

Review

Ultraviolet and environmental stresses involved in the induction and regulation of anthocyanin biosynthesis: A review

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Anthocyanins are the most conspicuous class of flavonoids which present a large class of secondary plant metabolites. Anthocyanins are important to many diverse functions within plants. Anthocyanin biosynthetic pathway is well established. Two classes of genes required for anthocyanin biosynthesis have been extensively characterized; the structural genes encoding the enzymes that directly participate in the formation of anthocyanins and other flavonoids, and the regulatory genes that control the transcription of structural genes. Light is one of the most important environmental factors regulating plant development and genes expression. Ultraviolet takes up 7% of sunlight and it stimulates distinct responses in plant. Both UV-A and low influence of UV-B can induce the accumulation of anthocyanin via induction of the expression of anthocyanin biosynthesis genes. Besides, the modulation of anthocyanin by environmental and developmental factors has been observed. A number of genes involved in anthocyanin biosynthesis are induced by abiotic stresses.

Key words: Anthocyanins, ultraviolet, photoreceptor, environmental stress.

INTRODUCTION

Flavonoids are a class of secondary metabolites widespread in various plants (Tahara, 2007). Flavonoids perform major roles in plants such as UV protection, defense against pathogens and pests, pollen fertility, signaling with microorganisms, auxin transport regulation, and pigmentation (Winkel-Shirley, 2001). Today more than 10,000 varieties of flavonoids have been identified (Tahara, 2007; Dixon and Paiva, 1995). Anthocyanins are the largest group of water-soluble pigments in the plant kingdom (Kong et al., 2003).

Flavonoids consist of anthocyanins, flavonoids, catechins, phenolic acids, secoiridoids, stilbenes, coumarins and isoflavones which are well known in vegetable crops (Corbett, 1974). Figure 1 shows that synthesis of flavonoids used the phenylpropanoid metabolic pathway in which the amino acid phenylalanine is used to produce 4-coumaroyl-CoA (Shih et al., 2008). Anthocyanins are commonly found in the red, blue, and purple colors of

fruits, vegetables, flowers, and other plant tissues or products and their amounts are especially high in berries and red wines (Mateus et al., 2001). Anthocyanins color shows variable differences- the aglycone, the pattern of glycosylation, and the amount of esterification of the sugars with aromatic acids or aliphatic, and the presence of co-pigments (Mazza et al., 2004). Approximately 500 individual anthocyanins have been identified (Ghosh, 2007).

In the plants, anthocyanidins can be classified to six categories according to the number and position of hydroxyl and methoxyl groups on the flavan nucleus, including pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin (Figure 2; Tahara, 2007). The main structures of anthocyanin consist of three main parts and three R groups (Mazza, 2007). The anthocyanidins compounds show various functions in counteracting the negative effects of nitrogen and oxygen reactive species, maintaining the redox homeostasis of biological fluids, and preventing human disease such as atherosclerosis, cardiovascular, and other degenerative pathologies such as diabetes, cancer, Parkinson's and Alzheimer's diseases (González-Gallego et al., 2007;

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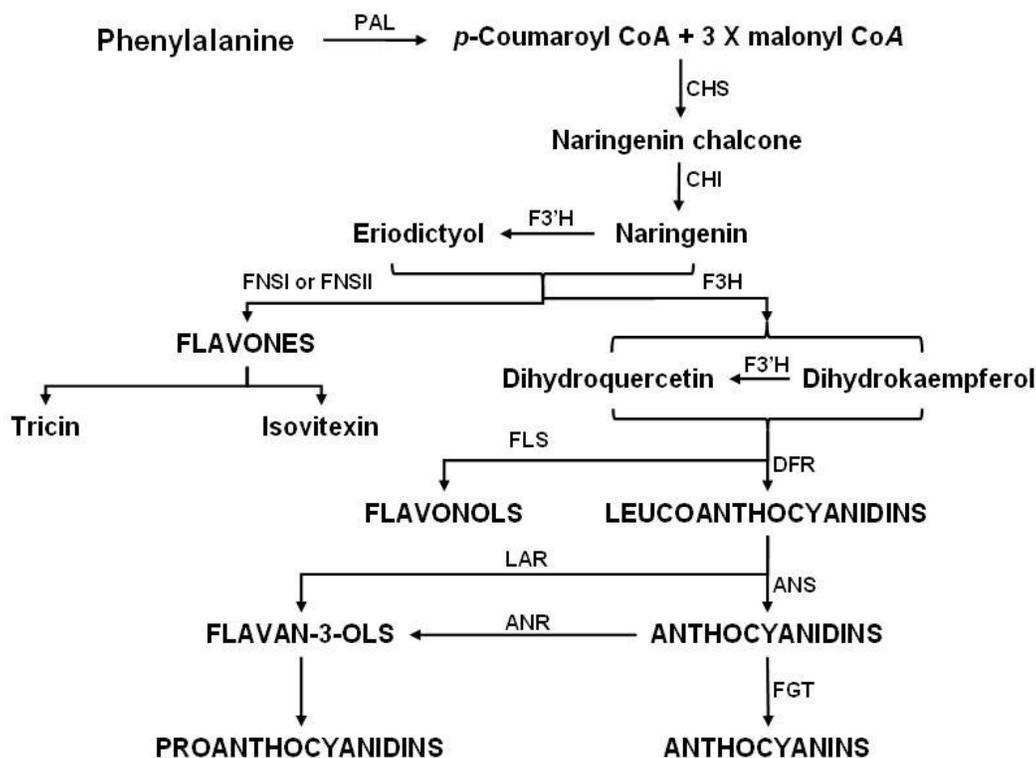


Figure 1. Pathways of flavonoid biosynthesis. The initial step is catalyzed by chalcone synthase (CHS) using malonyl CoA and p-coumaroyl CoA as substrates. Enzymes are abbreviated as follows: anthocyanidin synthase (ANS), anthocyanidin reductase (ANR), chalcone isomerase (CHI), dihydroxavonol 4-reductase (DFR), xavanone 3-hydroxylase (F3H), xavone synthases I or II (FNS I or FNS II), xavonoid 3-hydroxylase (F3H), xavonoid 3-O-glycosyltransferase (FGT), xavonol synthase (FLS), and leucoanthocyanidin reductase (LAR).

Harborne and Williams, 2000). In nature, the most commonly occurring anthocyanidin is cyanidin (Tahara, 2007). Researchers have used numerous strategies to determine the amounts of individual species and to estimate the antioxidative power of phenolic compounds in plants (Holton and Cornish, 1995). This review explains the anthocyanin and genes related to anthocyanin biosynthesis involved in ultraviolet irradiation and various environmental factors.

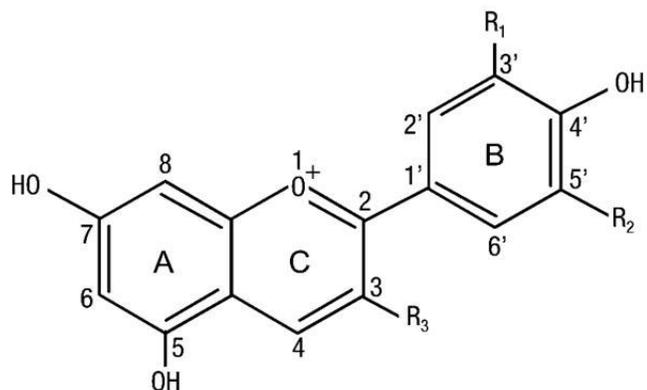
CHARACTERIZATION OF GENES INVOLVED IN ANTHOCYANIN BIOSYNTHESIS

Anthocyanin biosynthesis has been extensively studied in several plant species, and, therefore, detailed information of the course of reactions is available (Mol et al., 1989; Forkmann, 1991). Two classes of genes are required for anthocyanin biosynthesis, the structural genes encoding the enzymes that directly participate in the formation of anthocyanins and other flavonoids, and the regulatory genes that control the transcription of structural genes. The study of the genetic of anthocyanin synthesis began last century with Mendel's work on flower colour in peas

(Fairbanks and Schaalje, 2007).

Genetic loci were usually correlated with easily observed color changes in the early studies (Dooner et al., 1991; Gerats et al., 1982; Sparvoli et al., 1994). In the recent 70 years, genetics and biochemistry on anthocyanin metabolism were deeply studied. After the structures of anthocyanin and other flavonoids were determined, it was possible to correlate single genes with corresponding anthocyanins. Maize (*Zea mays*), snapdragon (*Antirrhinum majus*) and petunia (*Petunia hybrida*) are three important species for studying the anthocyanin biosynthetic pathway and isolating genes controlling the biosynthesis of flavonoids (Wiering and de Vlaming, 1973).

Two classes of genes are required for anthocyanin biosynthesis, the structural genes encoding the enzymes that directly participate in the formation of anthocyanins and other flavonoids, and the regulatory genes that control the transcription of structural genes. Over the last few years, many structural genes that encode enzymes related to anthocyanin biosynthesis and the transcription factors that activate or repress the structural genes have been cloned. These have contributed to an understanding of the molecular level of anthocyanin bio-



| | Substitution pattern | | |
|--------------|----------------------|------------------|----|
| | R1 | R2 | R3 |
| Delphinidin | OH | OH | OH |
| Cyanidin | OH | H | OH |
| Petunidin | OCH ₃ | OH | OH |
| Peonidin | OCH ₃ | H | OH |
| Malvidin | OCH ₃ | OCH ₃ | OH |
| pelargonidin | H | H | OH |

Figure 2. Structural classification of general anthocyanidin species (Tahara, 2007).

synthesis in higher plants.

Structural genes

A number of structural genes have been isolated from maize, snapdragon, and petunia through proteins purification, transposon tagging, PCR and differential screening. The first flavonoid biosynthetic gene isolated was CHS gene from parsley through a combination method of differential screening and hybrid-selected translation (Kreuzaler et al., 1983). Two years later, the parsley CHS clone was used as a molecular probe to isolate clones of two different CHS genes from petunia (Reif et al., 1985). A CHI cDNA was first isolated from French bean using antibodies made against the purified enzymes in 1988 (van Tunen et al., 1988).

A combination of differential screening and genetic mapping was used to isolate a cDNA clone corresponding to F3H (Martin et al., 1991). A cDNA encoding F3H was isolated from petals of petunia (Britsch et al., 1992). Using the petunia F3H cDNA clone as a heterologous probe, the corresponding cDNAs were cloned from carnation, China aster, and stock (British et al., 1993). F3'5'H cDNA was isolated from petunia via PCR amplification (Holton et al., 1993). DFR genes have been isolated from maize and snapdragon by transposon tagging and ANS gene was first isolated in maize by

transposon tagging, and then it was isolated from snapdragon and petunia (O'Reilly et al., 1985). A snapdragon DFR clone was used to isolate a homologous gene from petunia (Beld et al., 1989). The maize Bzl gene, encoding UDP glucose:flavonoid 3-Oglucosyltransferase (3GT), was isolated by transposon tagging with Ac (Dooner et al., 1985). A putative 3GT clone, pJAM338, was isolated from snapdragon using the maize gene as a probe (Martin et al., 1991). AMT clone was cloned in 1993 (Quattrocchio et al., 1993).

Regulatory genes

Regulatory genes influence the intensity and pattern of anthocyanin biosynthesis and generally control expression of many different structural genes. Isolation of these genes mainly made use of transposon tagging technology (Nevers and Saedler, 1977; Nevers et al., 1986). The *R* gene family determines the timing, distribution, and amount of anthocyanin pigmentation in maize. Afterwards, other regulatory genes B, C1, PI and Vpl genes were isolated successively (Chandler et al., 1989; Cone et al., 1993; Dellaporta et al., 1988; McCarty et al., 1989). Goodrich isolated Del gene from snapdragon in 1992 (Goodrich et al., 1992). Quattrocchio isolated An2 and An4 genes from petunia (Quattrocchio et al., 1993). *An2* controls the transcription of these anthocyanin genes in the flower limb and *An4* controls expression of the same set of genes in the anthers. Interestingly, gene *R*, *Sn*, *Lc* showed similar sequences to *B*; C1 and PI were also shown to be homologous; the Del and R family gene in maize share high sequence similarity; and An2 sequence was similar to C1 and PI. These observations suggest the synthesis of anthocyanin in different species was regulated by similar regulatory factors.

EFFECT OF ULTRAVIOLET ON ANTHOCYANIN

Light is one of the most important environmental factors regulating plant development and the expression of plant genes. A plant's ability to maximize its photosynthetic productivity depends on its capacity to sense, evaluate, and respond to light quality, quantity, and direction. The sun light comprises seven percent of UV range (200-400 nm). Most UV-A and a larger proportion of the UV-B spectrum reaches the Earth's surface with serious implications for all living organisms (Xiong and Day, 2001).

Ultraviolet-B

Elevated UV-B radiation (UV-B) has pleiotropic effects on plant development, morphology, and physiology. Low influence UV-B stimulates distinct responses, such as the accumulation of UV-absorbing pigments. Low influence of

UV-B was also found to stimulate the transcript levels of a robust set of genes involved in stress responses (Casati and Walbot, 2003; Ulm et al., 2004). UV-B also stimulates production of ROS (Casati and Walbot, 2003) and antioxidant defenses (Rozema et al., 1997; Jansen et al., 1998). It has been proposed that ROS not only function as destructive radicals, but also as signaling molecules during UV-B responses (Casati and Walbot, 2003). High influence of UV-B photons cause cellular damage by generating photoproducts in DNA (Sinha and Häder, 2002) and direct damage to proteins (Gerhardt et al., 1999). These responses are considered to play a protective role against potential damage by UV irradiation (Solovchenko and Schmitz-Eiberger, 2003). The most effective protection mechanism stimulated under such a light regime is the biosynthesis of flavonoids and other UV-B-absorbing phenolic components (Li et al., 1993; Landry et al., 1995). These flavonoids generally absorb in the 280-315 nm region and thus are capable of acting as UV filters, thereby protecting the underlying photosynthetic tissues from damage. Plants subjected artificially to UV-B radiation respond by changes in the pathway of flavonoid synthesis. Changes have been observed not only in the levels of flavonoids in epidermal cells of the adaxial leaf surface, but also in flavonoids in the leaf wax and in leaf hairs (Hirner et al., 2001).

Anthocyanins served as an important class of flavonoids; its accumulation stimulated by low fluence UV-B radiation was observed in maize (Sharma et al., 1999), rice (Reddy et al., 1994), apple fruits (Arakawa et al., 1985), kangaroo paw (Ben-Tal and King, 1997), apple flowers (Dong et al., 1998) *Arabidopsis* (Christie and Jenkins, 1996), and roses (Maekawa et al., 1980). UV-B increased the accumulation of anthocyanins via stimulating the expression of genes encoding enzymes in the anthocyanin biosynthetic pathway (Fuglevand et al., 1996). *CHS*, *DFR*, and *F3H* showed a positive correlation with anthocyanin accumulation in UV-B-irradiated lettuce leaves (Park et al., 2007). UV-B increased expression of stress response and ribosomal protein genes, whereas photosynthesis-associated genes were down-regulated, which is also a kind of protection mechanism of plants initiated by UV-irradiation. Genes involved in stress responses increasing upon UV-B exposure were observed in maize (Casati and Walbot, 2003) and *Arabidopsis* (Ulm et al., 2004). *CHS* and *PAL* transcripts were shown to accumulate in epidermal cells following UV irradiation using *in situ* hybridization and immunolocalization techniques (Schmelzer et al., 1988). Until now, many UV-activated signaling components have been identified (Stratmann et al., 2000); however, the actual signal transduction pathways activated by UV-B radiation are not yet well defined.

Ultraviolet-A

Since UV-A is hardly absorbed by ozone, UV-A radiation

reaching the earth's surface is much stronger than other wavelength of UV rays. Sometimes UV-A caused similar responses to low fluence UV-B such as necrosis observed in UV-sensitive *Arabidopsis* mutants defective in succinic-semialdehyde dehydrogenase (Bouché et al., 2003). Besides, UV-A induction of anthocyanin accumulation was also observed in grape (Kataoka et al., 2003), *Arabidopsis* (Christie and Jenkins, 1996), eggplant (Toguri et al., 1993), carrot cells (Hirner and Satz, 2000), and kalanchoë (Hoffmann, 1999). In addition, it is known that many horticultural crops, including fruits of eggplants (Matsumaru et al., 1971) and petals of *Primula malacoides* (Kashiwagi et al., 1977), show poor pigmentation in a greenhouse covered with UV-A-absorbing films, while the pigmentation was recovered when the UV-A cut film was replaced with UV-B cut films, which are commonly used for the production of these crops.

The accumulation of anthocyanin under UV-A was realized via induction the expression of anthocyanin biosynthesis genes. cDNA microarray made by unique gene fragments of subtraction library showed 81 genes were regulated including cytochrome P450, *PAL*, *F3H*, *ANS*, *CHS*, *DFR* and *GST* gene fragments related to anthocyanidin biosynthesis (Xu and Li, 2006). Phenylalanine ammonia lyase (*PAL*), chalcone synthase (*CHS*), flavanone 3-hydroxylase (*F3H*), dihydroflavonol 4-reductase (*DFR*), and anthocyanidin synthase (*ANS*) genes increased during a 24 h exposure to UV-A in turnip (Zhou et al., 2007) and in cell culture of carrot (Hirner et al., 2001). The production of anthocyanins by UV-A exposure may have contributed to the protection of the plant tissue from the potential damage by UV absorption, which may be responsible for the observation that UV-A did not have a strong impact on gene expression profiles as observed for low fluence UV-B response.

Photoreceptors

Photoreceptor evolved gradually when the plants evolved to adaptation light. Until now, three types of photoreceptors have been characterized: (1) A red, far-red-reversible chromoprotein, phytochrome for the absorption of red and far-red light (600-750 nm). (2) Cryptochromes that mediate several responses of blue light and UV-A (320-500 nm). More recently, another blue-light-absorbing chromoprotein, phototropin, has been identified as a photoreceptor mediating phototropism. (3) Photoreceptors for UV-B (282-320 nm). However, it has not been isolated until now (Giliberto et al., 2005; Briggs and Olney, 2001). All three types of these photoreceptors could potentially sense the UV-A signal (Mancinelli, 1986). UV-A responses in *Arabidopsis* could be replaced by blue light (Feinbaum et al., 1991; Fuglevand et al., 1996), indicating the involvement of UV-A/blue photoreceptors. However, some anthocyanin biosynthesis genes, i.e. *CHS* and *F3H*, are induced only upon UV-A exposure (Zhou et al., 2007). Both UV-B and

UV-A/blue pathways involve reversible protein phosphorylation and require protein synthesis. The UV-B and UV-A/blue light signaling are therefore different from phytochrome signal transduction pathway regulating CHS expression in other species (Christie and Jenkins, 1996).

OTHER ENVIRONMENTAL FACTORS

Biosynthesis of the flavonoid compounds were differentially modulated by environmental (exogenous) and developmental (endogenous) factors. Besides light, the factors involved include various stresses (wound, infection, low temperature), certain growth regulator (ethylene, auxin), and nutritional factors. Many flavonoid compounds are induced in response to wounding or to feeding by herbivores. These flavonoid compounds may act directly at defense compounds or may serve as precursors for the synthesis of lignin, suberin, and other wound-induced polyphenolic barriers (Hahlbrock and Scheel, 1989). Plants also produce phenolic compounds under fungal or bacterial attack or infection. They may also serve to prevent microbial infection in an otherwise nutrient-rich environment (Fukasawa-Akada et al., 1996). Levels of anthocyanins increase following cold stress (Christie et al., 1994) and nutritional stress (notably phosphate limitation). Water deficient increased anthocyanin accumulation in grape berries, which resulted from earlier and greater expression of the genes controlling flux through the anthocyanin biosynthetic pathway, including *F3H*, *DFR*, *UFGT* and *GST* (Castellarin et al., 2007). Low nitrogen induces flavonoid and isoflavonoid nod gene inducers and chemoattractants for nitrogen-fixing symbionts, whereas low iron levels can cause increased release of phenolic acids, presumably to help solubilize metals and thereby facilitate their uptake (Graham, 1991).

Phenylalanine ammonia-lyase (PAL) is a key enzyme in phenylpropanoid metabolism and catalyzes the conversion of phenylalanine to *trans*-cinnamic acid, the first step in the biosynthesis of phenylpropanoid. Changes in PAL activity can occur during growth or may follow traumatic or pathological events or the action of light (Brödenfeldt and Mohr, 1988). Red light, acting via phytochrome, stimulates PAL activity in the cotyledons and hypocotyls of tomato seedlings, and exposure to UV-B has been shown to stimulate PAL activity in rice, maize, and turnip (Reddy et al., 1994; Sharma et al., 1999). PAL activity can be induced by various stresses such as chilling, wounding, ozone, pathogen invasion (Lafuente et al., 2003), the plant hormone ethylene, and plant signal molecules, including jasmonic acid, salicylic acid, and MeJA (Campos-Vargas and Saltveit, 2002; Kim et al., 2007). Anthocyanin and PAL activity in strawberry plants is increased when treated with abscisic acid (ABA) (Jiang and Joyce, 2003). It was reported PAL is significantly induced by abiotic stresses, such as salt, cold and drought in tomato (Guo and Wang, 2008). Chalcone syn-

thase (CHS) is the first flavonoid biosynthetic gene isolated from parsley (Kreuzaler et al., 1983). Its synthesis can be induced either by long-wavelength light, UV-light or certain chemical and biological compounds, which are called elicitors (Lawton et al., 1983). However, PAL and CHS showed no accumulation after cold treatment in the dark, suggesting that the cold-induced accumulation of PAL and CHS mRNA is light dependent (Leyva et al., 1995). These observations suggest light plays a regulatory role in chilling and antioxidant signaling pathway.

Some environmental stresses induce the accumulation of flavonoid compounds in the plant by activating their biosynthesis as well as inhibiting their oxidation (Rivero et al., 2001). It was reported that the mechanism signal transduction of PAL depends on the adhesion of plasma membrane-cell wall (Wang et al., 2006). Some pathways regulated by defense, salt and oxidative stresses are shared with pathways regulated by UV radiation. However, UV-B radiation can activate additional pathways not shared with other stresses (Casati and Walbot, 2003).

FUTURE DEVELOPMENT

As anthocyanin are recognized for their diverse function in plant development and beneficial effects for human health, a lot of research is still needed to clarify the bioactive effects of different flavonoid compounds. Among them, an important research target would be to clarify the detailed course of reaction and controlling system involved in the anthocyanin biosynthesis.

Another challenging aspect would be to clarify the effects of various environmental factors on anthocyanin biosynthesis in various plants. Understanding of how anthocyanin functions in plant defense and environmental stress responses should provide insights into plant disease resistance and abiotic stress tolerance.

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