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Flavonoid Biosynthesis and Regulation: Keys to Understanding Plant Adaptation

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Abstract
Plant secondary metabolites, particularly polyphenolic compounds, play crucial roles in plant adaptation, defense mechanisms, and environmental interactions. This diverse group of bioactive compounds, known for structural complexity, has garnered significant interest due to its potential as candidate drugs and antioxidants.Flavonoids, a subclass of polyphenols, exhibit a 15-carbon skeleton structure and are involved in various physiological activities. The flavonoid biosynthetic pathway is complex, involving shikimic acid and phenylpropanoid pathways, with intricate regulation and transportation processes. Transcription factors like MYB, bHLH, and WD40-like proteins play pivotal roles in regulating flavonoid biosynthesis.Understanding the biosynthesis, regulation, and roles of these secondary metabolites provides insights into plant biology, with potential applications in drug development and health-promoting compounds.

Keywords: Polyphenols; secondary metabolism; flavonoids, regulation.

Introduction

Plant secondary metabolites are polyphenolic compounds that plays pivotal roles in plant adaptation, defense mechanisms, and interaction with the environment. This group of bioactive compounds, also known as specialized metabolites are a large group of phytochemicals, which are not directly involved in plant's vital processes such as growth, development, and reproduction (Fraenkel, 1959), but are major components in defense mechanism of (Stamp, 2003; Samuni Blank et al., 2012). Secondary metabolites are of special interest to the scientific community because of their structural diversity and their potency as a candidate drug and/or antioxidants.

Although several criteria, including chemical structure (presence of rings or sugars), composition (presence or absence of nitrogen), their solubility properties, have been considered for the classification of secondary metabolites, their biosynthetic pathway has been the most prominent one for their classification. On the basis of their chemical structures, secondary molecules have been broadly classified into terpenoids, alkaloids, phenolics, glycosides, tannins, and saponins (Verpoorte, 1998). On the basis of their biosynthetic pathway, secondary metabolites have been categorized into three broad groups: terpenes, phenolics, and alkaloids (Ávalos et al., 2009). With more than 40,000 different molecules, terpenes constitute the largest group of secondary metabolites in plants (Ávalos et al., 2009). From a chemical point of view, they are non-saponifiable lipids since fatty acids do not intervene in their

formation. They are also known as isoprenoids, since the basic structural unit that forms them is the isoprene molecule (Vranová et al., 2012). Terpenes are further classified on the basis of the number of isoprene units into monoterpenes, with three units in sesquiterpenes, with four in diterpenes, with six in triterpenes, with Chapter 2 Review of Literature 8 eight in tetraterpenes, and with more than 10 in polyterpenes. Many plants contain terpenes in their flowers and fruits as mixtures of volatile compounds with specific odors. As a constituent of the photosynthetic apparatus (carotenes), constituent of cell membranes (phytosterols), electron transport chain (ubiquinone and plastiquinone), and as regulators of plant growth and development (giberilins, strigolactones, brassinosteroids), defense (monoterpenes), pollination (sesquiterpenes α-farnesene and germacrene D.) terpenes have several biological functions. Alkaloids constitute another large and diverse group of secondary metabolites. Even while there is no uniform classification of alkaloids, several criteria, including biosynthetic origin, presence of basic heterocyclic nucleus in the structure, pharmacological properties, and distribution in plant families, have been used for their classification. Among these, biosynthetic origin of the alkaloids has been used most frequently for classification of alkaloids. On the basis of this criterion alkaloids are classified as pure alkaloids, protoalkaloids, and pseudoalkaloids. Majority of the alkaloids found in plants belong to this group. They contain intracyclic nitrogen, have basic character and are compounds of high reactivity. Lornithine, L-lysine, L-tyrosine, L-tryptophan, L-histidine, and L-arginine are often the precursor molecules for pure alkaloids. Protoalcaloides, in which the nitrogen atom is not part of the heterocycle, constitute a smaller class of alkaloids. This group of alkaloids is synthesized from L-tryptophan and L-ornithine and can also be considered as aromatic amines. Pseudoalkaloids contain heterocyclic rings with nitrogen but are not derived from amino acids. They are formed by subsequent incorporation of nitrogen into compounds originally free of this element. Terpenic alkaloids belong to this group. Alkaloids are known to play an important role in defence of plants against insects and herbivores. Due their therapeutic properties, alkaloids represent an important group of secondary plant products. Phenolics are aromatic compounds containing a hydroxyl group directly attached to an aromatic hydrocarbon. Chemically, phenolics are a very diverse group of secondary molecules; phenol being the simplest of all the phenolics. Simple phenolic compounds have C6 general skeleton representation (Fig.1), where “R” represents an organic group which could be alkyl, alkenyl, aryl etc. or hydroxy, alkoxy, amino etc, can be in the ortho (o), meta (m), or para (p) positions of the aromatic ring. Phenol itself is a benzene ring that is substituted with a hydroxyl group. Simple substituted phenol compounds include catechol, resorcinol, hydroquinone, pyrogallol, hydroxyquinol etc. Phenolic acids include phenols which contain a carboxylic acid. If the carboxylic acid functional group is directly bonded to the phenol ring, the phenolic compound is termed as hydroxybenzoic acid. When carboxylic acid functional group and the phenol ring are separated by two doubly bonded carbons (a C=C bond), phenolic compounds are termed as hydroxycinnamic acids. Phenolic compounds which contain more than one phenol unit are named as “polyphenol”. These molecules have a C15 general skeleton. Flavonoids and tannins are the two major groups of polyphenols present in plants. Flavonoids are polyphenolic secondary metabolites which occur naturally in plant tissues. Based degree of heterocyclic C-ring oxidation, the position of hydroxyl groups and the degree of polymerization, they can be classified into flavonols, flavones, flavonones, isoflavones, catechins, and anthocyanins (Winkel-Shirley, 2001). Currently more than 6000 flavonoids are known from different plant sources including leaves, fruits, nuts, seeds, and flowers. While all class of flavonoids play a central role to impart antioxidant potential in several plants such as fruits, vegetables, medicinal plants legumes etc, anthocyanins are class of flavonoids that also imparts color to fruits and vegetables as well.

Flavonoids are of particular interest to plant scientists as they perform a variety of roles in plants including pollination, protection against UV light, as defence molecules against pathogens and as signal molecules (Parr and Bolwell, 2000). Among the flavonoids, the anticancer and antioxidant activities of kaempferol and quercetin make them good candidates for inclusion in the human diet (Pietta, 2000; Ren et al., 2003; Williams et al., 2004).

Flavonoids are a class of polyphenolic compounds having the general structure of a 15-carbon skeleton which comprises two phenyl rings (A & B) and a heterocyclic ring (C) (Fig. 2). More than 10,000 compounds belonging to the flavonoid class have been reported based on their basic fifteen C_6-C_3-C_6 carbon framework or phenyl benzopyran moiety (Williams and Grayer, 2004; Tahara, 2007). On the basis of their structural diversity and redox state of the central pyran nucleus, flavonoids are broadly classified into 12 groups *viz.* chalcones, aurones, flavones, flavonols, flavanones, dihydrochalcones, catechins, flavan-3-4-diols, bioflavonoids, iso-flavonoids, proanthocyanidins, and anthocyanins (Fig. 3). Different compounds belonging to the above groups perform distinct chemical, physical and biological functions. While anthocyanins are brightly coloured and relatively stable molecules, flavonols are not so bright but are involved in several physiologically significant functions. Some of the important metabolites of these groups are cyanidin, malvidin, delphinidin, peonidin of anthocyanins; hesperidin, naringenin of flavanones; apigenin, baicalein of flavones; quercetin, rutin and myricetin among the flavonols. These compounds are reported to possess several bioactive properties including antioxidant, anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti- atherosclerosis, cardiovascular protection, improvement of the endothelial function, as well as inhibition of angiogenesis, and cell proliferation activity (Samanta et al., 2011; Panche et al., 2016). They are also known to be potent inhibitors for several enzymes, such as xanthine oxidase (XO), cyclo-oxygenase (COX), lipoxygenase, and phosphoinositide 3-kinase (Metodiewa et al., 1997; Walker et al., 2000). Flavonols, the most ancient and widespread flavonoids present in the plant kingdom, have a wide range of potent physiological activities including their responses to stress (Stafford, 1991; Pollastri and Tattini, 2011). Subramanian et al. (2007) have reported the role of flavonoids as symbiotic signal molecules that can directly interact with plant hormone signalling. The major classes of flavonoids and their subclasses with their source and function in plants are tabulated in Table 1.

Biosynthesis, cellular localization, and transport of flavonoids in plants:

Although the core set of reactions of flavonoid biosynthetic pathway are highly conserved, depending on the tissue and the species, isomerases, reductases, hydroxylases, and several dioxygenases modify the basic flavonoid skeleton to produce different types of flavonoids (Martens et al., 2010) (Fig. 2). Plants primarily have two general routes, *viz.* shikimic acid pathway and the phenylpropanoid pathway, for the biosynthesis of phenolic compounds. In the shikimic acid pathway phenol pyruvate and erythrose-4-phosphate react in a few steps to provide 3-dehydroquinate. Dehydration with shikimate dehydrogenase gives 3-dehydroshikimic acid which is again reduced to yield shikimic acid. Shikimic acid is then converted into chorismic acid which undergoes Claisen rearrangement to generate prephenic acid. The product is then converted in several steps into tyrosine which serves as a central point and a crucial precursor for the biosynthesis of various phenolic compounds. The other route *viz.* The phenylpropanoid pathway is essentially similar to the shikimic acid pathway till the synthesis of L-phenylalanine from where the phenylpropanoid pathway takes over. In this pathway, phenylalanine is converted to 4-

coumaroyl-CoA, which finally enters the flavonoid biosynthesis pathway. Phenylalanine ammonia-lyase (PAL) is the first key enzyme in the phenylpropanoid pathway (Liu et al., 2006). This enzyme catalyzes the conversion of L phenylalanine (L-Phe) to *trans*-cinnamic acid, a common substrate of different phenylpropanoid derivatives (Li et al., 2006). *trans*-cinnamate is hydroxylated by cinnamic-4-hydroxylases (C4H) and is finally activated by the 4- coumarate/cinnamate coenzyme and 4-coumaroyl-CoA-ligase (4CL), for the condensation of malonyl-CoA. As the major intermediates of the flavonoid biosynthetic pathway, chalcones are produced by the condensation of three molecules of malonyl-CoA and a single molecule of 4-coumaroyl-CoA. The condensation of 4-coumaroyl-CoA and malonyl-CoA is catalyzed by chalcone synthase (CHS) to form either tetrahydroxychalcone or trihydroxychalcone. Chalcone synthase, the first enzyme specific to the flavonoid pathway, produces chalcone scaffolds from which all flavonoids are derived. Chalcones are converted to the (2S)-flavanone naringenin by chalcone isomerases (CHIs) in a ring-closing step that forms the heterocyclic C-ring. These intermediates are further modified by a variety of hydroxylases, methyltransferases, reductases, and glycosyltransferases to form diverse flavonoids and isoflavonoids. For the biosynthesis of anthocyanins, dihydroflavonol reductase (DFR) catalyzes the stereospecific conversion of dihydroflavonols into the respective flavan-3, 4-diols (leucoanthocyanins) through NADPH-dependent reduction at the 4-carbonyl. Leucoanthocyanins are further converted to the anthocyanidins by anthocyanidin synthase (ANS; Winkel-Shirley, 2001; Pandey and Sohng, 2013) (Fig. 3).

Flavanone 3-hydroxylase (F3H) catalyzes the stereospecific 3-hydroxylation of (2S)-flavanone or naringenin, to form dihydroflavonol (dihydrokaempferol). Dihydroflavonols include dihydrokaempferol (DHK), dihydroquercetin (DHQ), and dihydromyricetin (DHM). Dihydrokaempferol (DHK) is further hydroxylated by flavonoid 3′-hydroxylase (F3′H) and flavonoid 3′5′-hydroxylase (F3′5'H), either at the 3' position or at both the 3' and 5' positions of the B ring, to form DHQ and DHM, respectively (Winkel-Shirley 2001; Andersen and Markham 2005). DHQ and DHM are further catalyzed to form cyanidin and delphinidin, respectively (Schijen et al., 2004). Dihydroflavonols serve as a common precursor for flavonols and anthocyanins and can either be oxidized by flavonol synthase (FLS) to form flavonols or reduced by dihydroflavonol reductase (DFR) using NADPH to synthesize leucoanthocyanins (flavan-3,4-diols)), which are subsequently converted to anthocyanidins by anthocyanidin synthase (ANS) (Kristiansen and Rohde, 1991). While flavonol synthase (FLS) catalyzes the conversion of DHK, DHQ, and DHM to various flavonols such as kaempferol, quercetin, and myricetin, dihydroflavonol reductase (DFR) catalyzes the conversion of DHK, DHQ, and DHM to various anthocyanins such as pelargonidin, cyanidin, and delphinidin (Tanaka and Brugliera 2013). UDP-glucose: flavonoid 3-O-glucosyltransferase catalyzes the transfer of the glucose moiety from UDP-glucose to the hydroxyl group at the 3' position of anthocyanidin's C ring. This interaction increases the aqueous solubility and stability of the final products in the vacuole. Lastly, transferases modify the flavonoid backbone with sugars, methyl groups, and/or acyl moieties, modulating the physiological activity of the resulting flavonoid by altering their solubility, reactivity, and interaction with cellular targets (Bowles et al., 2005; Ferrer et al., 2008).

There is strong evidence to show that enzymes of the phenylpropanoid and flavonoid biosynthesis pathways are organized into macromolecular complexes which are associated with endomembranes (Kutchan, 2005). Channelling of the flavonoids into subcellular compartments enables plants to effectively synthesize specific natural products and thus avoid metabolic interference. Winkel (2004) and

Ralston and Yu (2006) have demonstrated the involvement of cytochrome P450 monooxygenases (P450s)-related metabolons in phenylpropanoid, flavonoid, cyanogenic glucoside, and other biosynthetic pathways. Using transgenic tobacco plants expressing epitope-tagged versions of two phenylalanine ammonia-lyase isoforms (PAL1 and PAL2) and cinnamate-4-hydroxylase, Achnine et al. (2004) have provided additional evidence for the channelling of intermediates between specific isoforms of phenylalanine ammonia-lyase and cinnamate-4-hydroxylase. Moreover, the existence of a multienzyme complex has been proposed for the anthocyanin pathway in rice by yeast-two hybrid experiments (Shih et al., 2008). Recovery of flavonoid synthesizing enzymes from soluble cell fractions and immunolocalization experiments indicate a loose binding of the enzymes to the endoplasmic reticulum (ER), possibly in a multi-enzyme complex, whereas the pigments themselves accumulate in the vacuole (i.e. anthocyanins and proanthocyanidins) or the cell wall (i.e. phlobaphenes) (Winkel-Shirley, 2001).

The transport of flavonoids from the site of synthesis to various cell compartments and between tissues is a highly complex process and is poorly understood (Thompson et al., 2010 a,b; Zhao et al., 2011). However, the identification and characterization of flavonoid biosynthesis mutants in *A. thaliana* has provided some insight in this direction. Flavonoid transport is known to be associated with a complex vesicle trafficking system which is based on acyl, glycosyl, and methoxy substituting groups (Grotwold, 2004). Two major flavonoid transport mechanisms, referred to as "ligandin transport" and "vesicular transport", have been identified in *A. thaliana* (Grotewold and Davis, 2008; Zhao and Dixon, 2010). While the ABC proteins, a large family of transporters, are involved in ligandin transport of glycosylated flavonoids and xenobiotic aglycones (Frangne et al., 2002; Goodman et al., 2004), an antiporter bound with sugar and acyl residues has been reported to be responsible for vesicular uptake of flavonoids in secondary active transport (Marinova et al., 2007). In *A. thaliana*, vacuolar flavonoids/H+ antiporter reported in the seeds is a product of *tt12* mutant (Debeaujon et al., 2001, 2003; Marinova et al., 2007). The TT12 transporter belongs to the multidrug toxin efflux transporter family (MATE) found in *A. thaliana* tonoplast.

The ligandin transport model is based on genetic evidence that glutathione transferase (GST)-like proteins are required for vacuolar sequestration of pigments in maize, petunia, and *Arabidopsis* (Marrs et al., 1995; Alfenito et al., 1998). Vacuolar sequestration of anthocyanins in maize has been shown to require a multidrug resistance-associated protein-type (MRP) transporter in tonoplast, whose expression co-regulated strongly with the expression of structural anthocyanin genes (Goodman et al., 2004). MRP proteins are often referred to as glutathione S-X (GS-X) pumps because they transport a variety of glutathione conjugates. However, because anthocyanin–glutathione conjugate(s) have not been found, it is proposed that these GSTs might deliver their flavonoid substrates directly to the transporter, acting as a carrier protein (Koes et al., 2005). This hypothesis is supported by the fact that while *Arabidopsis*' GST (TT19), is localized in cytoplasm as well as tonoplast, it could bind to glycosylated anthocyanins and aglycones but not conjugate these compounds with glutathione (Sun et al., 2012). On the other hand, the vesicle-mediated transport model is based on observations that, before their import into vacuolar structures by an autophagic mechanism, anthocyanins and other flavonoids accumulate in the cytoplasm in discrete vesicle-like structures called anthocyanoplasts (Pourcel et al., 2010). Vesicle-mediated transport of anthocyanins in grapes has been shown to involve a GST and two multidrug and toxic

compound extrusion-type transporters (anthoMATEs). In plants more than 50 MATE proteins have been reported, among which TT12, FFT (flower flavonoids transporter), AFL5 (aberrant lateral root formation 5), and EDS5 (enhanced disease susceptibility 5) are the most prominent proteins which have been identified and functionally characterized (Braidot et al., 2008; Thompson et al., 2010b). These observations suggest the possibility of the coexistence of both mechanisms of transport, in which the participation of GSTs and transporters would be specific to cell and/or flavonoid-type (Gomez et al., 2011).

Regulation of flavonoid biosynthetic pathway:

Although flavonoid subgroups are derived from the same biosynthetic pathway, they accumulate differently in plant organs and tissues depending on developmental stage and environmental conditions. As a result, their distribution implies precise spatial and temporal regulation of the flavonoid biosynthetic pathway, which necessitates a specific combination of regulatory controls. Several transcription factors controlling the expression of known flavonoid biosynthetic genes have been isolated and studied in order to understand how flavonoid biosynthesis is regulated in plants. Table 2 lists these transcriptional factors, their sources, targeted plants, and the genes that regulate the flavonoid biosynthetic pathway. These factors are classified into six families viz. MYC, bHLH, MYB, WD40-like, WRKY, MADS Box, and TFIIIA-like proteins "WIP". In all the species analyzed to date, members of the R2R3-MYB domain protein family act as a common denominator in the regulation of the flavonoid biosynthetic pathway. While R2R3-MYB (M) acts in the MYB-bHLH-WDR (MBW) complex, which is composed of the bHLH (B) and WD40 (W) repeat families, to regulate the transcription of genes involved in anthocyanin and PA biosynthesis (Koes et al., 2005), R2R3-MYB (PFG) are known to directly bind to early flavonoid biosynthetic genes for regulation of their action (Mehrtens et al., 2005; Stracke et al., 2007). The MBW complex is highly organized, with each subunit performing a specific function such as DNA binding, activation of a target gene's expression, or stabilization of the complex of transcription factors. Genetic dissection of the flavonoid biosynthetic pathway has revealed the role of TRANSPARENT TESTA 4 (TT4), TT5, TT6, and TT7 in the control of chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), and flavanone 3′-hydroxylase (F3′H), respectively (Allan et al., 2008; Palapol et al., 2009). Successive reactions catalyzed by these enzymes generate dihydroflavonols, the last common precursors for the biosynthesis of flavonols, anthocyanins, and Proanthocyanidins. Dihydroflavonols are then oxidized by flavonol synthase (FLS) to produce flavonols, such as quercetin and kaempferol. These early biosynthetic steps are transcriptionally regulated by 3 closely related R2R3-MYB proteins *viz*. MYB11, MYB12, and MYB111, which activate the early flavonoid biosynthetic genes CHS, CHI, F3H, and FLS1 (Mehrtens et al., 2005; Stracke et al., 2007).

The ternary MYB-bHLH-WDR complex formed by TT2 (MYB family), TT8 (MYC family), and TTG1 (WD-like protein) has been demonstrated to regulate the expression of ANR in *Arabidopsis thaliana* (Gonzalez et al., 2008). Overexpression of *At*TT2, *At*TT8, and *At*TTG1 in *Fragaria ananassa* was demonstrated to increase the expression of F3'H, ANS, ANR, and LAR genes (Schaart et al., 2012). TT8, a basic helix-loop-helix transcription factor, has also been shown to activate *DFR* and *ANR* genes in *A. thaliana* (Nesi et al., 2000). WD40 repeats in the b unit of heterotrimeric G protein activate flavonoid-related Myb-like transcription factors like AN11 from *Petunia hybrida* and TTG1 from *Arabidopsis*

thaliana (Sompornpailin et al., 2002; Hichri et al., 2011). AN11 has been shown to indirectly regulate anthocyanin biosynthesis by regulating the MYB-like transcription factor anthocyanin2 following its binding with the MYB -like transcription factor GLABROUS1 (Hichri et al., 2011).

On the other hand, the flavonol branch of the pathway is regulated by the R2R3-MYBs PRODUCTION OF FLAVONOL GLYCOSIDE (PFG1/ MYB12, PFG2/ MYB11, and PFG3/ MYB111) (Mehrtens et al., 2005; Stracke et al., 2007). While the three flavonol-specific regulators MYB11, MYB12, and MYB111 (PFGs) activate CHS, CHI, F3H, and FLS1 in parallel, they do not regulate the expression of either DFR or UFGT (Mehrtens et al., 2005; Stracke et al., 2007). CHS, CHI, F3H, and FLS1 are co-regulated in *A. thaliana* and are required for the formation of the basic flavonol aglycone from p-coumaroyl-CoA (Hartmann et al., 2005) (Fig. 3). The WRKY family of transcription factors, such as *At*TTG2, has also been linked to flavonoid biosynthesis (Johnson et al., 2002; Ishida et al., 2007). The WRKY gene (TTG2) is a zinc finger protein that functions downstream of the WD40-like protein. As a result, WD40 is regulated by TT2 and Myb, as well as the MADS homeodomain genes. Furthermore, the TTG2 protein acts upstream of other regulatory genes to directly regulate BAN (*banyuls*), which encodes an ANR in the seed coat of *Arabidopsis thaliana*. Flavonol regulation in *A. thaliana* is governed by a different set of genes than the anthocyanin biosynthetic pathway, such as *At*MYB11 and *At*MYB12. The two proteins have been shown to regulate the expression of CHS, CHI, F3H, and FLS in different tissues in response to light (Mehrtens et al., 2005; Stracke et al., 2007). The MADS-box transcription factor family is also well-known for its role in the regulation of the flavonoid biosynthetic pathway. While Jaakola et al. (2010) linked the expression of *Vm*TDR4, a SQUAMOSA-class MADS-box transcription factor, to anthocyanin biosynthesis in bilberry, Lalusin et al. (2006) have correlated the expression of *Ib*MADS10, a sweet potato (*Ipomoea batatas*) SQUA transcription factor, with anthocyanin biosynthesis.

Apart from these major regulatory families, some other regulatory genes are known to have direct or indirect effects on flavonoid biosynthesis. These modulators belong to different transcription factor families such as R3-MYB, MYBL2, miR156- targeted SQUAMOSA PROMOTER BINDING-LIKE9 (SPL9), the WIP-type zinc finger protein TT1, class II CIN-TCP protein TCP3 and Anthocyaninless2 (ANL2). Anthocyaninless2 (*At*ANL2) gene from *Arabidopsis thaliana* is an example of a homeobox gene from the homeodomain leucine zipper (HD-Zip IV) family. This gene encodes a homeodomain protein of the HD-GL2 family, which is thought to be important in anthocyanin accumulation and root development (Vernoud et al., 2009). Some negative regulators from the MYB superfamily have also been shown to inhibit flavonoid biosynthesis. These regulators share a conserved motif in their C- terminal end (Vom Endt et al., 2002). These suppressor genes coding for MYB repressors/activators compete with endogenous MYB-related activators to inhibit flavonoid biosynthesis. Overexpression of the *Fa*MYB1 transcription factor has been shown to inhibit flavonoid biosynthesis in tobacco as well as the accumulation of anthocyanins and flavonols (Aharoni et al., 2001). Overexpression of *Am*MYB308 and *Am*MYB330 MYB transcription factors isolated from *Antirrhinum* has also shown to suppress the phenylpropanoid biosynthetic pathway in tobacco (Tamagnone et al., 1998). In addition, some signal transduction pathway components have also been shown to work upstream of transcription factors and bind to the promoters of flavonoid biosynthetic pathway genes, *Arabidopsis* ICX1 (increased chalcone synthase expression 1) mutant is one such example. In response to various stimuli, the mutants have been

shown to induce the expression of CHS and other flavonoid biosynthesis genes (Wade et al., 2003).

Understanding the intricate world of plant secondary metabolites raises a myriad of compelling questions, compelling scientists to employ diverse techniques for exploration. How do plants biosynthesize and regulate the production of secondary metabolites, and what ecological roles do these compounds play in interactions with other organisms? Unraveling the molecular mechanisms governing the biosynthetic pathways poses challenges that necessitate advanced genomic and transcriptomic analyses. How do environmental factors, such as climate change or nutrient availability, influence the qualitative and quantitative aspects of secondary metabolite profiles in plants? Addressing this question requires sophisticated metabolomics approaches, leveraging technologies like mass spectrometry and nuclear magnetic resonance spectroscopy. Furthermore, how can synthetic biology and metabolic engineering be harnessed to manipulate plants for optimal secondary metabolite production? Investigating these possibilities demands interdisciplinary collaboration, bringing together experts in plant biology, genetics, chemistry, and engineering. As researchers delve into these inquiries, the application of cutting-edge techniques will be paramount in unlocking the full potential of plant secondary metabolites, with implications for agriculture, medicine, and environmental sustainability.

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Table 1: Flavonoids, their classes and rich dietary sources

Flavonoid	Class	Dietary sources	References
Abyssinones	Flavanone	French bean seeds	Rathmell and Bendall (1971), Cruickshank et al.(1974)
Apigenin	Flavones	Milk, chocolate, commercial, reduced fat	Hertog et al.(1997)
Biochanin	Isoflavone	Red clover, soya, alfalfa sprouts, peanuts, chickpeas (Cicer arietinum), other legumes	Medjakovic & Jungbauer(2008)
Daidzein	Isoflavone	Soyabeans, tofu	Zhang et al.(2009)
Diosmetin	Flavone	Vetch	Andreeva et al.(1998)
Epicatechin	Flavan-3-ols	Milk, chocolate, commercial, reduced fat	Arts et al.(2000)
Eriodictyol	Flavanone	Lemons, rosehips	Hvattum(2002)
Fisetin	Flavonol	Strawberries, apples, persimmons, onions, cucumbers	Sahu et al.(2014)
Genistein	Isoflavone	Fats, oils, beef, red clover, soyabeans, psoralea, lupin, fava beans, kudzu,	Thompson et al.(2006); Umpress et al.(2005); Krenn et al.(2002);

		psoralea	Coward et al.(1993); Kaufman et al.(1997)
Hesperidin	Flavanone	Bitter orange, petit grain, orange, orange juice, lemon, lime	Khan et al.(2009)
Kaempferol	Flavonols	Apples, grapes, tomatoes, green tea, potatoes, onions, broccoli, Brussels sprouts, squash, cucumbers, lettuce, green beans, peaches, blackberries, raspberries, spinach	Calderon-Montaño et al. (2011); Liu(2013); Kim and Choi(2013)
Luteolin	Flavones	Celery, broccoli, green pepper, parsley, thyme, dandelion, perilla, chamomile tea, carrots, olive oil, peppermint, rosemary, navel oranges, oregano	Kayoko et al.(1998); López-Lázaro(2009)
Macluraxanthone	Xanthones	Maclura tinctoria (Hedge apple), Dyer's mulberry	Khan et al.(2009)
Myricetin	Flavonols	Vegetables, fruits, nuts, berries, tea, red wine	Ross & Kasum(2002); Basli et al.(2012)
Naringenin	Flavanone	Grapes	Felgines et al.(2000)
Peonidin	Anthocyanidin	Cranberries, blueberries, plums, grapes, cherries, sweet potatoes	Truong et al.(2010)
Quercetin	Flavonols	Vegetables, fruits and beverages, spices, soups, fruit, juices	Hertog et al.(1997); Justesen & Knuthsen(2001); Stewart et al.(2000); Zheng & Wang(2001)
Rutin	Flavonols	Green tea, grape seeds, red pepper, apple, citrus, fruits, berries, peaches	Atanassova and Bagdassarian (2009); Chang et al.(2000); Malagutti et al.(2006)
Rutin	Flavonol	Citrus fruits, apple, berries, peaches	Cruickshank et al.(1974); Chang et al.(2000)
Scopoletin	Coumarin	Vinegar, dandelion coffee	Gálvez et al.(1994)
Taxifolin	Flavanonol	Vinegar	Cerezoa et al.(2010)
Taxifolin	Flavanonol	Citrus fruits	Grayer & Veitch(2006); Kawaii et al.(1999)
Theaflavin	Catechins	Tea leaves, black tea, oolong tea	Leung et al.(2001)
Tricin	Flavone	Rice bran	Cai et al.(2005)

Table 2.: List of different type of transcription factors from plants with modified flavonoid biosynthetic pathway in genetically modified transgenic plants.

Type	Gene name	Source	Engineered Plants	Regulated genes	Branch pathway	Reference
	vMYBP1	*Vitis vinifera*	*Arabidopsis*	CHI, F3'5'H,	PAs	Bogs *et al.*

			thaliana tt2 mutant	ANS, LAR & ANR		2007
	VvMYB5a	*Vitis vinifera*	*Nicotiana tabacum*	CHS, CHI, F3H & DFR	ANs, FLs AND PAs	Deluce *et al.* 2006
	VvMYB5b	*Vitis vinifera*	*Nicotiana tabacum*	CHS, DFR & ANS	ANs	Hichri *et al*. 2011
	TT2	*Arabidopsis thaliana*	*Arabidopsis thaliana*	DFR & ANS	PAs	Nesi *et al.* 2001
	TT2+PAP1	*Zea Mays*	*Arabidopsis thaliana*	ANR	PAs	Sharma and dixon, 2006
	AtMYB11	*Arabidopsis thaliana*	*Arabidopsis thaliana*	CHS, CHI, F3H & FLS	FLs	Stracke *et al.* 2007
MYB	*AtMYB12*	*Arabidopsis thaliana*	*Nicotiana tabacum*	PAL, CHS, CHI, F3H & FLS	FLs	Pandey *et al.* 2012
	AN2	*Petunia hybrida*	*Petunia hybrida*	PAL, CHS & DFR	ANs	Yamagishi *et al*.2010
	DkMyb4	*Diospyros kaki*	*Actinidia deliciosa*	PAL, CHS, CHI, F3H, F3’5’H, DFR & ANR	PAs	Akagi *et al.* 2009
	BoMYB2	*Brassica oleracea*	*Arabidopsis thaliana*	F3’H, DFR & ANS	ANs	Chiu and Li, 2012
	R gene	*Glycine Max*	*Glycine Max*	ANS & GT	ANs	Gillman *et al.* 2011
	TaMYB14	*Trifolium repens*	*Trifolium repens*	F3’5’H, ANS, ANR & LAR	PAs	Hancock *et al.* 2012
	DkbZIP5	*Diospyros kaki*	*Diospros kaki*	Not documented	PAs	Akagi *et al.* 2009
	PAP1	*Arabidopsis thaliana*	*Rosa hybrida*	CHS, ANS & PAL	ANs	Zvi *et al*. 2012
	Sn	*Zea Mays*	*Lotus corniculatus*	DFR, ANS, LAR & PAL	ANs	Robbins *et al.*2003, Paolocci *et al.* 2007
	Rc	*Oryza sativa*	*Rice rc*	CHS & F3H	PAs	Sweeney *et*

			mutants			*al.* 2006, Furukawa *et al.*2007
	LC	*Zea Mays*	*Arabidopsis thaliana*	PAL, CHS, F3H, DFR, ANS, LAR & ANR	ANs	Sharma and dixon, 2006
MYC/HLH	*LC*	*Zea Mays*	*Medicago sativa*	DFR	ANs and PAs	Ray *et al.* 2003
	LC	*Zea Mays*	*Malus domestica*	Not documented	ANs and PAs	Li *et al.* 2007
	AN1	*Petunia hybrida*	*Petunia hybrida*	Not documented	ANs	Spelt *et al.* 2000
	NtAn1a & NtAn1b	*Nicotiana tabacum*	*Nicotiana tabacum*	CHS, CHI, F3H, DFR & ANS	ANs	Bai *et al.* 2011
	AN11	*Petunia hybrida*	*Petunia hybrida*	DFR	ANs	De Vetten *et al.*1997
	MtWD40	*Medicago trancatula*	*Medicago sativa*	Not documented	ANs and PAs	Pang *et al.* 2009
	ttg1	*Arabidopsis thaliana*	*Arabidopsis thaliana*	ANR, DFR & ANS	ANs and PAs	Bundry *et al.* 2004
	GhTTG1 & GhTTG3	*Gossypium hirsutum*	*Mathiola. incana*	CHS & DFR	ANs	Humphries *et al.*2005
WD40-like proteIns						
	PFWD	*Perilla frutescens*	*Arabidopsis thaliana*	DFR	ANs	Sompornpailin *et al.*2002
	C1 and R	*Zea Mays*	*Oryza sativa*	PAL, CHS, CHI, F3H, F3'H & DFR	ANs, FLs and PAs	Shin *et al.* 2006
	PAC1	*Zea Mays*	*Arabidopsis thaliana*	Not documented	ANs	Carey *et al.* 2004
	InWDR1	*Ipomoea nil*	*Ipomoea nil*	CHS, CHI, F3H, F3'H, DFR, ANS	ANs and PAs	Park *et al.* 2012

				& GT		
	InWDR1	*Ipomoea purpurea*	*Ipomoea purpurea*	ANS	ANs and PAs	Park *et al.* 2007
WRKY	TTG2	*Arabidopsis thaliana*	*Arabidopsis thaliana*	ANR	PAs	Ishida *et al.* 2007
	IbMADS 10	*Ipomoea batatas*	*Ipomoea batatas*	CHS, CHI, F3H, DFR & ANS	ANs	Lalusin *et al.* 2006
	IbMADS 10	*Ipomoea batatas*	*Arabidopsis thaliana*	CHS, CHI, F3H, DFR & ANS	ANs	Lalusin *et al.* 2006
MADS						
	TDR4	*Solanum lycopersicum*	*Arabidopsis thaliana*	CHS, DFR, ANS & ANR	ANs	Jaakola *et al.* 2010
	VmTDR4	*Vaccinium myrtillus*	*Vaccinium myrtillus*	Not documented	ANs	Jaakola *et al.* 2010
TFIIIA-like proteins	*TT1*	*Arabidopsis thaliana*	*Arabidopsis thaliana*	ANR	PAs	Sagasser *et al.* 2002

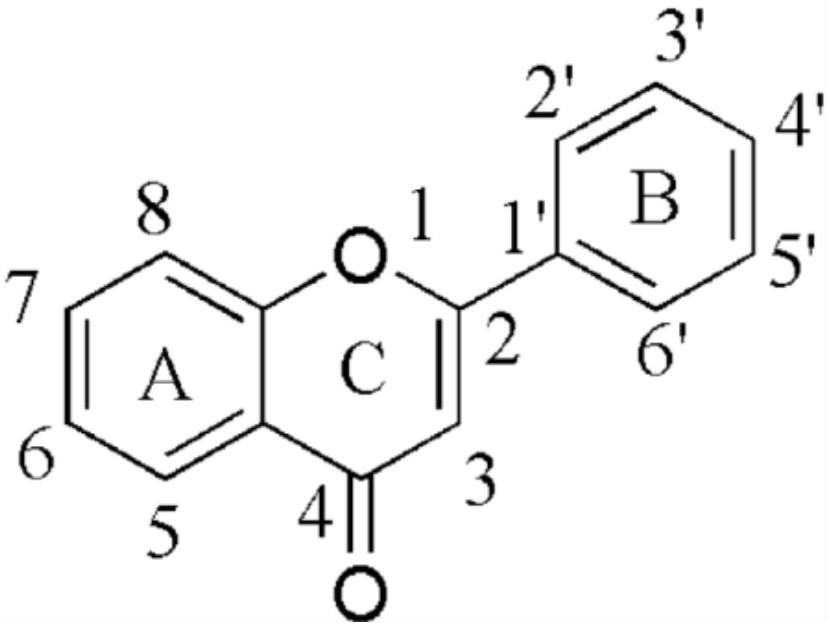

Fig. 1: Basic skeleton or structure of flavonoids.

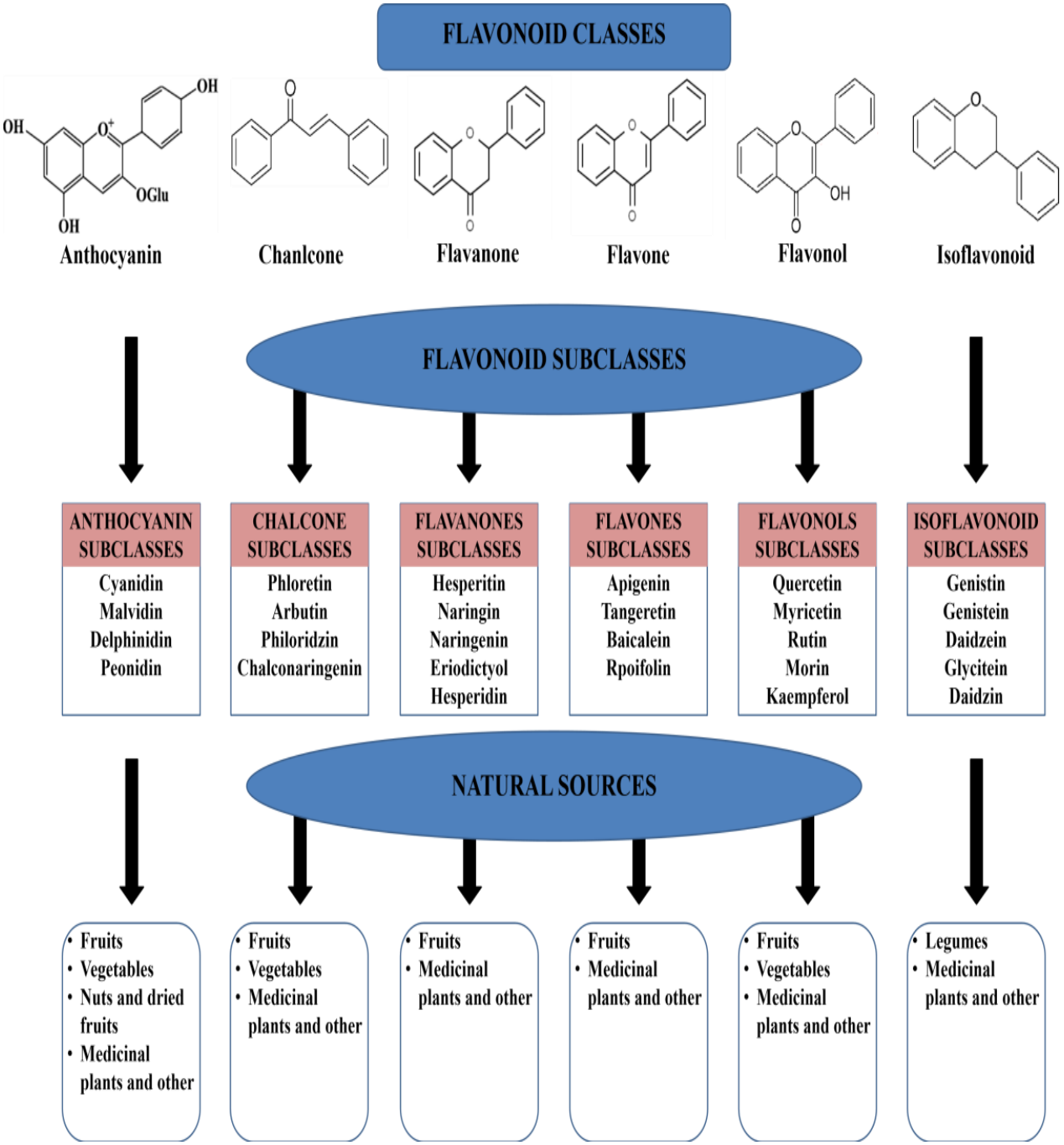
FLAVONOID CLASSES
Anthocyanin
Chanlcone
Flavanone
Flavone
Flavonol
Isoflavonoid
FLAVONOID SUBCLASSES
ANTHOCYANIN SUBCLASSES
Cyanidin
Malvidin
Delphinidin
Peonidin
CHALCONE SUBCLASSES
Phloretin
Arbutin
Philoridzin
Chalconaringenin
FLAVANONES SUBCLASSES
Hesperitin
Naringin
Naringenin
Eriodictyol
Hesperidin
FLAVONES SUBCLASSES
Apigenin
Tangeretin
Baicalein
Rpoifolin
FLAVONOLS SUBCLASSES
Quercetin
Myricetin
Rutin
Morin
Kaempferol
ISOFLAVONOID SUBCLASSES
Genistin
Genistein
Daidzein
Glycitein
Daidzin
NATURAL SOURCES
• Fruits
• Vegetables
• Nuts and dried fruits
• Medicinal plants and other
• Fruits
• Vegetables
• Medicinal plants and other
• Fruits
• Medicinal plants and other
• Fruits
• Medicinal plants and other
• Fruits
• Vegetables
• Medicinal plants and other
• Legumes
• Medicinal plants and other

Fig 2: Schematic representation of different classes of flavonoid, their basic skeleton structure and natural sources. (adapted from Panche et al., 2016)

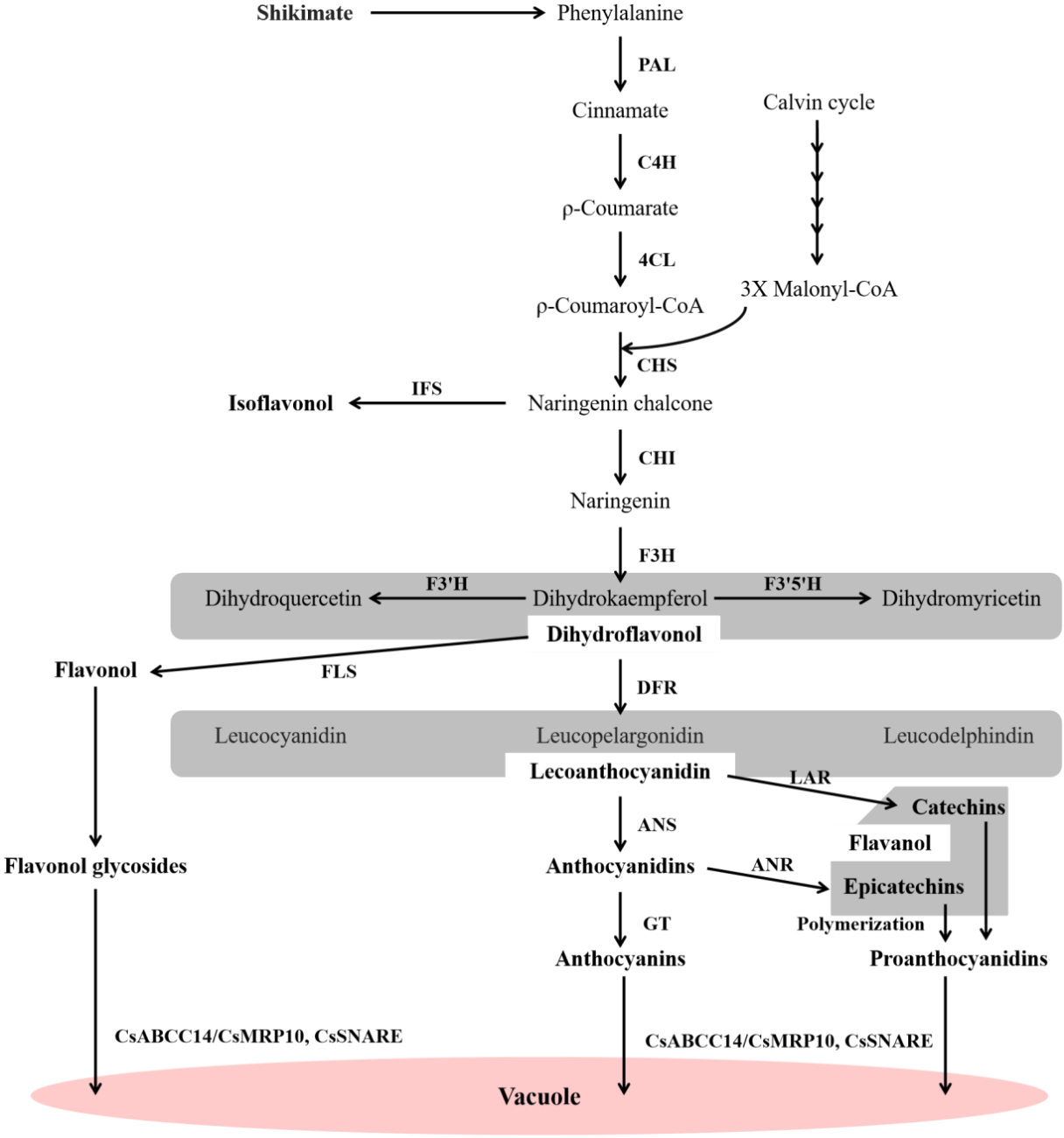

Fig 3The schematic representation of general flavonoid biosynthetic pathway in plants. PAL: phenylalanine ammonia-lyase, C4H: chalcone 4-hydrolase, 4CL: 4-courmarate:CoA ligase, CHS: chalcone synthase, CHI: chalcone isomerase, F3H: flavanone 3-hydroxylase, DFR: dihydroflavonol 4-reductase, ANS: anthocyanidin synthase, GT: gylcosyltransferase, CHR: chalcone reductase, IFS: isoflavone synthase, LAR: leucoanthocyanidin reductase, ANR: anthocyanidin reductase, F3'H: flavanone-3'-hydroxylase,

FS1: flavones synthase 1, FS2: flavone synthase 2, F3',5'H: flavonoid3',5'-hydroxylase, FLS: flavonol Synthase.