin and flesh carotenoid content and reveals associated transcription factors in apple (*Malus × domestica*). *Front. Plant. Sci.* 13, 967143. <https://doi.org/10.3389/fpls.2022.967143>.
- Anton, H., Bischof, S., Bringmann, G., Riederer, M., Hildebrandt, U., 2010. Very-long-chain aldehydes promote in vitro prepenetration processes of *Blumeria graminis* in a dose and chain length-dependent manner. *New. Phytol.* 188 (4), 907–1150. <https://doi.org/10.1111/j.1469-8137.2010.03419.x>.
- Bu, H., Gu, G., Hu, Y., Yang, Y., Yang, L., Yuan, H., Yu, W., 2025. Research advances in the synthesis and regulation of apple anthocyanins. *Biology* 14 (10), 1322. <https://doi.org/10.3390/biology14101322>.
- Chen, L., Hu, B., Qin, Y., Hu, G., Zhao, J., 2019. Advance of the negative regulation of anthocyanin biosynthesis by MYB transcription factors. *Plant. Physiol. Biochem.* 136, 178–187. <https://doi.org/10.1016/j.plaphy.2019.01.024>.
- Chen, L., Wang, X., Cui, L., Li, Y., Liang, Y., Wang, S., Chen, Y., Zhou, L., Zhang, Y., Li, F., 2022a. Transcriptome and metabolome analyses reveal anthocyanins pathways associated with fruit color changes in plum (*Prunus salicina* Lindl. PeerJ 10, e14413. <https://doi.org/10.7717/peerj.14413>.
- Chen, X., Gao, Q., Liao, S., Zou, Y., Yan, J., Li, Q., 2022b. Co-Pigmentation mechanism and thermal reaction kinetics of mulberry anthocyanins with different phenolic acids. *Foods* 11 (23), 3806. <https://doi.org/10.3390/foods11233806>.
- Chen, K., Pariyani, R., Li, Y., Li, J., Qiao, X., 2025. Metabolomics profiling of apples of different cultivars and growth locations. *Food Innov. Adv.* 4 (1), 116–126. <https://doi.org/10.48130/fia-0025-0012>.
- Cheng, C., Guo, Z., Li, H., Mu, X., Wang, P., Zhang, S., Yang, T., Cai, H., Wang, Q., Lü, P., Zhang, J., 2022. Integrated metabolic, transcriptomic and chromatin accessibility analyses provide novel insights into the competition for anthocyanins and flavonols biosynthesis during fruit ripening in red apple. *Front. Plant. Sci.* 13, 975356. <https://doi.org/10.3389/fpls.2022.975356>.
- Cliff, M., Sandford, K., Wismer, W., Hampson, C., 2002. Use of digital images for evaluation of factors responsible for visual preference of apples by consumers. *HortScience* 37 (7), 1127–1131. <https://doi.org/10.21273/HORTSCI.37.7.1127>.
- Dang, Q., Sha, H., Nie, J., Wang, Y., Yuan, Y., Jia, D., 2021. An apple (*Malus domestica*) AP2/ERF transcription factor modulates carotenoid accumulation. *Hortic. Res.* 8 (1), 223. <https://doi.org/10.1038/s41438-021-00694-w>.
- Das, P.R., Darwish, A.G., Ismail, A., Haikal, A.M., Gajjar, P., Balasubramani, S.P., Sheikh, M.B., Tsovala, V., Soliman, K.F.A., Sherif, S.M., El-Sharkawy, I., 2022. Diversity in blueberry genotypes and developmental stages enables discrepancy in the bioactive compounds, metabolites, and cytotoxicity. *Food Chem.* 374, 131632. <https://doi.org/10.1016/j.foodchem.2021.131632>.
- De Rosso, V.V., Mercadante, A.Z., 2007. The high ascorbic acid content is the main cause of the low stability of anthocyanin extracts from acerola. *Food Chem.* 103 (3), 935–943. <https://doi.org/10.1016/j.foodchem.2006.12.039>.
- Du Toit, D., Bravo Iniguez, A., Sun, Q., Zhu, M.J., 2024. Impact of cultivation practices and storage conditions on polyphenol content and antioxidant activity in ‘WA 38’, ‘Granny Smith’, and ‘Cripps Pink’ apples. *Postharvest Biol. Technol.* 217, 113100. <https://doi.org/10.1016/j.postharvbio.2024.113100>.
- Duan, W., Shao, W., Lin, W., Yuan, L., Lu, Q., Chen, L., Zagorchev, L., Li, J., 2021. Integrated metabolomics and transcriptomics reveal the differences in fruit quality of the red and white *Fragaria pentaphylla* morphs. *Food Biosci.* 40, 100896. <https://doi.org/10.1016/j.fbio.2021.100896>.
- Duan, S., Kim, C.K., Kim, J.H., Eom, S.H., 2025. Metabolic factors regulating the inhibition of anthocyanin biosynthesis in ‘Shinano gold’ apple. *Postharvest Biol. Technol.* 230, 113341. <https://doi.org/10.1016/j.postharvbio.2025.113341>.
- Duan, S., Kim, J.H., Kim, M.J., Gil, C.S., Kang, I.K., Eom, S.H., 2026. Effect of low-temperature storage regimes on the UV-B sensitivity of apple anthocyanin. *Postharvest Biol. Technol.* 232, 114013. <https://doi.org/10.1016/j.postharvbio.2025.114013>.
- El-Sharkawy, I., Liang, D., Xu, K., 2015. Transcriptome analysis of an apple (*Malus × domestica*) yellow fruit somatic mutation identifies a gene network module highly associated with anthocyanin and epigenetic regulation. *J. Exp. Bot.* 66 (22), 7359–7376. <https://doi.org/10.1093/jxb/erv433>.
- Felicitetti, E., Mattheis, J.P., 2010. Quantification and histochemical localization of ascorbic acid in ‘Delicious’, ‘Golden Delicious’, and ‘Fuji’ apple fruit during on-tree development and cold storage. *Postharvest Biol. Technol.* 56 (3), 215–221. <https://doi.org/10.1016/j.postharvbio.2010.01.010>.
- Fu, J., Liao, L., Sun, J., Yang, K., Lu, Z., Huang, Y., Lin, X., Zhu, K., Deng, X., Xu, Q., Wang, X., 2025. CsBBX24 regulates both carotenoid and anthocyanin biosynthesis in citrus. *Plant. Physiol.* 199 (4), 2645–2659. <https://doi.org/10.1093/plphys/kiaf539>.
- Giménez-Sanchis, A., Bermejo, A., Besada, C., 2024. Changes in the sugars and volatile compounds profiles associated with anthocyanin accumulation in oranges: Blood vs. blond varieties, and slightly pigmented vs. intensely pigmented blood fruit. *Food Res. Int.* 197 (1), 115199. <https://doi.org/10.1016/j.foodres.2024.115199>.
- Gong, J., Zeng, Y., Xu, Q., Chen, J., Yin, X., Xu, C., 2021. Red light-induced kumquat fruit coloration is attributable to increased carotenoid metabolism regulated by FcrNAC22. *J. Exp. Bot.* 72 (18), 6274–6290. <https://doi.org/10.1093/jxb/erab238>.
- Grosso, G., 2025. Anthocyanins for vascular health: plausible role of gut microbiota. *Int. J. Food Sci. Nutr.* 76 (2), 93–94. <https://doi.org/10.1080/09637486.2025.2475332>.
- Hu, Y., Gong, Z., Yan, Y., Zhang, J., Shao, A., Li, H., Wang, P., Zhang, S., Cheng, C., Zhang, J., 2024. ChBBX6 and ChBBX18 are positive regulators of anthocyanins biosynthesis and carotenoids degradation in *Cerasus humilis*. *Int. J. Biol. Macromol.* 282 (4), 137195. <https://doi.org/10.1016/j.ijbiomac.2024.137195>.
- Huang, J.J., Huang, Y.P., Li, W.F., Mao, J., Chen, B.H., 2024. Cloning of *PRE6-like* gene and analysis of its function in anthocyanin synthesis in apple. *J. Fruit. Sci.* 41 (5), 812–823. <https://doi.org/10.13925/j.cnki.gsxb.20240068>.
- Ibrahim, M.H., Jaafar, H.Z., Rahmat, A., Rahman, Z.A., 2012. Involvement of nitrogen on flavonoids, glutathione, anthocyanin, ascorbic acid and antioxidant activities of Malaysian medicinal plant *Labisia pumila* Blume (Kacip Fatimah). *Int. J. Mol. Sci.* 13 (1), 393–408. <https://doi.org/10.3390/ijms13010393>.
- Jaakola, L., Määttä, K., Pirttilä, A.M., Törrönen, R., Kärenlampi, S., Hohtola, A., 2002. Expression of genes involved in anthocyanin biosynthesis in relation to anthocyanin, proanthocyanidin, and flavonol levels during bilberry fruit development. *Plant. Physiol.* 130 (2), 729–739. <https://doi.org/10.1104/pp.006957>.
- Jaeger, S.R., Antúnez, L., Ares, G., Swaney-Stueve, M., Jin, D., Harker, F.R., 2018. Quality perceptions regarding external appearance of apples: Insights from experts and consumers in four countries. *Postharvest Biol. Technol.* 146, 99–107. <https://doi.org/10.1016/j.postharvbio.2018.08.014>.
- Jia, D., Fan, L., Shen, J., Qin, S., Li, F., Yuan, Y., 2019. Genetic transformation of the astaxanthin biosynthetic genes *bkt* and *crtr4* into apple tree to increase photooxidation resistance. *Sci. Hortic.* 243, 428–433. <https://doi.org/10.1016/j.scienta.2018.08.058>.
- Jia, D., Li, Z., Dang, Q., Shang, L., Shen, J., Leng, X., Wang, Y., Yuan, Y., 2020. Anthocyanin biosynthesis and methylation of the *MdMYB10* promoter are associated with the red blushed-skin mutant in the red striped-skin ‘Changfu 2’ apple. *J. Agric. Food Chem.* 68 (15), 4292–4304. <https://doi.org/10.1021/acs.jafc.9b07098>.
- Jia, D., Wu, P., Shen, F., Li, W., Zheng, X., Wang, Y., Yuan, Y., Zhang, X., Han, Z., 2021. Genetic variation in the promoter of an R2R3-MYB transcription factor determines fruit malate content in apple (*Malus domestica* Borkh.). *Plant. Physiol.* 186 (1), 549–568. <https://doi.org/10.1093/plphys/kiab098>.
- Kong, X., Xu, M., Wang, K., Chen, Q., Han, L., Li, Q., Guo, Q., Wan, H., Nie, J., 2024. Development of a comprehensive evaluation system for the sensory and nutritional quality of winter jujube (*Ziziphus jujuba* Mill. cv. Dongzao). *LWT-Food Sci. Technol.* 194, 115777. <https://doi.org/10.1016/j.lwt.2024.115777>.
- Kuang, L., Wang, Z., Cheng, Y., Li, H., Li, J., Shen, Y., Zhang, J., Xu, G., 2024. Cultivar and origin authentication of ‘Fuji’ and ‘gala’ apples from two dominant origins of China based on quality attributes. *Food Chem. X* 23, 101643. <https://doi.org/10.1016/j.fochx.2024.101643>.
- Lara, I., Heredia, A., Domínguez, E., 2019. Shelf life potential and the fruit cuticle: the unexpected player. *Front. Plant. Sci.* 10, 770. <https://doi.org/10.3389/fpls.2019.00770>.
- Li, X.J., Hou, J.H., Zhang, G.L., Wang, Z.R., Liu, S.C., Cheng, H.B., 2004. Comparison of anthocyanin accumulation and morpho-anatomical features in apple skin during color formation at two habitats. *Sci. Hortic.* 99, 41–53. [https://doi.org/10.1016/S0304-4238\(03\)00086-4](https://doi.org/10.1016/S0304-4238(03)00086-4).
- Li, S., Wang, W., Gao, J., Yin, K., Wang, R., Wang, C., Petersen, M., Mundy, J., Qiu, J.L., 2016. MYB75 phosphorylation by MPK4 is required for light-induced anthocyanin accumulation in *Arabidopsis*. *Plant. Cell.* 28 (11), 2866–2883. <https://doi.org/10.1105/tpc.16.00130>.
- Li, S., Yang, N., Chen, L., 2022. Paraffin section observation of flower bud differentiation of *Chimonanthus praecox* in Kunming and comparison of the differentiation processes in different regions, China. *Hortic. Plant. J.* 8 (2), 221–229. <https://doi.org/10.1016/j.hbj.2021.11.001>.
- Li, H.L., Xu, R.R., Guo, X.L., Liu, Y.J., You, C.X., Han, Y., An, J.P., 2024. The MdNAC72-MdABI5 module acts as an interface integrating jasmonic acid and gibberellin signals and undergoes ubiquitination-dependent degradation regulated by MdSINA2 in apple. *New. Phytol.* 243 (3), 997–1016. <https://doi.org/10.1111/nph.19888>.
- Li, F., Tahir, M.M., Yang, C., Weng, Z., Zhu, W., Zhang, Y., Zhou, K., Deng, Q., Qian, M., Wu, H., 2025a. Postharvest UV-A treatment promotes mango fruit pigmentation and

- ripening in a dose-dependent manner. *Postharvest Biol. Technol.* 230, 113836. <https://doi.org/10.1016/j.postharvbio.2025.113836>.
- Li, W., Lin, M., Li, Y., Liang, Y., Zhang, Z., 2025b. Comparative analysis of nutrition quality and volatile compounds in mature European and Asian red pears. *Food Chem.* 495 (2), 146448. <https://doi.org/10.1016/j.foodchem.2025.146448>.
- Lin, X., Lyng, J., O'Donnell, C., Sun, D.W., 2022. Effects of dielectric properties and microstructures on microwave-vacuum drying of mushroom (*Agaricus bisporus*) caps and stipes evaluated by non-destructive techniques. *Food Chem.* 367, 130698. <https://doi.org/10.1016/j.foodchem.2021.130698>.
- Mao, W., Han, Y., Chen, Y., Sun, M., Feng, Q., Li, L., Liu, L., Zhang, K., Wei, L., Han, Z., Li, B., 2022. Low temperature inhibits anthocyanin accumulation in strawberry fruit by activating FvMAPK3-induced phosphorylation of FvMYB10 and degradation of Chalcone Synthase 1. *Plant. Cell.* 34 (4), 1226–1249. <https://doi.org/10.1093/plcell/koac006>.
- Mao, Y., Luo, J., Cai, Z., 2025. Biosynthesis and regulatory mechanisms of plant flavonoids: A Review. *Plants* 14 (12), 1847. <https://doi.org/10.3390/plants14121847>.
- Meng, L.S., Li, Y.Q., Liu, M.Q., Jiang, J.H., 2016. The *Arabidopsis* *ANGUSTIFOLIA3-YODA* gene cascade induces anthocyanin accumulation by regulating sucrose levels. *Front. Plant. Sci.* 7, 1728. <https://doi.org/10.3389/fpls.2016.01728>.
- Miao, Y., Laun, T.M., Smykowski, A., Zentgraf, U., 2007. *Arabidopsis* MEK1 can take a short cut: it can directly interact with senescence-related WRKY53 transcription factor on the protein level and can bind to its promoter. *Plant. Mol. Biol.* 65 (1–2), 63–76. <https://doi.org/10.1007/s11103-007-9198-z>.
- Nadakuduti, S.S., Pollard, M., Kosma, D.K., Allen, C.J., Ohlrogge, J.B., Barry, C.S., 2012. Pleiotropic phenotypes of the sticky peel mutant provide new insight into the role of CUTIN DEFICIENT2 in epidermal cell function in tomato. *Plant. Physiol.* 159 (3), 945–960. <https://doi.org/10.1104/pp.112.198374>.
- Ning, J., Zhang, B., Wang, N., Zhou, X., Xiong, L., 2011. Increased leaf angle1, a Raf-like MAPKKK that interacts with a nuclear protein family, regulates mechanical tissue formation in the Lamina joint of rice. *Plant. Cell.* 23 (12), 4334–4347. <https://doi.org/10.1105/tpc.111.093419>.
- Rezende, F.M., Rossi, M., Furlan, C.M., 2025. Molecular control of flower colour change in angiosperms. *Plants* 14 (14), 2185. <https://doi.org/10.3390/plants14142185>.
- Shi, C., Liu, L., Wei, Z., Liu, J., Li, M., Yan, Z., Gao, D., 2022. Anthocyanin accumulation and molecular analysis of correlated genes by metabolomics and transcriptomics in sister line apple cultivars. *Life* 12 (8), 1246. <https://doi.org/10.3390/life12081246>.
- Sun, C., Wang, C., Zhang, W., Liu, S., Wang, W., Yu, X., Song, T., Yu, M., Yu, W., Qu, S., 2021. The R2R3-type MYB transcription factor MdMYB90-like is responsible for the enhanced skin color of an apple bud sport mutant. *Hortic. Res.* 8, 156. <https://doi.org/10.1038/s41438-021-00590-3>.
- Sun, X., Li, X., Wang, Y., Xu, J., Jiang, S., Zhang, Y., 2022. MdMKK9-mediated regulation of anthocyanin synthesis in red-fleshed apple in response to different nitrogen signals. *Int. J. Mol. Sci.* 23 (14), 7755. <https://doi.org/10.3390/ijms23147755>.
- Telias, A., Lin-Wang, K., Stevenson, D.E., Cooney, J.M., Hellens, R.P., Allan, A.C., Hoover, E.E., Bradeen, J.M., 2011. Apple skin patterning is associated with differential expression of MYB10. *BMC Plant. Biol.* 11, 93. <https://doi.org/10.1186/1471-2229-11-93>.
- Trouillas, P., Sancho-García, J.C., De Freitas, V., Gierschner, J., Otyepka, M., Dangles, O., 2016. Stabilizing and modulating color by copigmentation: insights from theory and experiment. *Chem. Rev.* 116 (9), 4937–4982. <https://doi.org/10.1021/acs.chemrev.5b00507>.
- Vimolmangkang, S., Han, Y., Wei, G., Korban, S.S., 2013. An apple MYB transcription factor, MdMYB3, is involved in regulation of anthocyanin biosynthesis and flower development. *BMC Plant. Biol.* 13, 176. <https://doi.org/10.1186/1471-2229-13-176>.
- Wang, Z., Meng, D., Wang, A., Li, T., Jiang, S., Cong, P., Li, T., 2013. The methylation of the *PcMYB10* promoter is associated with green-skinned sport in Max Red Bartlett pear. *Plant. Physiol.* 162 (2), 885–896. <https://doi.org/10.1104/pp.113.214700>.
- Wang, S., Wang, T., Li, Q., Xu, C., Tian, J., Wang, Y., Zhang, X., Xu, X., Han, Z., Wu, T., 2022. Phosphorylation of MdERF17 by MdMPK4 promotes apple fruit peel degreening during light/dark transitions. *Plant. Cell.* 34 (5), 1980–2000. <https://doi.org/10.1093/plcell/koac049>.
- Wang, Y., Li, S., Shi, Y., Lv, S., Zhu, C., Xu, C., Zhang, B., Allan, A.C., Grierson, D., Chen, K., 2024a. The R2R3 MYB Ruby1 is activated by two cold responsive ethylene response factors, via the retrotransposon in its promoter, to positively regulate anthocyanin biosynthesis in citrus. *Plant. J.* 119 (3), 1433–1448. <https://doi.org/10.1111/tpj.16866>.
- Wang, J., Zhao, Y., Sun, B., Yang, Y., Wang, S., Feng, Z., Li, J., 2024b. The structure of anthocyanins and the copigmentation by common micromolecular copigments: A review. *Food Res. Int.* 176, 113837. <https://doi.org/10.1016/j.foodres.2023.113837>.
- Wu, J., Chen, Y., Huang, Y., Hao, B., Dai, S., Zhao, L., Zhao, Z., Zhao, C., Zhang, L., Li, Y., Xu, X., Li, N., Huang, A.C., Zhou, J., Tan, M., Zhu, W., Zhao, Q., 2024. The cytosolic aminotransferase VAS1 coordinates aromatic amino acid biosynthesis and metabolism. *Sci. Adv.* 10 (2), eadk0738. <https://doi.org/10.1126/sciadv.adk0738>.
- Xing, Y., Sun, W., Sun, Y., Li, J., Zhang, J., Wu, T., Song, T., Yao, Y., Tian, J., 2023. MPK6-mediated HY5 phosphorylation regulates light-induced anthocyanin accumulation in apple fruit. *Plant. Biotechnol. J.* 21 (2), 283–301. <https://doi.org/10.1111/pbi.13941>.
- Xu, W., Dubos, C., Lepiniec, L., 2015. Transcriptional control of flavonoid biosynthesis by MYB-bHLH-WDR complexes. *Trends Plant. Sci.* 20 (3), 176–185. <https://doi.org/10.1016/j.tplants.2014.12.001>.
- Xue, X., Tian, S., Chen, R., Han, X., Wang, J., Zhao, X., 2022. Clarifying the mechanisms of the light-induced color formation of apple peel under dark conditions through metabolomics and transcriptomic analyses. *Front. Plant. Sci.* 13, 946115. <https://doi.org/10.3389/fpls.2022.946115>.
- Yang, T., Ma, H., Zhang, J., Wu, T., Song, T., Tian, J., Yao, Y., 2019. Systematic identification of long noncoding RNAs expressed during light-induced anthocyanin accumulation in apple fruit. *Plant. J.* 100 (3), 572–590. <https://doi.org/10.1111/tpj.14470>.
- Yang, T., Ma, H., Li, Y., Zhang, Y., Zhang, J., Wu, T., Song, T., Yao, Y., Tian, J., 2021. Apple *MPK4* mediates phosphorylation of MYB1 to enhance light-induced anthocyanin accumulation. *Plant. J.* 106 (6), 1728–1745. <https://doi.org/10.1111/tpj.15267>.
- Yang, R., Yang, Y., Hu, Y., Yin, L., Qu, P., Wang, P., Mu, X., Zhang, S., Xie, P., Cheng, C., 2023. Comparison of bioactive compounds and antioxidant activities in differentially pigmented *Cerasus humilis* fruits. *Molecules* 28 (17), 6272. <https://doi.org/10.3390/molecules28176272>.
- Yang, M., Xiang, Y., Luo, Z., Gao, Y., Wang, L., Hu, Q., Dong, Y., Qi, M., Li, D., Liu, L., Káčániová, M., Ban, Z., Li, L., 2024. Light-responsive transcription factors VvHYH and VvGATA24 mediate wax terpenoid biosynthesis in *Vitis vinifera*. *Plant. Physiol.* 196 (2), 1546–1561. <https://doi.org/10.1093/plphys/kiae366>.
- Zhang, L., Hu, J., Han, X., Li, J., Gao, Y., Richards, C.M., Zhang, C., Tian, Y., Liu, G., Gul, H., Wang, D., Tian, Y., Yang, C., Meng, M., Yuan, G., Kang, G., Wu, Y., Wang, K., Zhang, H., Wang, D., Cong, P., 2019. A high-quality apple genome assembly reveals the association of a retrotransposon and red fruit colour. *Nat. Commun.* 10, 1494. <https://doi.org/10.1038/s41467-019-09518-x>.
- Zhang, Q., Wang, L., Wang, Z., Zhang, R., Liu, P., Liu, M., Liu, Z., Zhao, Z., Wang, L., Chen, X., Xu, H., 2021. The regulation of cell wall lignification and lignin biosynthesis during pigmentation of winter jujube. *Hortic. Res.* 8 (1), 238. <https://doi.org/10.1038/s41438-021-00670-4>.
- Zhang, N., Jing, P., 2023. Red cabbage anthocyanins attenuate cognitive impairment by attenuating neuroinflammation and regulating gut microbiota in aging mice. *J. Agric. Food Chem.* 71 (41), 15064–15072. <https://doi.org/10.1021/acs.jafc.3c03183>.
- Zhang, M., Zhao, J., Wang, N., Hu, D.G., Borejsza-Wysocka, E., Cheng, L., 2025. Overexpression of a tonoplast malate transporter gene leads to enhanced anthocyanin biosynthesis in apple (in press.). *Plant. Biotechnol. J.* <https://doi.org/10.1111/pbi.70368>.
- Zhao, A.L., Xue, X.F., Wang, Y.K., Sui, C.L., Ren, H.Y., Li, D.K., 2016. The sugars and organic acids composition in fruits of different Chinese jujube cultivars of different development stages. *Acta Hortic.* 53 (6), 1175–1185. <https://doi.org/10.16420/j.issn.0513-353x.2015-0856>.
- Zhao, M., Zhang, M., Sun, W., Duan, S., Zhang, J., Song, T., Wang, Y., Yao, Y., Tian, J., 2025. Genome assembly reveals MdZAT5 coordinates anthocyanin biosynthesis in apple fruit peel and flesh by interacting with MdHY5 (in press.). *Plant. Biotechnol. J.* <https://doi.org/10.1111/pbi.70434>.
- Zheng, X.D., Wang, Z., Tan, M.N., Dong, M., Pan, S.X., Cao, N., Qin, L., 2025. A comprehensive study on the mechanism of apple flavor quality formation: Comparison of different apple cultivars in the Weihai region. *Food Chem.* 489, 144894. <https://doi.org/10.1016/j.foodchem.2025.144894>.
- Zhong, T., Olukolu, B., Bian, Y., Xue, S., Wissner, R.J., Ojiambo, P.S., Holland, J.B., Yang, Q., Balint-Kurti, P., 2026. The maize mitogen-activated protein kinase kinase gene *ZmMAPKKK45* is associated with multiple disease resistance. *New Phytol.* 249 (6), 3003–3020. <https://doi.org/10.1111/nph.70828>.
- Zou, Q., Bao, T., Yu, L., Xu, H., Liu, W., Li, Z., Zhu, Y., Chen, R., Hou, X., Zhang, Z., Wang, N., Chen, X., 2025. The regulatory module MdCPL-MdILR3L mediates the synthesis of ascorbic acid and anthocyanin in apple. *Plant. Biotechnol. J.* 23 (4), 1101–1117. <https://doi.org/10.1111/pbi.14567>.