Content, Composition, and Biosynthesis of Anthocyanin in *Fragaria* Species: A Review

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Abstract. **Anthocyanins are responsible for fruit coloration and are beneficial to human health. The fruits of cultivated strawberry (*Fragaria ×ananassa*) varieties are colorful, a trait that attracts consumers. The fruits of wild *Fragaria* species, close relatives of the cultivated strawberry, vary in color. In this review, we describe the content and composition of anthocyanins in cultivated and wild strawberry varieties. We also explore the biosynthetic pathway of anthocyanins, including their transcriptional regulation mechanisms. Additionally, we discuss the effect of environmental factors on anthocyanin accumulation. This review will inform further studies toward developing anthocyanin-rich strawberries via environmental control and exogenous application of compounds.**

Anthocyanins are one of the pigments contributing to fruit color (Li et al. 2019a). In plants, anthocyanins attract pollinators and seed dispersers and enhance plant tolerance to biotic and abiotic stress (Krüger et al. 2021; Treutter 2005). Moreover, anthocyanins are flavonoids with strong antioxidant and free radical scavenging properties (Garcia and Blesso 2021; Kong et al. 2003). Fruit color is a crucial attribute influencing consumer preferences (Li et al. 2018). Anthocyanins also possess beneficial health effects against various disorders (Belwal et al. 2017; Hartman et al. 2006; Henriques et al. 2020; Jayaprakasam et al. 2005, 2006; Seeram et al. 2006; Li et al. 2021a, 2021b; Mattioli et al. 2020; Shishtar et al. 2020). Therefore, anthocyanins have received significant research focus in recent years (Li et al. 2019b).

Cultivated strawberries (*Fragaria ×ananassa* Duch.), belonging to the genus *Fragaria*, are famous for their colorful appearance and delectable flavor. Currently, strawberries are grown worldwide, second only to grapes in global production (Dzhanfezova et al. 2020; Pillet et al. 2015; Qin et al. 2008; Sirijan et al. 2020; Zhao et al. 2021). Anthocyanin accumulation is responsible for the colorful appearance of strawberries (Sirijan et al. 2020; Zhao et al. 2021). Thus, various varieties of strawberries differ in appearance ranging from white to red, depending on the anthocyanin content and composition in their receptacles and achenes (Cheel et al. 2005; Lin et al. 2018; Wang et al. 2014). The wild *Fragaria* species, a close relative of cultivated strawberry, exhibits diverse appearances attributed to variations in anthocyanin accumulation in its fruits. For example, *F. nilgerrensis* produces white fruits (Shen et al. 2020; Zhang et al. 2020a), whereas *F. pentaphylla* exhibits two morphs, red and white fruits (Duan et al. 2017, 2021). Therefore, there is a need for comprehensive exploration of the content, composition, and regulation of anthocyanin biosynthesis in *Fragaria* species. This will facilitate the development of anthocyanin-rich strawberries to meet the increasing demand for health-promoting compounds in the human diet (Xu et al. 2018).

Here, we describe the content and composition of anthocyanins in cultivated and wild strawberry species. Furthermore, we discuss the biosynthetic pathway of anthocyanins and their transcriptional regulation mechanisms. Finally, we highlight the effects of environmental factors, including abiotic stresses, on anthocyanin accumulation. This review summarizes vital information needed to develop anthocyanin-rich strawberries via environmental manipulations.

Anthocyanin Biosynthesis

Anthocyanin is synthesized via the phenylpropane pathway, and phenylalanine is the initial precursor in anthocyanin synthesis. Phenylalanine is catalyzed by phenylalanine ammonialyase (*PAL*), cinnamic acid 4-hydroxylase (*C4H*), and 4-coumarate-CoA ligase (*4CL*) (Ariza et al. 2016). Subsequently, it enters the anthocyanin biosynthetic pathway, an extension of the general flavonoid pathway. Anthocyanin biosynthesis starts with the chalcone synthase (*CHS*)-mediated synthesis of naringenin chalcone from 4-coumaroyl-CoA and malonyl-CoA. Chalcone isomerase (*CHI*) then isomerizes naringenin chalcone to naringenin. Flavanone 3-hydroxylase (*F3H*) converts naringenin into dihydrokaempferol that is further hydroxylated by flavonoid 3′-hydroxylase (*F3′H*) or flavonoid 3′,5′-hydroxylase (*F3′5′H*) into two other dihydroflavonols: dihydroquercetin and dihydromyricetin. The three dihydroflavonols are then converted by dihydroflavonol 4-reductase (*DFR*) into colorless leucoanthocyanidins, which are subsequently converted to colored anthocyanidins by anthocyanidin synthase (*ANS*) (Liu et al. 2018). Ultimately, colored anthocyanidins are glycosyltransfered by various flavonoid 3-O-glucosyltransferase (*UFGT*), whereas some are further acylated with aromatic acyl groups by acyltransferases (Liu et al. 2018).

Fruits contain six main anthocyanin pigments; cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin (Chen et al. 2017). Cyanidin, pelargonidin, and delphinidin are synthesized from phenylalanine by various enzymes, and peonidin is synthesized from enzyme-modified cyanidin. Malvidin is synthesized from delphinidin (Fig. 1) (Shen et al. 2020). Anthocyanins are biosynthesized differently in various *Fragaria* species and
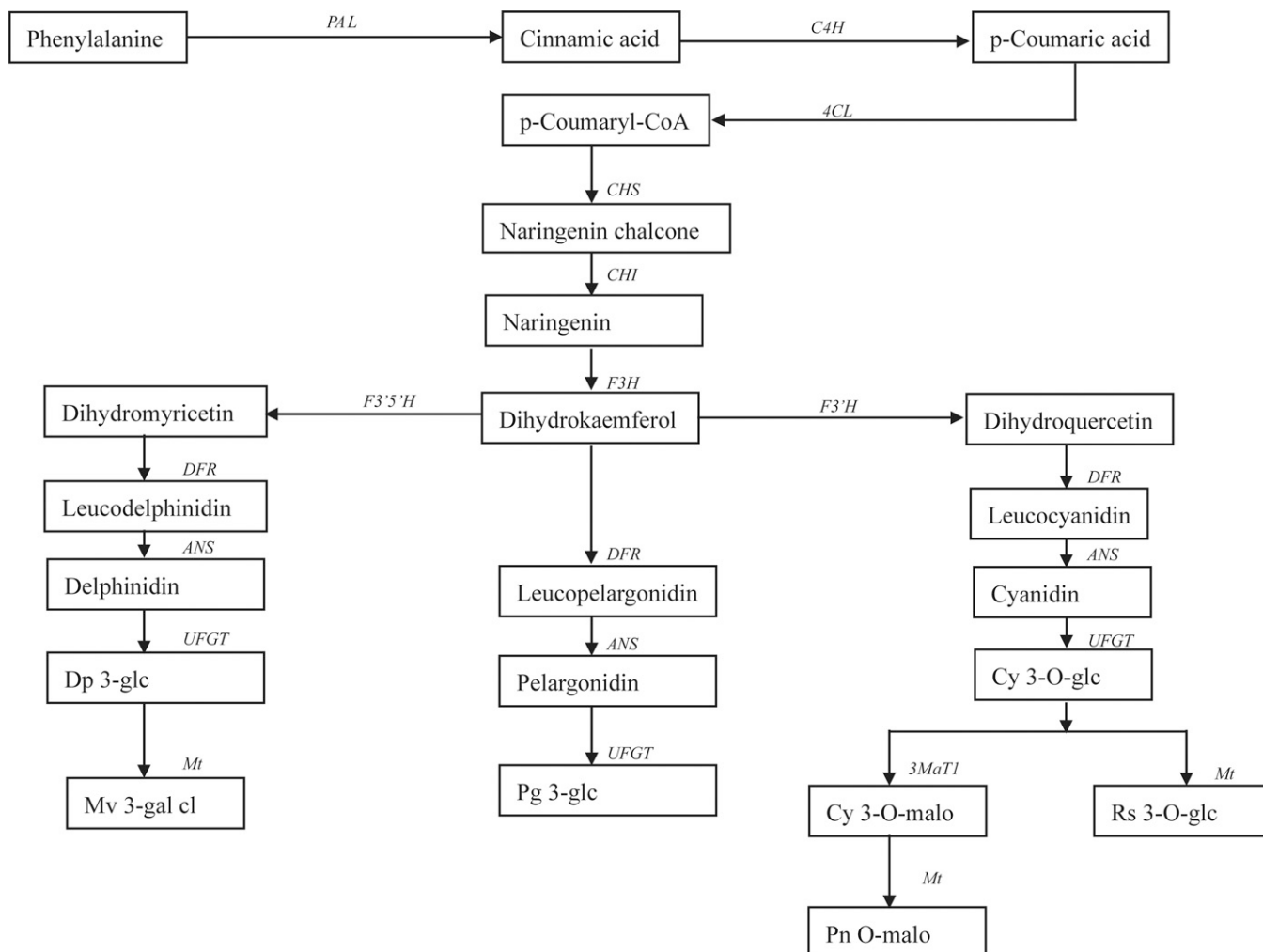
Fig. 1. Schematic representation of the anthocyanin biosynthetic pathway in *Fragaria* species (Shen et al. 2020). *PAL*: phenylalanineammonialyase; *C4H*: Cinnamate 4-hydroxylase; *4CL*: 4-coumarate coenzyme A ligase; *CHS*: chalcone synthase; *CHI*: chalcone isomerase; *F3H*: flavanone 3-hydroxylase; *F3'H*: flavonoid 3'-hydroxylase; *F3'5'H*: flavonoid 3',5'-hydroxylase; *DFR*: dihydroflavonol 4-reductase; *ANS*: anthocyanidin synthase; *UFGT*: flavonoid 3-O-glucosyltransferase; FLS: flavonol synthase; Cy: cyanidin; Dp: delphinidin; Pg: pelargonidin; Mv: malvidin; Pn: peonidin; Rs: rosinidin; glc: glucoside.

varieties (Kim et al. 2015; Salvatierra et al. 2014; Shen et al. 2020; Simirgiotis et al. 2009). The most commonly occurring anthocyanins in strawberries are cyanidin and pelargonidin derivatives (Salvatierra et al. 2014). Cyanidin, pelargonidin, and delphinidin were identified in the red fruits of *F. pentaphylla*, whereas only cyanidin and delphinidin were detected in the white fruits of *F. nilgerrensis* (Shen et al. 2020). Additionally, rosinidin synthesized from enzyme-modified cyanidin was detected in *F. pentaphylla* and *F. nilgerrensis*, whereas petunidin was not detected (Fig. 1) (Shen et al. 2020).

Anthocyanin Content and Composition

Anthocyanin content and composition in cultivated strawberries. The content of anthocyanins varies among different varieties of cultivated strawberries (*F. ×ananassa*) (Buendía et al. 2010; Meyers et al. 2003; Skupień and Oszmiański 2004). More than 25 anthocyanins have been detected in various strawberry cultivars

via a high-performance liquid chromatography–diode array coupled to mass spectrometric detection (Silva et al. 2007). Notably, pelargonidin-3-glucoside, pelargonidin 3-O-rutinoside, and cyanidin-3-glucoside are the three main anthocyanins in strawberries (Dzhanfezova et al. 2020). Silva et al. (2007) studied the anthocyanin content of five strawberry cultivars (Camarosa, Carisma, Eris, Oso Grande, and Tudnew) and found that pelargonidin-3-glucoside accounted for 77% to 90% of the total anthocyanins, followed by pelargonidin-3-rutinoside (6% to 11%), and cyanidin-3-glucoside (3% to 10%). Dzhanfezova et al. (2020) evaluated anthocyanin levels in 12 noncommercial strawberry cultivars and found that pelargonidin-3-glucoside was the most abundant, accounting for 50% to 90% of the total anthocyanin content, followed by cyanidin-3-glucoside, which accounted for 1% to 47%, depending on the cultivar. Cyanidin-3-glucoside content has reduced significantly in modern cultivars (Kelebek and Selli 2011; Lin et al. 2018; Tonutare 2015). In addition, pelargonidin-3-

rutinoside and pelargonidin-3-malonylglucoside accounted for 12.5% of the total anthocyanins, while pelargonidin-3-rutinoside only accounted for 3.1% in *F. ×ananassa* cv. *Senga Sengana* (Kjersti et al. 2005). Despite several reports on anthocyanin biosynthesis in strawberries, the studies were limited to fewer than 20 accessions. Transcriptome analysis of red and white strawberry cultivars revealed 70 differentially expressed genes involved in the anthocyanin pathway, which could potentially explain the white coloration of strawberry fruits (Zhao et al. 2021). Thus, additional studies are needed to identify the key genes responsible for anthocyanin accumulation and color formation in strawberry fruits.

Anthocyanin content and composition in wild strawberries. Anthocyanin levels and composition vary remarkably among wild and cultivated strawberries. Cyanidin-3-glucopyranoside is the most abundant anthocyanin in *F. chiloensis* (Cheel et al. 2005). Sondheimer and Karash (1956) and Tonutare (2015) reported that the major anthocyanins in

F. vesca are glycosylated pelargonidin and cyanidin, produced at a ratio of around 1:1. Simirgiotis et al. (2009) found that the content ratio of pelargonidin 3-glucoside and cyanidin 3-glucoside was 1.88 in *F. chiloensis* ssp. *chiloensis* f. *patagonica*, but 0.68 in *F. chiloensis* ssp. *chiloensis* f. *chiloensis*. Furthermore, Kim et al. (2015) examined the contents of pelargonidin 3-glucoside, pelargonidin-3-rutinoside, and cyanidin 3-glucoside of 14 *F. orientalis* varieties and concluded that the varieties had varying anthocyanin composition. Shen et al. (2020) detected 26 anthocyanins in *F. pentaphylla* and *F. nilgerrensis* fruits. In *F. pentaphylla*, cyanidin and pelargonidin accounted for 83.13% and 14.81% of the total anthocyanins, respectively. In *F. nilgerrensis*, cyanidin accounted for 97.66% of the total anthocyanin content, while pelargonidin level was undetectable (Shen et al. 2020). Cyanidin 3-glucoside chloride, cyanidin 3-galactoside, and cyanidin 3-glucoside were the most abundant anthocyanins in *F. pentaphylla* and *F. nilgerrensis* fruits, accounting for 60.70% and 94.24% of the total anthocyanins in *F. pentaphylla* and *F. nilgerrensis*, respectively (Shen et al. 2020).

Anthocyanin content and composition in strawberry achenes. Achenes of strawberries also contain substantial amounts of anthocyanins. Notably, anthocyanin content and composition in strawberry achenes also vary depending on the genotype. For instance, Salvatierra et al. (2014) found that cyanidin 3-glucoside was 1.5 times higher in achenes of *F. chiloensis* ssp. *chiloensis* than those of *F. chiloensis* ssp. *patagonica*. The study also found that cyaniding 3-glucoside content was almost 25 times higher than pelargonidin 3-glucoside content in *F. chiloensis* ssp. *chiloensis* (Salvatierra et al. 2014). Kjersti et al. (2005) measured the anthocyanin content of receptacles and achenes of cultivated strawberry varieties and found that pelargonidin-3-glucoside was the most abundant anthocyanin in the receptacles, while cyanidin was only available in trace amounts. However, the levels of cyanidin-3-glucoside and pelargonidin-3-glucoside in achenes were almost the same, accounting for 80% of total anthocyanins in the fruits. These results revealed that anthocyanin composition varies between receptacles and achenes (Kjersti et al. 2005). Although strawberry achenes constitute a small portion of the fruit, they contribute more than 41% of the total antioxidant content, accounting for 81% of total anthocyanins (Ariza et al. 2016). Therefore, consuming strawberry achenes contributes more anthocyanins to the diet than other parts of the fruit (Ariza et al. 2016).

Role of Structural Genes in Anthocyanin Content and Composition

The structural genes related to anthocyanin biosynthesis can be divided into two groups: the early-regulated biosynthesis genes (EBGs) (*CHS*, *CHI*, *F3H*, *F3'H*, and *F3'5'H*) and the late-regulated biosynthesis genes (LBGs) (*DFR*, *ANS*, and *UFGT*) (Dubos et al. 2010).

The LBGs regulate anthocyanin accumulation in *Fragaria* species (Duan et al. 2017;

Giampieri et al. 2018; Hossain et al. 2018; Salvatierra et al. 2014; Shen et al. 2020). *ANS* overexpression has been shown to increase anthocyanin content in *F. ×ananassa* (Giampieri et al. 2018). Shen et al. (2020) also found that the upregulation of LBGs (*DFR*, *ANS*, and *UFGT*) enhances the synthesis of cyanidin derivatives and delphinidin in *F. pentaphylla*. Conversely, the inhibition of *UGT75C1* and *UGT79B1* genes was found to impede the synthesis of cyanidin O-hexoside-O-hexoside-O-hexoside in the red fruits of *F. pentaphylla* (Shen et al. 2020). Lin et al. (2013) found that *FaDFR* silencing significantly reduced glycoside pelargonidin and cyanidin levels in *F. ×ananassa* by 93.3% and 97.2%, respectively. Additionally, Duan et al. (2017) identified 27 single nucleotide polymorphisms within the *FpDFR* gDNA sequences between red and white fruits of *F. pentaphylla*, contributing to anthocyanin accumulation. The substrate specificity of *DFR* is linked to the variations in anthocyanin composition in different *Fragaria* species (Miosic et al. 2014). Two *DFR* genes (*DFR*1 and *DFR*2) were identified in *F. ×ananassa* and *F. vesca*. Notably, *DFR*1 cannot catalyze dihydrokaempferol (the substrate for pelargonidin synthesis), whereas *DFR*2 exhibits a high affinity for dihydrokaempferol. Variations in the ratios of the two *DFRs* lead to different cyanidin to pelargonidin ratios between *F. ×ananassa* and *F. vesca* (Miosic et al. 2014). For example, the upregulation of *DFR*1 and silencing of *F3'H* results in the accumulation of pelargonidin-based pigments in *F. ×ananassa*, whereas the enhanced expression of *DFR*2 and *F3'H* promotes the synthesis of cyanidin-based pigments in *F. vesca* (Miosic et al. 2014).

EBGs appear to regulate anthocyanin biosynthesis in strawberries indirectly. Specifically, the silencing of *CHS* or *F3H* genes has been shown to decrease the anthocyanin content of strawberry fruits significantly (Hoffmann et al. 2006; Jiang et al. 2013). *F3'H* and *F3'5'H* catalyze the synthesis of cyanidin-based and delphinidin-based anthocyanins, respectively (Seitz et al. 2007). These enzymes facilitate the hydroxylation of the anthocyanin B-ring, synthesized from 4-coumaroyl-CoA produced from phenylalanine via the shikimate pathway (Nabavi et al. 2020). Increasing hydroxylation of the B-ring affects the hue of the pigment and causes a shift from the red end of the visible spectrum to the blue (Schwinn et al. 2014; Tanaka 2006). *F3'H* expression varies significantly between different *Fragaria* species/genotypes during fruit development, impacting the composition of the two major anthocyanins (cyanidin and pelargonidin derivatives) (Thill et al. 2013).

Glutathione S-transferase (*GST*) is an enzyme involved in cellular detoxification processes and has been implicated in plant stress responses (Allocati et al. 2018). Lin et al. (2020) found that *FaGST1*, *FaGST37*, *FaGST39*, *FaGST73*, and *FaGST97* may indirectly promote vacuolar anthocyanin accumulation in cultivated strawberries. Moreover, the expressions of these five *FaGST* genes were significantly correlated

with the expressions of other anthocyanin biosynthetic structural genes (e.g., *FaCHI*, *FaCHS*, and *FaANS*), indicating their involvement in anthocyanin accumulation in strawberries.

Transcriptional Regulation of Anthocyanin Biosynthesis in *Fragaria* Species

The role of R2R3-MYB transcription factors in anthocyanin biosynthesis. R2R3 MYB transcription factors are essential in regulating the structural genes related to anthocyanin biosynthesis in *Fragaria* species (Espley et al. 2007; Hichri et al. 2011). Specifically, *MYB10* transcripts levels have been shown to increase during the development of strawberry fruits, indicating that *MYB10* may participate in anthocyanin synthesis in strawberries (Aharoni et al. 2001; Castillejo et al. 2020; Roy et al. 2018). Studies have shown that allelic variation of *MYB10* is the major factor controlling the natural variation of the skin and flesh color in strawberry fruits (Castillejo et al. 2020). In red-fleshed accessions, the insertion of the CACTA-like transposon (*FaEnSpm*-2) in the *MYB10-2* promoter enhanced *MYB10-2* expression and anthocyanin biosynthesis. However, in white flesh fruit, the *gypsy*-transposon and two additional loss-of-function mutations in *MYB10* truncated the protein and knocked out anthocyanin biosynthesis (Castillejo et al. 2020). Silencing the *FaMYB10* gene in *F. ×ananassa* downregulated all anthocyanin structural genes except *ANS* (Medina-Puche et al. 2014). However, *FaMYB10* overexpression significantly enhanced the expression of anthocyanin structural genes, including *ANS* (Lin et al. 2018). Knockout of *FvMYB10* in *F. vesca* inhibited anthocyanin synthesis, while *FvMYB10* overexpressing lines exhibited significantly higher anthocyanin levels than the control. Similar results were also observed in *F. ×ananassa* (Wang et al. 2014). In *F. nilgerrensis*, a mutation in the upstream regulatory region of *FnMYB10* downregulated the *FnMYB10* gene, resulting in the white fruit phenotype (Zhang et al. 2020a). Notably, *MYB10* is expressed throughout the fruit flesh and skin of *F. ×ananassa*, whereas in *F. vesca*, it is only expressed in the outer cell layers of the mature fruit (Lin-Wang et al. 2010). A correlation between *FaMYB10* overexpression and cyanidin 3-glucoside accumulation has also been found in *F. ×ananassa* cv. *Fengguang* (Xu et al. 2018).

The repressors of anthocyanin synthesis have also been identified, including *FaMYB1* (Aharoni et al. 2001). Flowers of transgenic tobacco lines overexpressing *FaMYB1* showed a severe pigmentation (cyanidin 3-rutinoside) reduction, accompanied by reduced expression of LBGs and suppressed enzyme activities (Aharoni et al. 2001). Overexpression of *FaMYB1* has also been shown to inhibit the expression of *ANS* and *UFGT*, resulting in decreased anthocyanin content, whereas silencing of *FaMYB1* results in the upregulation of white anthocyanin reductase (*LAR*) in *F. ×ananassa* (Kadomura-Ishikawa et al. 2015b). Silencing the *FcMYB1* gene in *F. chiloensis* results in the upregulation

of *ANS* and downregulation of *ANR* and *LAR*, leading to increased anthocyanin content (Salvatierra et al. 2014). However, *FvMYB1* did not repress anthocyanin synthesis in *F. vesca* (Roy et al. 2018) as it does in *F. ×ananassa* (Aharoni et al. 2001; Hu et al. 2018) and *F. chiloensis* (Salvatierra et al. 2014). Researchers have revealed that the regulatory mechanism of *MYB1* on anthocyanin biosynthesis varies between *F. ×ananassa* and *F. chiloensis*. However, silencing of the *MYB1* gene suppresses the expression of *LAR*, an enzyme that catalyzes proanthocyanidin synthesis, in both *Fragaria* species (Salvatierra et al. 2014). These results indicate that *MYB1* negatively controls anthocyanin biosynthesis in strawberry fruits by regulating the expression of anthocyanin/proanthocyanidin biosynthetic structural genes. This further affects the synthesis direction of the intermediate metabolites (Salvatierra et al. 2014).

Moreover, genome-wide analysis of the *MYB* gene family in the octoploid strawberry revealed that *FaMYB28*, *FaMYB54*, and *FaMYB576* potentially regulate anthocyanin biosynthesis (Liu et al. 2021). Meanwhile, *FaMYB5*, *FaMYB11*, and *FaMYB9* were demonstrated to repress anthocyanin biosynthesis in the octoploid strawberry (Hossain et al. 2018; Schaart et al. 2013).

The role of basic helix-loop-helix transcription factors in anthocyanin biosynthesis. The basic helix-loop-helix (*bHLH*) transcription factor family is the second-largest transcription factor family in plants. It is characterized by the conserved *bHLH* domain and plays a central regulatory role in many plant biological processes (Zhao et al. 2018). Four *bHLHs* (*FabHLH25*, *FabHLH29*, *FabHLH80*, and *FabHLH98*) have been predicted to regulate anthocyanin biosynthesis in strawberries (Zhao et al. 2018). Furthermore, Li et al. (2020) found that *FvbHLH9* is a positive regulator of anthocyanin biosynthesis in *F. vesca*. However, *FvbHLH9* can only promote the transcription of *FvMYB10* to activate *FvDFR* expression in the presence of WD40 (Li et al. 2020). This is consistent with the previous reports that the genes coding for the key enzymes in the anthocyanin biosynthesis are mainly regulated at the transcriptional level by the MYB-bHLH-WD40 (MBW) complex (An et al. 2012; Espley et al. 2007; Yao et al. 2017; Zhou et al. 2019).

HY5 belongs to the basic leucine zipper (bZIP) transcription factor and is light-inducible (Li et al. 2020). Under light conditions, *HY5* and *MYB* aggregate and directly bind the promoters of anthocyanin biosynthesis genes to promote anthocyanin synthesis (Takos et al. 2006; Stracke et al. 2010). Li et al. (2020) further confirmed that *FvHY5* and *FvbHLH9* specifically bind the promoter region of some key enzyme genes, including *FvDFR*, to activate their expression. For example, *FvDFR* expression is activated through the heterodimer formed between *FvHY5* and *FvbHLH9*. Wang et al. (2014) also found that *FvbHLH33*, a potential partner of *FvMYB10*, can regulate anthocyanin structural genes, resulting in anthocyanin accumulation. Besides, *FabHLH3*

and *FabHLH3*-delta are potential positive regulators, whereas *FabHLH33* is a potential negative regulator of anthocyanin biosynthesis in the high-anthocyanin strawberry cultivars (Hossain et al. 2018).

The role of other transcription factors in anthocyanin synthesis. More recently, many other families of transcription factors have been demonstrated to modulate anthocyanin (An et al. 2017; Duan et al. 2017). The RAV group (related to ABI3/VP1) of transcription factors (TFs) plays multifaceted roles in plant growth, development, and responses to environmental stresses. Zhang et al. (2020b) found that *FaRAV1* had the highest transcriptional activation effect on the promoter of *FaMYB10*, a key activator of anthocyanin biosynthesis. The study also reported that silencing *FaRAV1* through transient RNA interference decreased *FaMYB10* expression and anthocyanin content in *F. ×ananassa* fruits. Additionally, transcriptome analysis of *FaRAV1*-overexpressing strawberry fruit revealed that transcripts of phenylpropanoid and flavonoid biosynthetic pathway genes were upregulated. *FaRAV1* stimulates anthocyanin accumulation in strawberries by either directly activating anthocyanin pathway gene promoters or upregulating *FaMYB10* (Zhang et al. 2020b).

The plant-specific transcription factor Teosinte branched1/Cycloidea/Proliferating cell factors (*TCP*) is crucial in plant growth and development. *FvTCP9* participates in the biosynthesis of abscisic acid (ABA) and anthocyanins to regulate fruit ripening. Transcription analysis showed that *FvTCP9* could affect the expression of ABA signaling-related genes (*FaNCED1*, *FaPYR1*, *FaSnRK2*, and *FaABI5*). A yeast two-hybrid assay revealed that *FvTCP9* interacts physically with *FaMYC1* to modulate anthocyanin biosynthesis, indicating that *FvTCP9* promotes fruit ripening by regulating the biosynthesis of ABA and anthocyanins (Xie et al. 2020). Notably, *FaWD44–1* is a potential negative regulator of anthocyanin biosynthesis in high-anthocyanin strawberry cultivars (Hossain et al. 2018).

The role of long noncoding RNAs in anthocyanin synthesis. Long noncoding RNAs (lncRNAs) are a class of functional RNAs stretching longer than 200 nucleotides and lack protein-coding capacity. Substantial evidence indicates that lncRNAs play critical regulatory roles in diverse biological processes in plants, including stress response (Wang et al. 2017), flower and fruit development (Zhu et al. 2015), and ripening (Kang and Liu 2015). Lin et al. (2018) found that the competitive intensity of microRNAs (miRNAs) and lncRNA for the same mRNA targets was lower in white-fleshed strawberries than in red-fleshed strawberries, indicating that downregulating lncRNAs might modulate anthocyanin biosynthesis by acting as targets for miRNAs.

Factors Affecting Anthocyanin Biosynthesis in *Fragaria* Species

Light. Light intensity and quality, including shading (Anttonnen et al. 2006), ultraviolet-B radiation (Josuttis et al. 2010; Ordidge et al. 2010; Tsormpatsidis et al. 2011), and blue and red LED light (Zhang et al. 2018a, 2018b, 2018c), significantly affect anthocyanin biosynthesis (Cominelli et al. 2008; He and Giusti 2010). Anthocyanins protect plants from ultraviolet damage (Zoratti et al. 2014). Light treatment (blue, green, and red light), especially blue light, can increase anthocyanin synthesis in the postharvest strawberry fruits during storage by inducing the activities of *CHS*, *F3H*, *DFR*, *ANS*, and *UFGT* (Kadomura-Ishikawa et al. 2013). Blue light-irradiated (40 μmol·m^{-2}·s^{-1} blue light irradiation) fruits of *F. ×ananassa* cv. *Fengguang* had higher anthocyanin content accompanied by increased activities of *TAL*, *PAL*, *C4H*, and *4CL* (Xu et al. 2018). Light treatment also increased the expression of *MYB10* in *F. ×ananassa* and *F. vesca*, thereby increasing the expression of anthocyanin structural genes and anthocyanin biosynthesis (Kadomura-Ishikawa et al. 2015a; Xu et al. 2018). Recent studies have shown that photoreceptors, such as Phototropin 2 (PHOT2), are crucial in mediating anthocyanin accumulation in response to light (Sharma et al. 2021; Zhang et al. 2016). Overexpression of PHOT2 increases anthocyanin content in its leaves and fruits in *F. ×ananassa* (Sharma et al. 2021).

Temperature. The synthesis of anthocyanin is also affected by temperature. When exposed to cold temperatures, plants increase anthocyanin biosynthesis to improve their survival under cold stress (Catala et al. 2011). Conversely, suppressing anthocyanin biosynthesis might enhance survival under high-temperature stress (Kim et al. 2017). In addition, Kim et al. (2017) discovered that high temperature induces the degradation of *HY5* protein through COP1, enhancing the expression of the negative regulator and reducing the expression of anthocyanin biosynthetic genes.

Studies have shown that temperature affects anthocyanin accumulation in strawberry fruits. A positive correlation between anthocyanin contents and the temperature has been observed in strawberries grown in controlled environments (Balasooriya et al. 2020; Josuttis et al. 2011; Wang and Zheng 2011) and under natural conditions (Cervantes et al. 2020; Josuttis et al. 2012). Wang and Zheng (2011) found that increasing the night temperature from 12 to 22 °C, with the day temperature kept constant at 25 °C, significantly increased anthocyanin content in *F. ×ananassa*. Balasooriya et al. (2020) also found that a day temperature of 30 °C could significantly increase anthocyanin content in strawberry fruits than 25 °C. Low-temperature stress (4 °C) increased the expression of anthocyanin structural genes and anthocyanin accumulation in *F. ×ananassa* fruits by regulating the expression of *FaMYB10* and *FaMYB1* (Zhang et al. 2018c). Furthermore, low temperature induced anthocyanin production

in strawberries, alleviating oxidative damage (Zhang et al. 2018c). The increase in anthocyanin content at low temperatures can be attributed to the modulation of *ANS* and *UFGT* structural genes by the regulatory genes (*MYB10* and *MYB1*) (Zhang et al. 2018c).

Temperature also affects anthocyanin composition. For example, fruits grown at higher temperatures exhibited increased levels of total anthocyanin and pelargonidin-3-glucoside, the most abundant anthocyanin in strawberries (Balasooriya et al. 2020).

CO_2. Different studies have reported inconsistent findings regarding the effect of CO_2 on anthocyanin biosynthesis in strawberries. Some studies have shown that elevated CO_2 concentration can increase anthocyanin accumulation in *F. ×ananassa* (Balasooriya et al. 2020; Choi and Kang 2018; Wang et al. 2003). Shin et al. observed higher levels of anthocyanins in air-stored strawberries than in CO_2-stored strawberries (Shin et al. 2008). A high concentration of CO_2 can increase the carbon supply in the growth environment, leading to higher carbon availability in plants. High carbon availability is associated with more carbohydrate accumulation and enhanced synthesis of carbon-based secondary products, especially soluble phenols, and condensed tannins (Balasooriya et al. 2020).

However, some studies have reported that high CO_2 levels can negatively affect anthocyanin accumulation. Li et al. (2019a) found that elevated CO_2 (20% CO_2 treatment) lowers anthocyanin content and the activity of *PAL*, *C4H*, *4CL*, and *CHS*. The elevated CO_2 levels also suppressed the expression of 13 genes involved in the phenylpropanoid and flavonoid biosynthetic pathways, suggesting that high CO_2 concentration may suppress anthocyanin biosynthesis by regulating the flavonoid pathway. In contrast, Blanch et al. (2012) found that anthocyanin accumulation was not reduced in strawberries treated with 20% CO_2; however, flavonoid production was redirected toward catechin and procyanidin B3 accumulation in 40% CO_2-treated fruits, with a sharp decrease in anthocyanin levels. The study also showed that catechin induction by high CO_2-treated could protect strawberries from fungal decay.

In addition, the effect of CO_2 on anthocyanin biosynthesis has been shown to vary among different tissues. For instance, Gil et al. (1997) reported that CO_2 had a minimal effect on the anthocyanin contents of the external tissues but induced a remarkable decrease in anthocyanin accumulation in the internal tissues.

Water and salt. Plants growing in the field experience multiple stresses during their development, compounded by the dramatic changes in global climate. A recent study revealed that water stress could increase anthocyanin content in *F. ×ananassa* fruits (Adak et al. 2017). Similar results were observed in strawberry plants subjected to drought stress (Terry et al. 2007) or salt stress (Keutgen and Pawelzik 2008). Mild drought and salt stresses have also been shown to increase the content of phenolics, anthocyanins, and L-ascorbic acid. Drought and salt stresses are also associated with increased anthocyanin content and antioxidant activity through an ABA-dependent mechanism in *F. ×ananassa* fruits (Perin et al. 2019). Perin et al. (2019) found that drought or salt stress-induced anthocyanin accumulation and increased levels of ABA and its derivatives (phaseic and dehydrophasic acids), associated with the phenylpropanoid and flavonoid pathways, activate the expression of several anthocyanin synthesis genes.

Exogenous compounds. Exogenous compounds, such as sucrose (Li et al. 2019b) and ABA (Ji et al. 2012; Jia et al. 2011), were found to induce anthocyanin synthesis. Li et al. (2019b) found that exogenous sucrose application on strawberry fruits during storage can increase the accumulation of four pelargonidin derivatives, including pelargonidin 3-glucoside, pelargonidin 3-rutinoside, pelargonidin 3-malonylglucoside, and pelargonidin 3-methylmalonyglucoside. Moreover, the accumulation of pelargonidin derivatives was involved in activating the pentose phosphate, shikimate, phenylpropanoid, and flavonoid pathways (Li et al. 2019b).

Furthermore, Cao et al. (2010) found that applying 0.2 g/L of benzo-thiadiazole-7-carbothioic acid S-methyl ester (BTH) on strawberry fruits for 10 days at 1 °C increases anthocyanin content. This can be attributed to the activation of BTH-related enzymes, including *G6PDH*, *SKDH*, *TAL*, *PAL*, *C4H*, and *DFR* (Cao et al. 2010).

Conclusion

Anthocyanin content and composition vary among cultivated and wild strawberry varieties. The three major anthocyanins in strawberries are pelargonidin-3-glucoside, pelargonidin 3-O-rutinoside, and cyanidin-3-glucoside. However, the biosynthetic pathways of anthocyanins in strawberries need further exploration. Anthocyanin biosynthesis in strawberries is regulated by structural genes, including EBGs and LBGs. Transcription factors, specifically those belonging to the *R2R3-MYB*, *bHLH*, and *WD40* families, are crucial in anthocyanin biosynthesis regulation. However, more novel transcription factors involved in anthocyanin biosynthesis need to be identified and characterized (Lin et al. 2018). Genetic engineering and hybrid screening are increasingly being used to enhance anthocyanin content in strawberries for improved fruit quality (Qin et al. 2008; Sirijan et al. 2020). Therefore, more studies on the molecular regulation mechanisms involved in anthocyanin biosynthesis will help optimize the genetic breeding programs. Anthocyanin accumulation is also affected by environmental factors (abiotic stresses and exogenous compounds), indicating that manipulating environmental factors can improve the anthocyanin contents of fruits. However, further studies should be conducted to validate these findings. Furthermore, CRISPR/Cas9 (Hu et al. 2019) has been widely used as a highly valuable tool for both basic and applied studies on various organisms and may be useful for researching the functional genes related to anthocyanin synthesis of *Fragaria* in the future (Gao et al. 2020; Tang et al. 2018).

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