

Naturally Occurring Anthocyanin, Structure, Functions and Biosynthetic Pathway in Fruit Plants

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Received Date: April 25, 2017; Accepted Date: April 29, 2017; Published Date: May 06, 2017

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Abstract

Anthocyanins are naturally occurring compounds, member of the flavonoid groups of photochemical, involved in defense against the damaging effects of UV irradiation in plants and protect from many oxidants. The anthocyanins, group of pigments are relatively small and diverse flavonoid family in nature, and responsible for the attractive colors, red and purple to blue in many plants. Presence of pigments in flowers and fruits seems to provide attraction for pollination and aiding seed distribution, it also provides antiviral and antimicrobial activities, however their occurrence in the vacuoles remains ambiguous. During the last decades, anthocyanin gene expression and many structural genes encoding enzymes has been extensively studied in fruits, flowers and leaves in many plants.

In addition, the genetic regulating mechanism, their biosynthesis and other factors involved are well described. The biosynthesis pathway of anthocyanin is a complex with diverse branches responsible to produce variety of metabolites. In general Anthocyanins, production through the flavonoid path, are a class of vital phenolic compounds. Over six thousand diverse anthocyanins have been reported from various species. So far, the potential health benefits of anthocyanins have been reported in the contexts of their antioxidant properties. Anthocyanins are also extensively studied for their several positive effects on body. Based on these facts, the present review briefly summarizes recent advances, highlighting the importance of biosynthetic pathway of anthocyanins, thus will serve to encourage advance investigation in this field.

Keywords: Anthocyanins; Biosynthetic pathway; Flavonoid; Anthocyanin regulating genes; Phenolic compounds

Background

Anthocyanins are the vital coloring pigments evident, are present in all plant tissues throughout the plant kingdom [1]. The word Anthocyanin is a combination of two Greek words Anthos and Kyanos, (Anthos=Flower, and Kyanos=Blue). Anthocyanins are naturally occurring compounds having member of the flavonoid class of phytochemicals, predominantly found in wines, tea, nuts, fruits, cocoa, cereals, honey, olive oil, vegetables, blackcurrant, red cabbage, red radish, and black carrot.

These pigments are widespread in nature and responsible for colors in different organs of plant such as stem, leaf, flower, fruit, root and tubers (orange, red, purple, and blue) [2,3]. In addition anthocyanins also have dietary importance for human health due to their marked use (US 180-215 mg/day), is much higher than the intake on a daily basis (23 mg/day) expected for additional flavonoids, [4] and reported encouraging results in the treatment of different syndromes like cancer and other cardiac diseases [5].

For more over than a century biologists, biochemists, and physiologists have conjectured for the promising biological effectiveness of anthocyanins during plant growth and human consumption [6]. Anthocyanin apparently plays mainly two major roles at different developmental stages and biological functions,

attracting pollinators for the purpose of pollination and absorbs strong UV (ultraviolet) radiation, insects using UV wavelengths and particularly flower colors are more prominent; these pigments having important roles in predation in carnivorous plants and pollination magnetize insects to crops and grasp apparatus.

The subsequent and significant role of anthocyanin-related pigments, to preventing photo-oxidative damage and serves as a defensive UV screen. These pigments are formed in relation to UV spotlight, and shelter the DNA from injury by ultraviolet radiation of DNA double helix paired strands of inherited substances in cells and become cross-linked and also prevents cell multiplication and other pivotal cellular mechanism like protein synthesis [1,7,8].

Besides anthocyanins, other pigments such as carotenoids and flavonols are influenced by final pigmentation of phenotype by vacuolar pH, metal complexes and cell shape [2]. Apart from imparting coloring agent in plants, anthocyanins have group of health-promoting benefits, and also defend from diver's oxidants through multiple mechanisms. Though, anthocyanins have received not as much interest than new flavonoids [1].

In addition to the functions of anthocyanin in plants, they having other applications, for example, one of the significant tasks is neural dysfunction and cognitive decline has been examined. Joseph, et al. [9] reported that anthocyanins in fruit juices were efficient in overturn age-related difficulties in human body, several behavioral and neural parameters, e.g. carbachol-stimulated GTPase activity, striatal Ca

buffering in striatal synaptosomes, motor behavioral performance on the rod walking, oxotremorine enhancement of a K1-evoked release of dopamine from striatal slices, accelerando tasks, and Morris water maze efficiency. However, the bioavailability of anthocyanins may be underestimated since the metabolites formed in the course of digestion could be responsible for the health benefits associated with anthocyanins [10].

Chemistry

Although the chemical structure of anthocyanin is an important in determining their potential role, and because of specific chemical structure, anthocyanins are described by an electron deficiency, and makes extremely reactive towards ROS (reactive oxygen species), also recognized as free radicals; therefore consider to be influential natural antioxidants. There are numerous structures and anthocyanin profile has been reported in different plant species [11,12].

Over 600 anthocyanins were isolated from various plants species. They are based on a single basic core structure, the flavylium ion. Therefore C15 skeleton based on with a chromane ring bearing a 2nd aromatic ring B in position 2 (C6-C3-C6) through single or additional sugar molecules bonded at diverse hydroxylated positions on the basic formation. The sugar molecules are generally conjugate to the anthocyanidin structure by the C3 hydroxyl in C ring.

Hundreds of anthocyanins are known varying in the basic anthocyanidin skeleton and the position are extent the glycosides are appended to the structure are alternative glycosides of salts of phenyl-2- benzopyrylium (anthocyanidins) [13]. The anthocyanins basic chromophore is the 7-hydroxyflavylium ion, naturally present usually have hydroxyl substituent's at positions 3 (constantly glycosylated, provide thermal constancy) and 5 (occasionally glycosylated), and the 2-phenyl- or B-ring has single or additional hydroxy or methoxy substituent [14].

Natural occurring anthocyanins in which the 7-hydroxy groups are glycosylated or substituted by a methoxy group are unusual. While large number of anthocyanin structural forms have been predicted [15], they are differ in primarily in the alternative pattern in the B-ring and the sugar nature and additional molecules that build up the glycosylated segment. The shades of naturally occurring and artificial anthocyanins have ranges (yellow to purple) and depends on the substituent's in the B-ring, the limited pH, the state of accumulation of the anthocyanins, particularly organic molecules, or complexation in the case of blue shade, in addition complication by metal cations [9,16].

The number and nature of bonded sugars to basic structure, the aromatic or aliphatic carboxylates bonded to the sugar molecule and location of the preset bonds [1]. Chemical structure of anthocyanins are glycosides and anthocyanidins, acylglycosides, and the aglycones flavyliums (2-phenylbenzopyrylium) varied in the diverse hydroxyl or methoxyl replacement in the central arrangement [17,18]. The center of the anthocyanidin, the flavylium, has the specifically C6-C3-C6 flavonoid structure, surround by one fused aromatic ring (A ring) one heterocyclic benzopyran ring (C ring), and one phenyl component (B ring) (Figure 1).

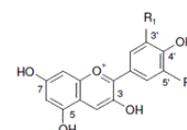


Figure 1: General chemical and with basic structure of Anthocyanin and Anthocyanidin in plants.

Classification

Anthocyanins can be classified into two major groups on the basis of their chemical composition, flavonoid (concerned to isoflavone/ flavone, C₁₅H₁₀O₂) and phenolics (phenol, C₆H₅OH). They are nearly universal, water-soluble, terrestrial plant pigments. They are glycosides of polymethoxy and polyhydroxy derived of flavylium salts or 2-phenylbenzopyrylium [19]. So far 600 dissimilar entities of anthocyanins have been discovered from different species and this quantity is still rising with the recent advancements in research [11,12,15]. In the plant kingdom, it was documented that through methylation of the six ordinary anthocyanidins, approximately 90% of the anthocyanins were modified of three methylated anthocyanidins, malvidin, petunidin and peonidin, comprised of 20% of the overall described anthocyanidins [17]. Authors reported in grapes that the anthocyanin sketch afford sufficient knowledge to create an isolation of classes in the studied grapes or wines [20]. With the development of technology and introduction of new techniques such as HPLC-ESI-MS/MS and new determining methods, more than 600 structure/kinds of anthocyanins were discovered in nature [21].

In particular, flavonoids are a group of secondary metabolites, responsible for the widest color range, (pale-yellow to blue), in number of seeds, flowers, fruits and leaves and other tissues are belongs to the class of phenylpropanoids [22]. Though, there are some different substitutions patterns are also naturally found in several other plant species, such as 3-deoxyanthocyanidins, 5-methoxycyanthocyanidins, 6-hydroxyanthocyanidins and 7-methoxycyanthocyanidins [15]. In most plant species have only O-glycosylation. However, some current reports have also worked out the presences of C-glycosylation in diverse species [23].

Anthocyanins Distribution and Diversified Biological Function

Anthocyanins ubiquitously extensively distributed in the plant kingdom particularly in all seed plants and stored in vacuoles. In many fruits, pro-anthocyanidins and flavonols are the major phenolic compounds at the commencement of fruit development, with the flavonoid pathway typically switching to the synthesis of anthocyanins at the beginning of fruit ripening [24-26]. Betalains, yellow-to-red nitrogen included compounds, are derived of tyrosine in the plant kingdom [22], are subclass of flavonoid, a hug class of phenolic secondary metabolites that are extensive along with different species and involved in many plant developmental functions [24,27]. Anthocyanin is often produced in vegetative plant tissues [28]. Fruits, such as, pear, apple, citrus, Chinese bayberry, peach, play an vital function in nutrition and fitness benefits for its rich sources of vitamin C, flavonoids, carotenoids (provitamin A), and other nutraceutical compounds. Because of light color and having higher solubility, this

group is considering a prospective natural tincture to replace synthetic colorants. Due to its significance to the coloring quality of harvested and processed vegetables and fruits, they have been comprehensively inspected by food scientists and horticulturists [21,29]. Of these healthful materials, anthocyanins belonging to the flavonoids compound family are water soluble pigments are present in fruits and flowers, as attractants for insects and animal. These compounds have been suggested to reduce cancers, oxidative stress and heart diseases [4,21,30,31].

Anthocyanin pigments have free radical scavenging and anti-oxidative properties and protection against various pathogens, attraction of pollinators for pollination and predators for seed dispersal, as well as the new projected modulation of signaling cascades and give their antioxidant capacity, they are believed to protect plant cells against ultraviolet (UV) radiation and high light intensity, cold temperature, water stress, wounding and to defend against microbes and phytopathogens [28,32]. The major function of anthocyanins is the antioxidant activities and fortification against DNA injury, these composite being capable to detain hazardous free radicals as singlet oxygen ($1O_2$), superoxide radical (O_2^-), hydroxyl radical (HO) and hydrogen peroxide (H_2O_2), chemical groups that direct to lipid peroxidation of cell membranes. [33]. UV-B radiation (280-320 nm) can direct to the inhibition of photosynthesis, peroxidation of lipids, degradation of proteins, as well as reduced plant biomass and development. Most of radiation is absorbed in DNA, is oxidised by UV-B is 240 to 310 nm waveband. Expression of gene might be changed through the development of pyrimidine dimers [34]. Anthocyanins have been discovered as strong inhibitors and novel antioxidants of lipid peroxidation in contrast with other standard antioxidants [1,35] and its phenolic structure is dependable for antioxidant actions; i.e., capability to search reactive oxygen species (ROS) such as superoxide (O_2^-), peroxide (ROO^-), singlet oxygen ($1O_2$), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2), [36]. Due to many reasons, anthocyanin biosynthesis in fruits becomes indispensable aspect of currently active research area, which is supportive to understand the mechanism better and develop novel fruit cultivars with higher anthocyanin content [31].

The anthocyanins distribution in grapes branches are unpredictable, depending on climatic and physiological factors [37]. In a number of red grapevines, for instance Pinot Noir and Cabernet Sauvignon, the anthocyanins accumulation primarily in the hypodermal cell layers following veraison in grapes skin [38,39]. Though, there are still a number of exceptional grapes of *V. vinifera* also produce high content in their fruit pulp [40]. The 3-glucoside anthocyanins: Cyanidin, Petunidin, delphinidin and malvidin are available in red wines. Though, malvidin 3-glucoside coumarate and malvidin 3-glucoside acetate are the major compounds of all edible plant berries [41].

Factors Affecting Anthocyanin Biosynthesis

Over the last few decades, investigation to understanding of the molecular mechanism underlying the ecological regulation of the anthocyanin biosynthesis in fruit plants has been increased. Factors such as growth conditions, growing region and variety can influence the intensity of anthocyanins produced and the profile of various pigments [42]. However, in all fruits, environmental cues affect the qualitative and quantitative composition of anthocyanins in the maturation of fruits. In general, it depends on the genetic background of the species/variety determine which type of anthocyanins will produce in respected fruits, yet environmental factor can affect the

concentration of diverse anthocyanins in different ways [43]. The development in anthocyanins color depends on the level of pH, metal ions *in vitro* and with coexisting monochrome compounds (co-pigments, typically flavonols and flavones), anthocyanidins are redder and extra unvarying as the flavilium cation produce at low pH (pH <3), under slightly acidic conditions it is colorless (pH 3–6), and at pH 6 and above bluer and unbalanced as the quinonoidal stand [37,44].

Anthocyanins reversibly undertake skeleton transformation with alteration in pH, which has a most important effect on color shades (Figure 2). The oxonium appearances that dominate at pH 1.0 are colored. Thus at pH 3.01, cyanidin-3-glucoside form, 50% will be colorless and 50% colored. Glycosidic substitution at the 5 location having low pKa whereas acylation along with cinnamic acids distinctly strengthen the pKa. Acylated 3-glycosides predominate in blackcurrant anthocyanins. These strengthen the pH array where blackcurrant can efficiently be useful for food.

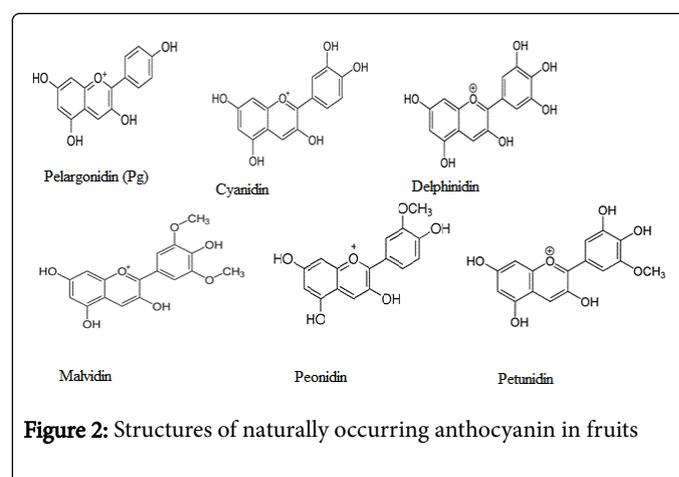


Figure 2: Structures of naturally occurring anthocyanin in fruits

Whereas variation in anthocyanin skeleton can affect pigment permanence, concentration and the foodstuff matrix can also have a wide range effects on pigment strength [29]. There are also other possible factors impart in synthetic pathway such as co-pigmentation and pH that affect the perceived hue of a tissue [45]. Anthocyanin biosynthesis can be affected by environmental and internal factors, such as temperature fluctuation, UV-B, light and ABA through changes in expression of regulatory and structural genes [31]. In recent studies it has been reported that, non-climateric fruits, in the regulation of ripening ABA is involved in the related anthocyanin biosynthesis [46,47]. Effect of Jasmonates (JAs) have been studied to color formation in grapevine and apples, it might be possibly via interaction with ethylene biosynthesis [48].

Several current predictions are associated with the effect of the environmental factors with changes in the regulatory complex controlling anthocyanin biosynthesis in crops [49]. Many genes concerned in anthocyanin synthesis have pleiotropy and are affected by many external and internal factors. Recently, most of new regulatory genes have been acknowledged in apple, grape and other fruits [50]. Some of them are responsive to environmental stimuli, for example hormones, nutrition, temperature and light intensity [4,51,52]. The regulations of enzymatic activities in the various pathways are extremely controlled by genes. Transcriptional controls having crucial function in controlling the generally activities of flavonoides biosynthesis. The passageway is also regulated in response to various environmental and developmental cues [2,53].

MYB proteins are major components in the provision of definite gene expression patterns, while the bHLH proteins and supplementary component of the regulatory compound and might have beyond controlling targets [26]. It has been revealed that certain *R2R3-MYB* genes interrelated to flavonoid biosynthesis way in fruits respond to light or other environmental stimulus in a diverse manner [54]. Azuma, Yakushiji, Koshita and Kobayashi [49] reported expression pattern of anthocyanin biosynthesis-related MYB genes (*VIMYBA1-3*, *VIMYBA2*, *VIMYBA1-2*) in grapevine berries and recorded variation in the skin of berries kept in darkness or light at low (15°C) or high (35°C) temperature, whereas some other examined *MYB* genes (*MYB5a*, *MYB5b*) of the pathway did not react to the treatments. The highest anthocyanin production levels were measured in grapevine berries kept in light at 150°C [55]. Zhou, Shi and Xie [55] also reported In Arabidopsis that higher nitrogen concentration decrease in the expression of *PAP1* and *TT8*, genes from the TTG1–GL3/TT8–PAP1 (WD40–bHLH– MYB) anthocyanin biosynthesis regulatory complex, and an increase in the expression levels of three lateral organ-boundary domain genes (*LBD37*, *LBD38*, *LBD39*) that perform as negative regulators for biosynthesis.

Anthocyanin regulating genes and enzymes

The biosynthetic pathway leading to anthocyanins is well known and the main regulatory genes controlling the pathway have been studied and isolated in many plant species [26]. The enzymes belongs to diverse families, such as cytochromes (P450), 2-oxoglutarate-dependent dioxygenases (OGD) and glucosyl transferases (GT), which propose that plants recruit enzymes from preexisting metabolic passageway. Phylogenetic analysis specified that genes encoding enzymes with the similar activities had deviated previous to the speciation of flowering plants [56]. Two different classes of genes are involved in anthocyanin synthesis, the structural genes encoding the enzymes that directly contribute in the production of flavonoids and anthocyanins, and the dictatorial genes that direct the structural genes transcription [24]. The transcription factors that specially involved in regulating the expression of the structural genes of the pathway have been reported in many plants. The regulatory elements are still under investigation [57]. Consequently, there are very exceptional reports are available about the control of anthocyanin genes in plants root system [58]. There is indication of the enzymes implicated in flavonoid metabolism acting as membrane linked with the multi-enzyme complexes, which have connotation on generally competence, regulation and specificity, of the pathway [53]. These regulators interact with each other and the partnership seems to be complex, they build transcription complexes through the promoters of the structural genes. For instance, in maize MYB domain C1 protein regulate the anthocyanin synthesis pathway needs a bHLH partner (B/R) to make active dihydroflavonol reductase (DFR) promoter and the flavonoid structural genes, while MYB domain P protein controlling the phlobaphene, which activate the promoter lacking a bHLH partner [59]. Furthermore, new dictatorial genes, such as TT1, a zinc finger protein [60], TTG2, a WRKY transcription factor [61], ANL2, a home domain (HD) protein [62] and TT16, a MADS domain protein [63], have been documented. A number of genes are implicated in a variety of features of plant growth, besides flavonoid synthesis, including growth and development of the seed (*TT16*, *MADS*; *TT1*, *zinc finger*; *AN11*, *WD40*; *ANI*, *bHLH*) [64], root hair and trichome (*WRKY*, *TTG2*; *GL3* and *EGL3*, *bHLH* *TTG1*, *WD40*) [65], root (*ANL2*, *HD*), and mucilage formation (*TTG1*, *TTG2*, *EGL3* and *GL3*) or acidification of vacuoles (*ANI* and *AN11*).

MYB proteins plays pivotal role in the regulation of the biosynthetic pathways of assorted secondary metabolites, growth and developmental changes, disease resistance and signal transduction [66]. *MYB* genes contain 100–160-bp DNA-binding domains comprising single or multiple repeats are structurally conserved. The *R2R3 MYB* genes related with the flavonoid pathway have two repeats and characterize the most abundant group of *MYB* genes in plants. 126 members of the R2R3 subfamily in Arabidopsis have been characterized and, out of these, 13 members are associated to the regulation of flavonoid metabolism [66,67].

A comprehensive judgment of anthocyanin gene expression is convoluted by not only the number of genes involved in the pathway, but also fact that most of these genes present in the form of multiple copies in the genome [68]. In various studies it was pointed out that the structural genes contributed in anthocyanin synthesis were coordinately expressed and their levels of expression were certainly related to the degree of anthocyanin concentration [4,52]. In fruits, number of key genes that are implicated in the regulating fruit ripening have been reported, many of them are SEPALLATA- and SQUAMOSA-class MADS box or SBP box transcription factors [69,70]. Particularly in grapes, the flavonoid biosynthesis regulation has been investigated thoroughly and 14 flavonoid biosynthesis related R2R3 MYB family members have been identified [67]. In *V. vinifera*, there are at about three genes encoding CHS, Chs1 (AB015872), Chs2 (AB066275) and Chs3 (AB066274), which are transcribed under diverse controls [71,72]. While in apple, coordinated expression of MdF3H, MdANS, MdCHS, pDFR and pUFGluT together was correlated well with anthocyanin biosynthesis [73]. Furthermore, the transcription levels of UFGT were highly expressed in red fruits compared to white skinned in grape [74] and ANS, UFGT and CHS, in citrus [75]. From the above mentioned information, the corresponding expression pattern of structural genes related to improving anthocyanin synthesis is reliant on species.

Anthocyanin Biosynthetic Pathway

Anthocyanin biosynthesis initiates at the start of ripening of the fruits begins and usually continues throughout the ripening phase [38,76,77], examined some enzymatic activities throughout anthocyanin biosynthetic pathway and measured during the development of De Chaunac in berries. Accumulation of anthocyanin begins just at the start to accumulate sugars and increased rapidly until the concentration leveled off late in ripening, till total soluble solids were approximately 24 °Brix (a refractive index measure of the total dissolved solids). The basic upstream pathway of flavonoid has been evaluated comprehensively at genetic and biochemical levels in different species, and demonstrated in a number of model species, such as soybean, Arabidopsis and barley, as well in many other fruit plants, especially in grapes and apple [78,79]. During the flavonoid biosynthesis enzymes have pivotal role in each step and enzymatic activities have been investigated, these enzymes are present in the cytosol. Flavonoid pathway are encoded by a number of gene families, while enzymes action is delayed in the anthocyanin pathway were usually encoded by distinct active genes. After synthesis of pigments, flavonoids are transported to cell walls or vacuoles [80].

The synthetic pathway of anthocyanin is also well implicated and conserved among most of seed plants [3,81]. Roubelakis-Angelakis and Kliewer [82] found in Cardinal grapes that as the ripening progressed, PAL activities increased paralleled with anthocyanin accumulation. In various *Vitis* species and varieties a unique set of anthocyanins and the

profiles has been reported [42]. Usually *Vitis vinifera* varieties produce 3-acetylglucoside, 3-monoglucoside and 3-p-coumarylglucoside derivatives of the aglycones, petunidin, peonidin, malvidin, cyaniding and delphinidin with malvidin derivatives often being the main structures are found [38,76]. In the beginning of the series of chemical reactions, phenylalanine is the initial and direct predecessor for the synthesis of anthocyanidins (Figure 3). Flavonoids are produced in the cytosol, while synthetic enzymes form a super-molecular complex (metabolon) *via* interface among proteins and are secure in the endoplasmic reticulum (ER) membrane [3,81]. The conversion from phenylalanine to anthocyanines requires a series of reactions catalyzed by enzymes, initiated by ammonia catalyzed and the action of enzyme called phenylalanine ammonia-lyase (PAL), PAL is significant catabolic agent, producing nitrogen and carbon. In plants it is a major enzyme that catalyzes the initial steps in the production of a diverse polyphenyl compounds (Figure 3) and is mainly involved in defense mechanisms [83]. Meanwhile in this step phenylalanine transformed into trans-cinnamic acid by transamination of ammonia. During transformation of ammonia many structural genes are involved and PAL is the primary gene but its role is not yet specified and controversial in previous studies [36]. Followed by transformation of trans-cinnamic acid mediated changes into 4-coumaroyl-CoA with the action of C4H and 4CL which also leads to synthesis of Hydroxyphenyl lignin in fruit trees [31]. While lignin, stilbenes, amides, benzoic acids and esters were produced in some species, whereas lignin acts as structural support and stilbenes are helpful in defense against biotic and abiotic stress. Subsequently flavonoid pathway initiated by three molecules of malonyl CoA (at the same time acetic acid with acetyl CoA carboxylase (ACC) reacts with malonyl-coA) with the single molecule of 4-coumaroyl-CoA produced naringenin chalcone; stereo specifically and rapidly isomerized, in addition to converts into Naringenin by chalcone isomerase (CHI), the I gene regulating CHI enzyme activities while in some plants such as in bilberry Fruit naringenin chalcone will produce (Figure 3) [24]. This reaction is accomplished with the help of CHS (chalcone synthase), plays as catalytic enzyme and pathway diverges into different branches which lead to the formation of different classes of flavonoids. In the biosynthetic pathway single enzyme is reacting to catalyzing agent, overall stability of the pathway have impact on the synthesis of anthocyanidin because of over expression, heterologous expression or silencing of one genes direct to significant changes in the flavonoid composition of the target tissues [22,31]. In some fruit crops CHS elements are essential to be identified. Several studies, have identified the expression of the CHS in tissue was passively linked with fruit coloring as demonstrated in grape [71], apple [73], bilberry [24], citrus [75], pear [8] etc.; and was up-regulated by low temperature [4,52], light [50], and UV-B [51], and was unchanged or inhibited with the ethanol application [84]. Thus, the CHSs in fruit trees appeared to be under different transcription controls, respectively. With the increase in enzymatic activities cinnamate-4-monooxygenase, p-coumarate:UFGT paralleled increase in anthocyanin content while CoA-ligase catalyzes reactions before CHS in the biosynthetic pathway [51,77]. Naringenin chalcone deviated into two main pathways, produce flavones glycoside and dihydroflavonols, somewhere flavones glycoside is colorless compound. In various fruit crops flavanone 3-hydroxylase reacts with Naringenin and switch into dihydrokaempferol along with direct to three major products (Figure 4), which leads to development of different forms of anthocyanin as end product of this pathway. However, as accumulation began the activities of PAL and CHI decreased and then subsequently increased (Figure 3).

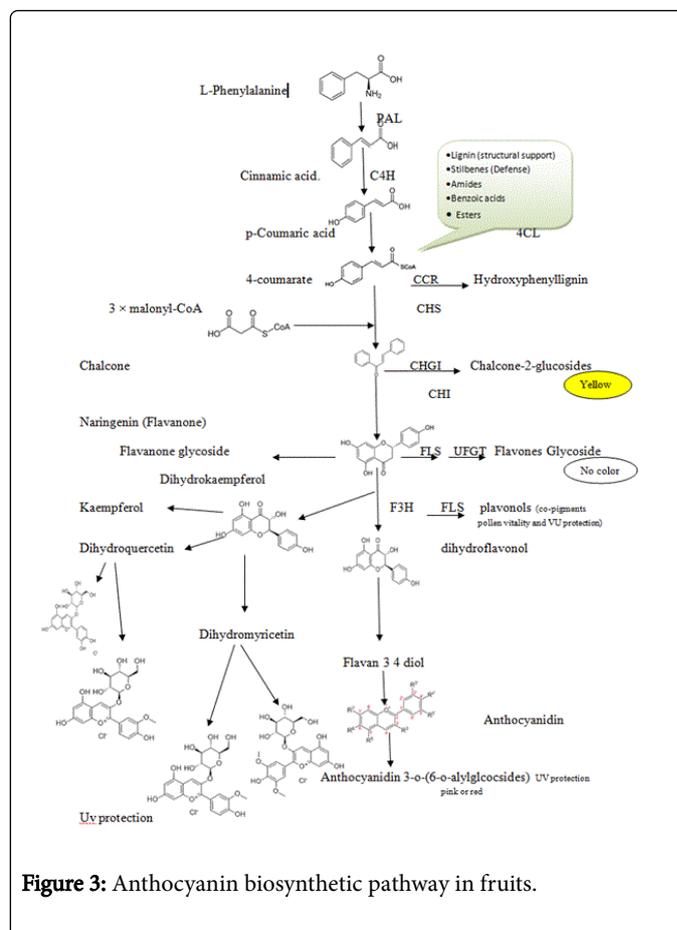


Figure 3: Anthocyanin biosynthetic pathway in fruits.

CHS activity is very difficult to predict until anthocyanins were detected after third week of developmental stages [77]. Kaempferol is the first product is the consequence of flavonol synthesis and dihydrokaempferol. Kaempferol has been identified in number of plant, generally used in traditional medical treatments. While the other two main products are produced by F3H and F35H are Dihydroquercetin and Dihydromyricetin respectively. Dihydromyricetin is unstable compound and converts into leucocyanidin or Dihydroquercetin as side product in some fruits. Later on hydroxylated at the 3-position by F3H, naringenin converts to dihydro-flavonols, which subsequently reduced to leucoanthocyanidin by k, While in various plants the same pathway flavanone diverts into two, first is to synthesis of Flavonol glycosides, where FLS and UFGT act as enzymes to convert dihydroflavonol, mostly have no color or pale yellow; second one is to production of flavan-3, 4 Diol with the reaction of DFR, leads to the anthocyanidin and anthocyanidin 3-o-(6-o-allylglucoside), its color is pink or red (Figure 4).

Following the synthesis of dihydrokaempferol at each reaction there are side branches or products were synthesized, having specific biological functions. As early studies showed that dihydrokaempferol sidetracked into two branches and produce dihydroquercetin and dihydromyricetin while both of them leads to the production of same compound with the identical regulating enzyme (DFR) [24,85]. In fruit plants, pro-anthocyanidins and flavonols are the most important phenolic compounds produced at the commencement of fruit growth (Figure 4), with the flavonoid pathway typically switching to the synthesis of anthocyanins at the beginning of

maturation stage [24,25]. It is the most common and present in over 82% of examined fruits and berries [85]. Leucoanthocyanidin at both sides degraded into procyanidin and prodelfinidin with action of leucoanthocyanidin reductase (LCR). Leucoanthocyanidin at both ends ANS directs to cyanidin and Delphinidin. Delphinidin and its methylated derivatives, malvidins and petunidins are sources of purple and dark bluish colors, whereas bright red colored fruits have pelargonidins and cyanidins are main pigments [24,85]. In the majority fruits Cyanidin 3-glycosides are found and main pigments of anthocyanidins, such as in red-skinned apples (Figure 4). In some fruits like cherries, raspberries and strawberries have pelargonidin glycosides, in addition to cyanidin glycosides. Dark skin colored fruits such as certain grapevine and blueberries also contain petunidin, malvidin, delphinidin, glycosides and peonidin [26].

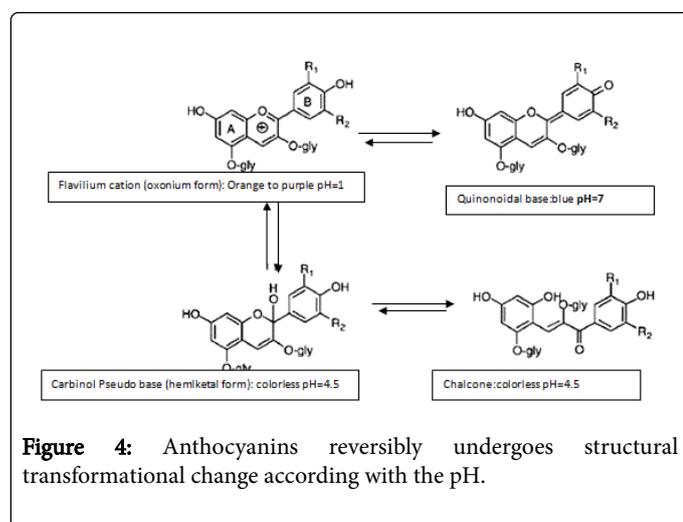


Figure 4: Anthocyanins reversibly undergoes structural transformational change according with the pH.

During the next phase Flavanone 3-hydroxylase (F3H), flavonoid 3- hydroxylase (F3OH), and flavonoid 30, 50-hydroxylase (F3050H) direct the synthesis of cyanidin, delphinidin and anthocyanidins. Delphinidin and its methylated derivatives, malvidins and petunidins, are sources of dark bluish and purple colors, whereas cyanidins and pelargonidins are the main pigments in bright red color present in fruits [24].

ANS catalyzes using leucoanthocyanidin as substrate and produce corresponding colored anthocyanidins. At the end, glycosylated hydroxyl group at C3 of anthocyanidins by glycosyl moieties from UDP trigger sugar donor molecules by the action of UFGT to yielded consequent anthocyanins, flavonoids are synthesized in the cytosol and finally stored in the vacuole, with an acidic environment in the subcellular compartment of cell. Glycosyl moieties transformed anthocyanidins in various synthetic ways in a specific to family or species, and its color pattern significantly rely on hydroxyl groups on the B-ring; as the number of larger groups, the darkens the blue color [22,31]. In grape berries anthocyanins is synthesized through the flavonoid path, which split the similar upstream pathway throughout proanthocyanidins, in anticipation of the development of anthocyanidins by the catalysis of anthocyanidin synthase (ANS), also known as leucoanthocyanidin dioxygenase (LDOX) [11,79].

Meanwhile in some plant species FLS assist to produce Dihydroflavonol and Flavonol glycoside, Flavonol glycoside have no color or some time pale color, these co-pigments are helpful against UV protection and pollen viability (Figure 4). Leucoanthocyanins by leucoanthocyanidin dioxygenase/antho-cyanidin synthase (LDOX/

ANS) converted into anthocyanidins and promote glycosylated by UDP (uridine diphosphate)-glucose: UFGT. O-methyl transferases (OMTs) catalyze and produce O-methylated anthocyanins such as peonidin, malvidin and petunidin [80].

In addition anthocyanidin is advance made to order with methyl, acyl or glycosyl classes, catalyzed by methyltransferases (MT) glucosyltransferases (GT) and acyltransferases (AT), [22], the variation of anthocyanidins is family or species dependent on diversity. Diverse UDP-glucose-dependent glycosyl transferases belongs to glycosyltransferase family (<http://www.cazy.org/fam/accGT.html>), acyltransferases (ATs) mainly belonging to the BAHD family [86].

Conclusion

Anthocyanins correspond to a class of an important antioxidants, are common in plants and foods. Recently, many researchers have been reported on the biosynthetic pathway and there function in plant as well in human body. There is need for further investigation about regulation of anthocyanin, relationship among their chemical structure and antioxidant capacity and limited enzymatic studies of anthocyanin accumulation and control of biosynthetic pathway, because of what anthocyanin biosynthesis to be switched on during fruit ripening. Thus, the limited information about enzymatic studies on accumulation of anthocyanin in grapes has not revealed a deep knowledge about the control of biosynthetic pathway and main causes of anthocyanin biosynthesis to be switched on during ripening. During this event, MYB-bHLH-WD40 transcription factor complexes direct the transcriptional transformation of the flavonoid biosynthesis genes. Though, the signaling network following the ripening associated anthocyanin biosynthesis remains unclear. However, many key players in the complicated regulatory network remain to be identified, PAL is the primary gene but its role is contradictory and not yet specified in previous studies [26, 33]. However, little is known about anthocyanin accumulation in roots of carrots or other species. Consequently, there are very exceptional reports available about the control of anthocyanin genes in plants root system [58].

Anthocyanins contribute to the quality trait of fruits and vegetables in a significant way and are therefore targets in many plant breeding programs. The chemistry of anthocyanins in fruits is formed via the process of complicated metabolic networks regulated by genetic, environmental and developmental factors. However, need further clarification of other environmental factors associated with biosynthesis mechanisms. New insights have also been gained regarding the contribution of fruit development related transcription factors to anthocyanin biosynthesis, but more in-depth studies are required for an understanding of the nature of the interaction as well [26]. However more investigation about anthocyanins is necessary to establish the wide spectrum of human health benefits, the recently existing data and current reports are extremely cheering [87].

Future perspectives

Although during last decades of research work on important role of anthocyanins in human and plants, integrated findings remains ambiguous. A number of speculations were articulated to elucidate the key role in plant leaves. Nevertheless, none accommodates the extent of variability in pigmentation outline over time, space, or the multiple environmental signals. Anthocyanins biosynthetic pathway and its production in fruit tree crops is one of the important aspects under recent active research area not only due to visual appeal but also

nutritional value. Identification of transcription factors in fruit crops is limited, and their response to cooperatively respond to internal and external factors, and then regulate structural genes needs to be further investigated. Anthocyanin is increasingly used with new enology practices to production and stability of anthocyanin derivatives in the future, in human consumption and functional foods products. However, need of more detailed investigation of new flavonoids and significance *in vivo* of anthocyanins require thorough investigation, furthermore, there are still many facets to be known during biosynthesis and regulation of anthocyanins, such as the association of genetic control among proanthocyanidins and anthocyanins, precise method of anthocyanin intracellular transport, the specific mechanism that normalize the period of anthocyanin accumulation. We believe such findings will be discovered through the ongoing research work in future.

Competing Interests

The authors declared that they have no competing interests.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

TP, JS and FF searched material and participated in drafting the manuscript. ZC and JF conceived of the study and reviewed the English correction, and participated in coordination. All authors read and approved the final manuscript.

Acknowledgment

This work is supported by Important National Science and technology Specific Projects (No. 2012FY110100-3).

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