Structural Protein Genes of the Maize Flavonoid Pathway

Subjects: Plant Sciences

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Maize is one of the most important crops for human and animal consumption and contains a chemical arsenal essential for survival: flavonoids. Moreover, flavonoids are well known for their beneficial effects on human health. A total of twenty-one genes for the flavonoid pathway of maize was described. The first three genes participate in the general phenylpropanoid pathway. Four genes are common biosynthetic early genes for flavonoids, and fourteen are specific genes for the flavonoid subgroups, the anthocyanins, and flavone C-glycosides.

Zea mays L. anthocyanins biosynthesis flavonoids

1. Introduction

The comprehension of the maize flavonoid pathways is necessary for plant breeders who want to develop new pigmented maize varieties with better nutraceutical properties and for any health and food scientists working with phenolic compounds. The diversity in the palette of color in maize seeds correlates with differences in the pigment, including carotenoids and flavonoids. Researchers will deepen into these aspects to explain the impressive correlation between plant color, plant survival, and human health.

In maize (*Zea mays* L.), flavonoids act as deterrents against herbivores, regulate pollen development, and have defensive roles against UV-B radiation [1][2][3]. Flavonoids are a large family of phenolic compounds that share a biosynthetic pathway and, therefore, a common chemical arrangement. The basic structure consists of a C15 skeleton arranged in a C6-C3-C6 where one of the C6 corresponds to a phenyl that is bound to a benzopyran (C6-C3) denominated chromene according to the IUPAC nomenclature (**Figure 1**). Flavonoids originate from the mevalonate and phenylpropanoid pathways converging in C6-C3-C6 compounds. Some flavonoid molecules differentiate themselves by the chemical changes in the pyran ring, also known as the flavonoid's C ring [4][5]. For example, anthocyanidins have a modified benzopyran structure with a double bond between the oxygen atom and C2, forming the flavylium cation. Meanwhile, flavones have a double bond between carbons 2 and 3 and a carbonyl group at the C4 position.

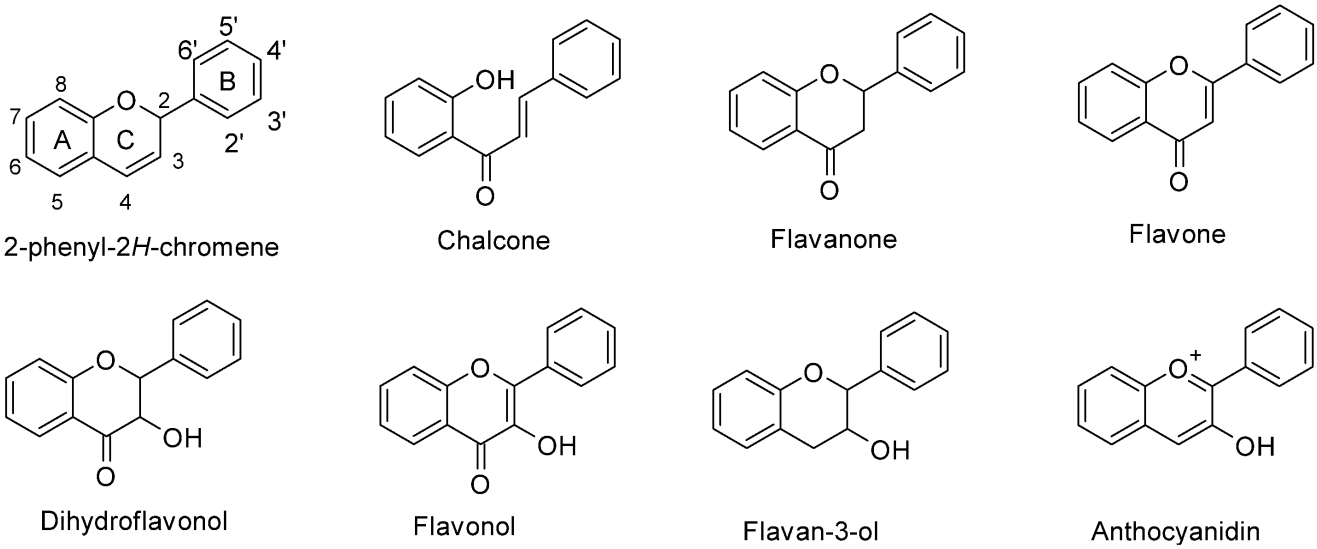

Figure 1. Chemical structure of flavonoid subgroups and the basic C6-C3-C6 skeleton (2-phenyl-2*H*-chromene). A, B, and C refer to a specific ring of the flavonoid skeleton.

2. Structural Protein Genes of the Maize Flavonoid Pathway

2.1. Phenylpropanoid Pathway

The first enzymatic steps in the flavonoid pathway are from three genes of the phenylpropanoid pathway (**Table 1**). These three enzymes direct the transformation of phenylalanine to coumaroyl-CoA. Those genes are *ZmPAL* (phenylalanine ammonium lyase, multiples genes, EC 4.3.1.24) [6], *ZmC4H* (cinnamic acid 4-hydroxylase, Zm00001d009858, EC 1.14.14.91) [6][7], and *Zm4CL* (4-coumarate CoA ligase, *bm5*, EC 6.2.1.12) [8]. The three genes share a similar expression profile of downstream genes in the flavonoid pathway in anthocyanin-pigmented tissues [9][10]. Recent analyses have demonstrated multiple gene families in flavonoid biosynthesis with a tissue-specific expression. In addition, some genes such as *Zm4CL* codify various isoforms, each of which has specific functions [11]. The research on these genes focuses on their roles in lignin biosynthesis [8]. For example, under sugarcane mosaic virus (SCMV) infection, *ZmPAL* and *ZmC4H* genes are upregulated, generating the substrate for lignin production [6]. Meanwhile, studies on a brown *midrib5* maize line demonstrated that a *Zm4CL* mutant was responsible for defective lignin biosynthesis [12]. There is a correlation between anthocyanins and lignin where the fungi *Ustilago maydis* activates the anthocyanin but reduces the lignin biosynthesis, thus facilitating its invasion into the maize seed [13].

Table 1. Summary of genes involved in the early steps of the maize flavonoid pathway.

Gene Name	Locus	Enzyme/Protein Name	EC	Reference
(*ZmPAL*)	*m**	Phenylalanine ammonium lyase	4.3.1.24	[6]
(*ZmC4H*)	8L	Cinnamic acid 4-hydroxylase	1.14.14.91	[6][7]
bm5 (*Zm4CL*)	5	4-Coumarate CoA ligase	6.2.1.12	[12]

Gene Name	Locus	Enzyme/Protein Name	EC	Reference
c2 (*ZmCHS*)	4L	Chalcone synthase	2.3.1.74	[14]
whp1 (*ZmCHS*)	2L	Chalcone synthase	2.3.1.74	[15]
chi1 (*ZmCHI*)	1L	Chalcone isomerase	5.5.1.6	[16]
fht1 (*ZmF3H*)	2S	Flavonoid 3-dioxygenase	1.14.11.9	[17]
pr1 (*ZmF3′H*)	5L	Flavonoid 3′-monooxygenase	1.14.14.82	[18]

EC code and locus were obtained from BRENDA [19] and MaizeGDB [20], respectively. The *m** means multiple loci.

2.2. Early Biosynthetic Genes of Flavonoids

2.2.1. Chalcone Synthase (*ZmCHS*, *c2*, EC 2.3.1.74)

The first crucial step in flavonoid biosynthesis (**Figure 2**) is the production of the naringenin chalcone (C6-C3-C6) from the condensation of three molecules of malonyl-CoA (3 × C2) using a 4-coumaroyl-CoA (C6-C3) as substrate [21]. This gene is also known as polyketide synthase (PKS) type III. The chalcone synthase (CHS) works similarly to other PKS enzymes from the mevalonate/acetate pathway [4]. The reaction extends the aliphatic chain from the coumaroyl-CoA three times using two carbon units from a malonyl-CoA. Then, an intramolecular Claisen condensation occurs to form the second aromatic ring.

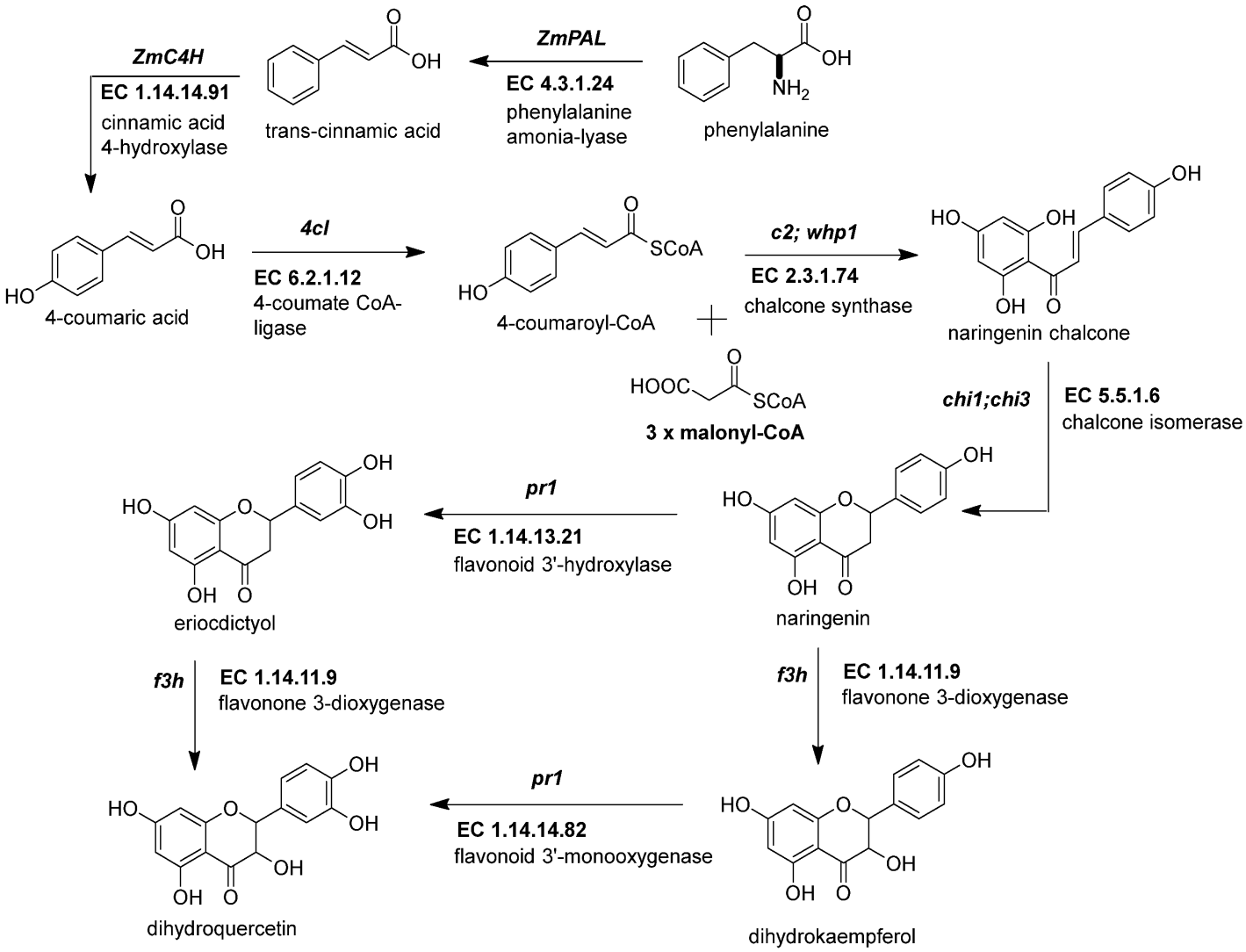

Figure 2. Early genes in the flavonoid pathway. The flavonoid pathway begins with the transformation of phenylalanine to coumaroyl-CoA. The last steps end with the intravacuolar accumulation of acylated anthocyanins. The genes responsible for supplying the coumaroyl-CoA into the flavonoid pathway are phenylalanine ammonium lyase (*ZmPAL*, EC 4.3.1.24), cinnamic acid 4-hydroxylase (*ZmC4H*, EC 1.14.14.91), and 4-coumarate CoA ligase (*Zm4CL*, *bm5*, EC 6.2.1.12). The flavonoid genes are divided into early biosynthetic genes (*EBGs*) and late biosynthetic genes (*LBGs*). EBGs comprise four genes: chalcone synthase (*ZmCHS*, *c2*, EC 2.3.1.74), chalcone isomerase (*ZmCHI*, *chi1*, EC 5.5.1.6), flavonoid 3-dioxygenase (*ZmF3H*, *fht1*, EC 1.14.11.9), and flavonoid 3′-monooxygenase (*ZmF3′H*, *pr1*, EC 1.14.14.82). References: [20][22].

Genome-wide analysis revealed up to 15 *ZmCHS* genes in the maize genome (Han et al., 2016). However, the members more consistently studied are the duplicated *c2* (*ZmCHS01*) and *whip1* (*ZmCHS02*) genes (**Table 1**) [14]. Multiple tissues, including tassels, ear husks, and the aleurone layer of endosperm at different developmental stages, express the genes *c2* and *whip1* [23][24]. Indeed, functional alleles for genes *c2* and *whip1* are vital to increasing the biosynthesis of any flavonoids downstream, such as apigenin and tricin, essential for lignin formation, and C-glycosyl flavones [15][25]. Meanwhile, members of the chalcone synthase family, such as *ZmCHS013* and *ZmCHS014*, compared to *ZmCHS01*, had a lower expression in most tissues and different responses under the stimuli of salicylic acid [26].

2.2.2. Chalcone Isomerase (*ZmCHI*, *chi1*, EC 5.5.1.6)

This enzyme catalyzes an intramolecular Michael-type addition from the chalcone 2-*O* to its α,β-unsaturated carbonyl (**Figure 2**). The final product is the typical phenyl-chromanone or flavanone structure [4]. The first gene sequenced from this family in maize was *ZmCHI* (**Table 1**) [16]. Interestingly, mutants have not been reported in maize for this gene, due to the multiple homologous sequences found for *ZmCHI* in the maize genome. An experiment designed to find QTLs for resistance to *Fusarium* corn fungi detected *ZmCHI3* as a second member of the family [27]. Indeed, a transformed maize callus with a copy of *ZmCHI3* from a resistant inbred was less susceptible to maize plagues.

2.2.3. Flavonoid 3-Dioxygenase (*ZmF3H*, *fht1*, EC 1.14.11.9)

ZmF3H is a Fe^{2+} and 2-oxoglutarate-dependent dioxygenase that introduces a hydroxyl group in position 3 of the chalcone structure, generating a dihydroflavonol [17]. There is just one gene copy known in the maize genome. In a previous report, *ZmF3H* was found to be the only gene in the flavonoid pathway in which mRNA expression levels correlate with the synthesis of flavonols in anthers [28]. Moreover, its expression increases in pigmented kernels compared to white seeds [9].

2.2.4. Flavonoid 3′-Monooxygenase (*ZmF3′H*, *pr1*, EC 1.14.14.82)

This *pr1* or purple aleurone1 gene has been studied in maize because its alleles are responsible for changes in color pigmentation caused by a difference in anthocyanin profile [29][30]. *ZmF3′H* is monooxygenase hydroxylate in the 3′ position from the phenyl ring B (**Table 1**). When the gene is functional, its enzyme can produce blue/violet-colored anthocyanidins (cyanidin and peonidin). If not, it generates a red/orange mono-hydroxylated pelargonidin [18]. Red kernels are homozygous for the recessive alleles *pr1* that do not produce functional enzymes, resulting in the pelargonidin-base anthocyanins predominating over the anthocyanin profile. The dominant *Pr1* alleles have a gene dose effect in the purple kernel pigmentation, which means that each *Pr1* allele in diploid (vegetative) or triploid (endosperm) tissues increase the cyanidin-base anthocyanins (**Figure 2**) in the pigmented tissue [31].

Moreover, *ZmF3′H* has a role in the biosynthesis of 3-deoxyflavonoids and phlobaphene; as occurs with the anthocyanins, the precursor transforms into a di-hydroxylated phenyl ring B compound [32]. A *Pr1* allele is essential for the resistance against biotic stress depending on C-glucosyl flavone (maysin) accumulation in salmon-colored silks [33].

2.3. Late Biosynthetic Genes of Maize Anthocyanins

2.3.1. Dihydroflavonol 4-Reductase (*ZmDFR*, *a1*, EC 1.1.1.219)

This enzyme converts the dihydroflavonol (or flavanonol) to a flavan-3,4 diol by reducing the 4-carbonyl (**Figure 3** and **Table 2**) [34]. There is a hypothesis that this enzyme has a role in phlobaphene biosynthesis by transforming the 4-carbonyl into flavanones to produce 4-flavan-4-ol [35]. The gene locus of *ZmDFR*, a1, has been deeply studied for two reasons. The first is its linkage to the *sh2* gene, responsible for the shrunken seed phenotype, that

made possible the studies on transposable elements and meiotic recombination hotspots in the a1-sh2 interval [36][37]. The second reason is that the gene product is a vital enzyme in the flavonoid pathway, favoring which flavonoid subgroup could be biosynthesized [38]. If there is a functional allele, it can produce anthocyanidins (**Figure 3**) and phlobaphenes (see Section 2.4.1). However, two copies of a non-functional allele would redirect it to flavanol and flavone biosynthesis [39].

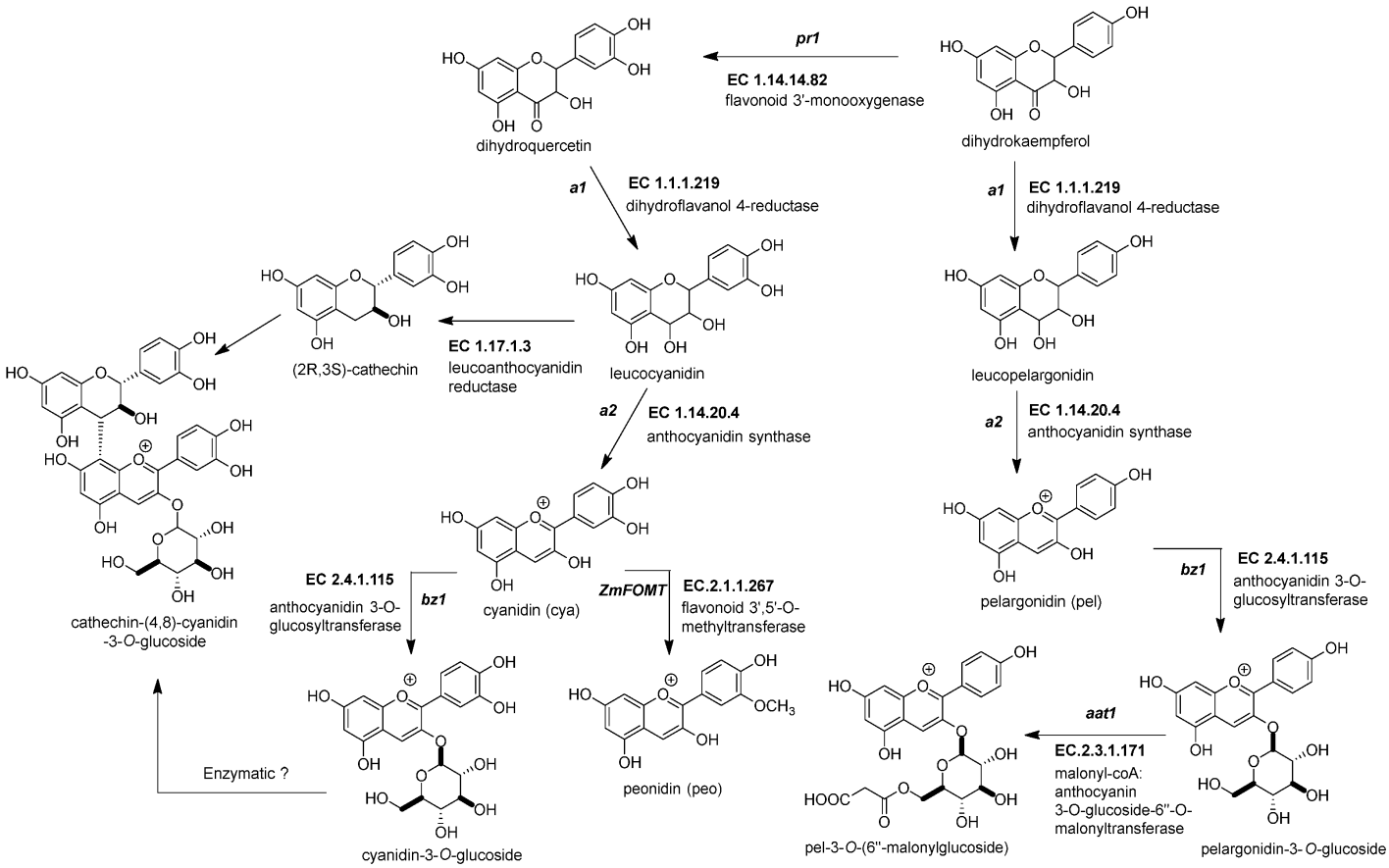

Figure 3. Biosynthetic genes for maize anthocyanin pathway. After the formation of the dihydroflavonol, five enzymatic steps catalyze its biotransformation into acylated maize anthocyanins. Those genes are the following: dihydroflavonol 4-reductase (*ZmDFR*, *a1*, EC 1.1.1.219), anthocyanidin synthase (*ZmANS*, *a2*, EC 1.14.20.4), anthocyanidin 3-*O*-glucosyltransferase (*ZmAGT*, *bz1*, EC 2.4.1.115), malonyl-CoA: anthocyanin 3-*O*-glucoside-6″-*O*-malonyltransferase (*Zm3MAT*, *aat1*, EC 2.3.1.171), and flavonoid 3′,5′-*O*-methyltransferase (*ZmAOMT*, EC 2.1.1.267). The glutathione S-transferase (*ZmGST*, *bz2*, EC 2.5.1.18) and multidrug resistance protein (*ZmABCC3* and *ZmABCC4*, MRP3 and MRP 4, EC 7.6.2.2) are required to deliver them inside the vacuole. References: [20][22].

Table 2. Summary of anthocyanin genes in the maize flavonoid pathway.

Gene Name	Locus	Enzyme/Protein Name	EC	Reference
pr1 (*ZmF3′H*)	5L	Flavonoid 3′-monooxygenase	1.14.14.82	[18]
a1 (*ZmDFR*)	3L	Dihydroflavonol 4-reductase	1.1.1.219	[34]

Gene Name	Locus	Enzyme/Protein Name	EC	Reference
-(*ZmLAR*)	-	Leucoanthocyanidin reductase	1.17.1.3	- *
a2 (*ZmANS*)	5S	Anthocyanidin synthase	1.14.20.4	[40]
bz1 (*ZmAGT*)	9S	Anthocyanidin 3-*O*-glucosyltransferase	2.4.1.115	[41]
aat1 (*Zm3MAT*)	1L	Malonyl-CoA: anthocyanin 3-*O*-glucoside-6″-*O*-malonyltransferase	2.3.1.171	[42]
omt1 and omt4- (*ZmAOMT*)	4L	Anthocyanin S-adenosyl-l-methionine-dependent *O*- methyltransferase	2.1.1.267	[43][44]
bz2 (*ZmGST*)	4L	Glutathione-S-transferase	2.5.1.18	[45]
mrpa3 (*ZmABC3*) *mrpa4* (*ZmABC4*)	9S 1S	Multidrug resistance-associated protein or ATP-binding cassette transporter	7.6.2.2	[46]

* Not found yet in maize. EC code and locus were obtained from BRENDA [19] and MaizeGDB [20], respectively.

multiple transcription factors [9][10]. *ZmDFR1* has a gene duplication in the maize genome, known as *a4*. Nevertheless, it is not clear if there is an active protein in the tissue from the genomic sequences alone [34]. Both genes have a higher expression in pigmented kernels than in anthocyanin-less seeds [9].

2.3.2. Anthocyanidin Synthase (*ZmANS*, *a2*, EC 1.14.20.4)

The dioxygenase *ZmANS* oxidizes at the C-3 position of a flavan-3,4 diol, generating a flavan-3,3,4 triol (**Figure 3**) [17]. After oxidation, two water molecules are removed, producing an anthocyanidin molecule [47]. Moreover, the *ZmANS gene* expression is upregulated in pigmented kernels compared to white seeds through elements that conserve the promoter region for the MBW complex [9][40]. The *a2* is the unique copy known in the maize genome.

2.3.3. Anthocyanidin 3-*O*-Glucosyltransferase (*ZmAGT*, *bz1*, EC 2.4.1.115)

This enzyme is also known as UDP-flavonoid glucosyltransferase (*ZmUFGT*). It catalyzes the transference of glucose to the C-3 position of anthocyanidins (**Figure 3**) [48][49]. This locus is named bronze1 since *bz1* alleles cannot produce a functional gene product and are responsible for the bronze-colored aleurone [41][50]. Glycosylated anthocyanidins (anthocyanins) accumulate in a vacuole only when the *ZmAGT* is functional. If not, the anthocyanidins are prone to oxidation, turning into brown pigments in the cell wall [51]. The expression occurs in all anthocyanin pigmented tissue because it contains conserved elements in its promoter, as other genes are upregulated simultaneously by the MYB-bHLH-WD40 (MBW) complex [50][52].

The locus *bz1* is located in the intergenic region *bz1-stc1*, known for the varying copies of transposable elements [53][54]. A relevant study included the first discovery of the first DNA transposable element, the Ac/Ds transposon, that resulted in a Nobel Prize being awarded to Dr. McClintock [55]. The Ds activation by marker Ac produces a chromosome rupture of chromosome 9 short arm region, which was recognized phenotypically by the apparition of bronze-colored spots in the kernel [56].

2.3.4. Malonyl-CoA: Anthocyanin 3-*O*-Glucoside-6″-*O*-Malonyltransferase (*Zm3MAT*, *aat1*, EC 2.3.1.171)

Two types of acyl moieties can modify the glycosidic part of the anthocyanins in the *Plantae* kingdom, aromatic and aliphatic dicarboxylic acids. *Zm3MAT* (**Figure 3**) was the first anthocyanin acyltransferase (AAT) discovered not only in maize but also in monocots [42][57]. *Zm3MAT* is necessary to produce mono-malonylated anthocyanins, the most common type of anthocyanins in the aleurone layer [58]. *Zm3MAT* was selected as a QTL for the reduced acylation phenotype and then corroborated through a knockout by Mu transposon insertion [42]. Further research showed that *Zm3MAT* exerts a dimalonyl transferase activity and can utilize both acyl moieties malonyl-CoA and succinyl-CoA, but it is more specific for malonyl-CoA [57]. The spectrum of anthocyanin selectivity ranges from the most preferable to the least preferable as follows: cyanidin-3-*O*-glucoside, pelargonidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, and delphinidin-3-*O*-glucoside.

2.3.5. Flavonoid 3′,5′-*O*-Methyltransferase, or Anthocyanin S-Adenosyl-l-Methionine-Dependent *O*-Methyltransferase (*ZmFOMT* or *ZmAOMT*, EC 2.1.1.267)

This enzyme catalyzes the methylation of a hydroxyl group in the -3′ or -5′ position of the 3-hydroxyflavonoid's phenyl B-ring (**Figure 3**) [43]. The enzyme uses several flavonoids as substrates, not just anthocyanins. These include aglycone and glycosylated forms of flavonols or anthocyanidins. However, every member has a specific affinity that favors some substrate above others [58]. Unfortunately, in maize, this enzyme has not been characterized yet. However, Chapman and collaborators mentioned two candidate genes, namely *omt1* (Zm00001d052841) and *omt4* (Zm00001d05284), for anthocyanin *O*-methyltransferases related to QTLs for peonidin-base anthocyanins [44].

2.3.6. Glutathione-S-Transferase (*ZmGST*, *bz2*, EC 2.5.1.18)

The glutathione S-transferase (GST) family in maize includes more than 40 GST gene sequences [59]. This family of enzymes detoxifies cells affected by xenobiotics, such as herbicides, by conjugating a glutathione (GSH) molecule [60][61][62]. After being labeled with glutathione, these molecules are sent out of the cell by an ATP-dependent glutathione conjugate export pump [63]. However, the *bz2* gene, a GST type III, is supposed to label the anthocyanin to be recognized by a vacuolar glutathione pump, and then the labeled anthocyanin is transported into the vacuolar lumen [45][63]. Until now, there is no evidence that shows that anthocyanins are conjugated with GSH. However, the role of *bz2* in the accumulation of anthocyanins is accepted. Other researchers suggested that this enzyme may function as a carrier protein for vacuolar anthocyanin sequestration [64].

When *ZmGST* is not functional, the anthocyanins are not transported to the vacuole interior. Then, the intravacuolar pH and environment contribute to maintaining these molecules without degradation [65]. As described for *bz1*, a maize plant without functional alleles will develop a bronze-colored kernel [51]. *ZmGST* is upregulated in pigmented tissue because it shares conserved binding sites in the promoter region for the MBW complex interaction, a characteristic shared with other upstream genes in the flavonoid pathway [9][66].

2.3.7. Multidrug Resistance Protein (*ZmABCC3* and *-4*, *mrpa3*, EC 7.6.2.2)

ZmABCC3 is part of a broader ATP-binding cassette (ABC) superfamily protein containing up to 130 open reading frames [62]. In maize, this superfamily of transmembrane proteins anchored to the cell membrane is highly specialized in expelling xenobiotics from the intracellular environment [67]. However, *ZmABCC3* and *ZmABCC4* are present in the tonoplast of vegetative tissues and in the aleurone layer, respectively [46].

This protein follows a similar expression profile to other genes related to anthocyanin biosynthesis [9][68]. Recent research in species such as *Vitis vinifera* and *Arabidopsis thaliana* shows that their homologous sequences to *ZmABBC3* are GSH/anthocyanin co-transporters [69][70].

2.3.8. Flavanol-Anthocyanin Condensed Forms

The flavanol-anthocyanin condensed forms are compounds found in maize; however, there is still no description of a known enzyme producing them [71]. Their biosynthesis starts with the generation of the flavan-3-ol unit (**Figure 3**). The leucoanthocyanidin reductase (E.C. 1.17.1.3) participates in a reduction reaction in the C-3 position of the leucoanthocyanidin [44][72]. This enzyme is yet unidentified in maize. Then, a linkage occurs between the anthocyanin and the flavan-3-ol, but there is no recognized enzyme for this process (**Figure 3**). However, it is known that a QTL for the flavanol-anthocyanin condensed form was mapped near the *p1* locus [44].

In wine, the presence of flavanol-anthocyanin condensed forms is related to aging. However, in maize, there is evidence of natural formation [71]. The production of flavanol-anthocyanin condensed forms consumes monomeric anthocyanin, therefore reducing the total concentration [57].

2.4. Biosynthesis of Flavonols, Flavones C-Glycosides, and Phlobaphenes in Maize

2.4.1. Flavonol Synthase (*ZmFLS1*, *fls1*, EC 1.14.20.6)

The flavonols are important in maize due to their effects on male fertility and UV-B protection [73]. Flavonol synthesis depends on flavanone 3-dioxygenase and flavonol synthase, a Fe^{2+}/2-oxoglutarate dependent dioxygenase (**Figure 4** and **Table 3**). The transcription factors that regulate the expression of anthocyanins and C-flavone glycosylated biosynthetic genes can also upregulate the expression of *ZmFLS1* [1][17]. In the maize genome are two copies (*ZmFLS1* and *ZmFLS2*) in tandem in the long arm of chromosome 5. The expression of both enzymes was augmented under UV-B light and in high-altitude landraces compared to the inbred lines through an increased *p1* expression [1][3].

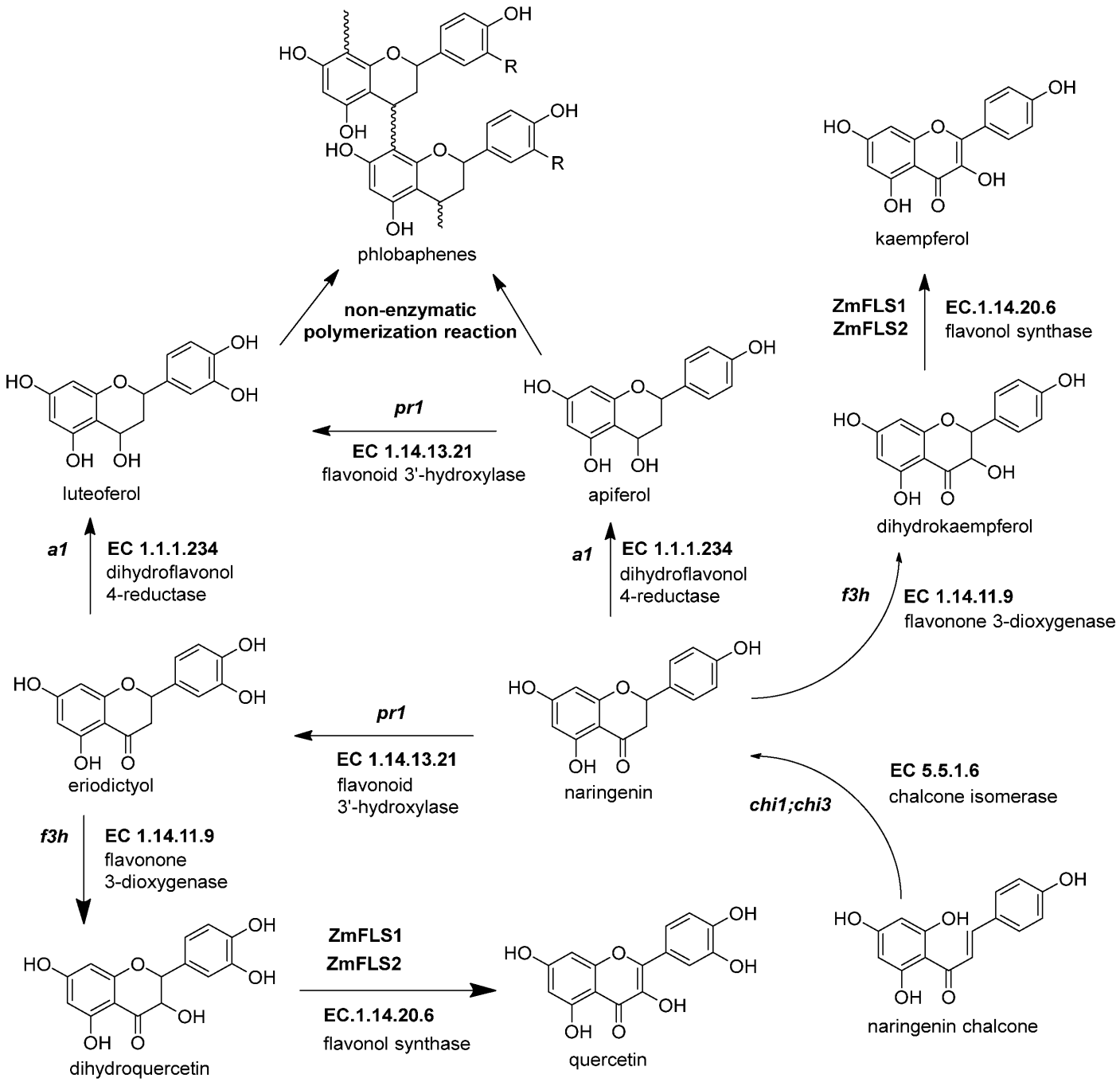

Figure 4. The biosynthetic genes of flavonol and phlobaphenes. The flavanones naringenin and eriodyctiol are the starting substrates for the other flavonoid subgroups. Flavonol synthesis depends on flavanone 3-dioxygenase (*ZmF3H*, *fht1*, EC 1.14.11.9) and flavonol synthase (*ZmFNS1*, *fns1*, EC 1.14.20.5). Phlobaphene synthesis begins with the action of dihydroflavonol 4-reductase (*ZmDFR*, *a1*, EC 1.1.1.219) on flavanones, generating flavan-4-ol molecules that undergo a non-enzymatic polymerization into phlobaphenes. References: [20][22][74].

Table 3. Summary of flavonol and flavone C-glycoside genes in the maize flavonoid pathway.

Gene Name	Locus	Enzyme/Protein Name	EC	Reference
fls1 (*ZmFLS1*) *fls2* (*ZmFLS2*)	5L 5L	Flavonol synthase	1.14.20.6	[1]

Gene Name	Locus	Enzyme/Protein Name	EC	Reference
fnsi1 (*ZmFNSI1*) *fnsi2* (*ZmFNSI2*)	1S 1S	Flavone synthase I	1.14.20.5	[75]
fnsii1 (*ZmFNSII1*)	10L	Flavone synthase II	1.14.19.76	[2]
fns1 (*ZmF2H1*)	9L	Flavanone 2-hydroxylase	1.14.14.162	[76]
cgt1 (*ZmCGT*)	6L	UDP-glucose:2-hydroxyflavanone C-glucosyltransferase	2.4.1.360	[77]
sm2 (*UGT91L1*)	2L	flavonol-3-*O*-glucoside L-rhamnosyltransferase	2.4.1.159	[78]
sm1 (*ZmRHS1*)	6L	Glucose-4,6 dehydratase	4.2.1.76	[33]

EC code and locus were obtained from BRENDA [19] and MaizeGDB [20], respectively.

2.4.2. Flavone Synthase I (*ZmFNSI1-2*, *fnsi1*, EC 1.14.20.5) and Flavone Synthase II (*ZmFNSII-1*, *fnsii1*, EC 1.14.19.76)

Maize possesses three enzymes that can synthesize flavones from a flavanone, flavone synthases I and II, and flavone 2-hydroxylase (**Figure 5**) [2][77]. The flavone synthase produces a desaturation in the C2–C3 bond in the flavanone through an oxidation reaction. The oxidative mechanism in *ZmFNSI* is a Fe^{2+}/2-oxoglutarate-dependent dioxygenase, like in *ZmFLS1*, whereas that in *ZmFNSII* is CYP450 [2]. In addition, *ZmFNSI1* is upregulated more in tassels than in silks compared to *ZmF2H* [79]. The *p1* transcription factor regulates the expression of *ZmFNSI*. Meanwhile, the anthocyanin MBW complex regulates the expression of *ZmFNSII*. Both types of flavone synthases generate apigenin, which defends the plant against UV-B radiation-induced damage [2].

2.4.3. Flavanone 2-Hydroxylase (*ZmF2H1*, *fns1*, EC 1.14.14.162)

In maize, this is the third known enzyme that can produce the flavone backbone of the flavone C-glycosides in the salmon-colored silks [76]. This enzyme is phylogenetically closer to FNS type II, both being CYP proteins [77][80]. Flavanone-2-hydroxylase adds a hydroxyl group into the flavanone C-2, producing the opening of the C-ring and finally generating the 3-oxo-dihydrochalcone (**Figure 5**). After this opening, it can be glycosylated in either of the two positions of the A-ring, closing the C-ring, eliminating water (spontaneous or not), and then generating in vitro a mixture of C-6 or C-8 glycosylated flavones [77].

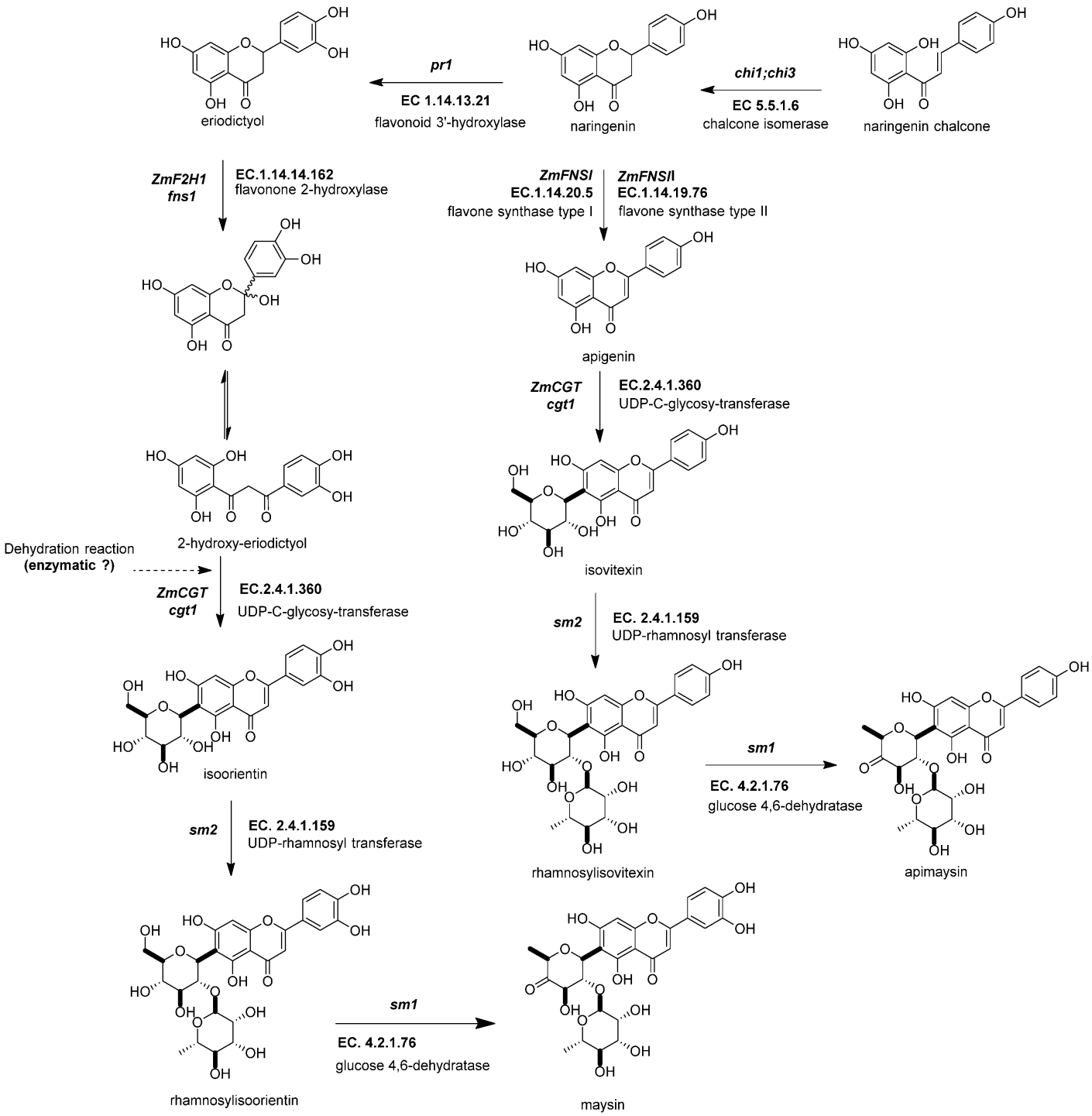

Figure 5. Biosynthetic genes of flavone C-glycosides. The flavanones naringenin and eriodictyol are the initial substrates for the other flavonoid subgroups. There are two possible ways to generate C-glycosyl flavones, indirectly or directly, from any flavanone. The indirect pathway begins through flavanone-2-hydroxylase (*ZmF2H*, *fnsii1*, EC 1.14.14.162) opening the C-ring, producing a 3-oxo-dihydrochalcone. Then, UDP C-glycosyl transferase (*ZmCGT*, *cgt1*, EC 2.4.1.360) generates a glycosidic bond in the A-ring. Then, there is a dehydration reaction (spontaneous or enzymatic) that produces the C6-flavone glycoside. The direct pathway firstly involves flavone synthase I (*ZmFNSII-2*, fnsii2, EC 1.14.20.5) and flavone synthase II (*ZmFNSII-1*, *fnsi2*, EC 1.14.19.76) producing the same reaction by the addition of a double bond between C2 and C3 in the flavanone. Then, a flavone functions as a substrate for the UDP C-glycosyl transferase (*ZmCGT*, *cgt1*, EC 2.4.1.360). The enzymatic

action of UDP-rhamnosyl transferase (*ZmCGT*, *sm2*, EC 2.4.1.159) and glucose 4,6 dehydratase (*sm1*, EC 4.2.1.76) produces either apimaysin or maysin. References: [20][33][81].

2.4.4. UDP-Glucose:2-Hydroxyflavanone C-Glucosyltransferase (*ZmCGT*, *cgt1*, EC 2.4.1.360)

UDP-glucose:2-hydroxyflavanone C-glucosyltransferase generates a glycosidic bond in the A-ring from the C-1 of the glucose to the C-6 in the C-glycosyl flavones (**Figure 5**) [33]. In vitro and in vivo experimental evidence has demonstrated that the *ZmCGT* enzyme has a bifunctional capacity to form glycosidic bonds with C or O atoms. On the contrary, there is only in vitro evidence for C-8 flavone glycosides [76]. The likely reason for that is the possibility of an enzyme that only selects C-6 glycosylated 2-hydroxyflavanone for dehydration into C-6 glycosyl flavones [82].

2.4.5. UDP-Rhamnosyl Transferase (*sm2*, *UGT91L1*, EC 2.4.1.159)

The UDP-rhamnosyl transferase enzyme forms the glycosidic bond between the glucose C-2 and the rhamnose C-1 (**Figure 5**) [78]. Functional alleles confer a characteristic salmon color to the silks due to the accumulation of maysin/apimaysin in the silks. This is due to *p1* upregulating *sm2* and is expressed principally in silks [33] but also in non-vegetative tissues such as pollen, tassels, and seeds [79].

2.4.6. Glucose-4,6 Dehydratase (*ZmRHS1*, *sm1*, EC 4.2.1.76)

The biosynthesis of C-flavones glycosides in maize ends with a modification to the glucose structure of the rhamnosylisoorientin (or rhamnosylisovitexin) to produce maysin/apimaysin (**Figure 5**) [83]. These metabolites give the ear of maize the ability to deter the herbivore *Helicoverpa zea*, commonly known as corn earworm [15][33]. This locus was found to be responsible for producing the last step in the maize flavone pathway and found to be a putative UDP-rhamnose synthase (*ZmRHS1*) [33][78]. The gene has two putative domains; the first domain is a UDP-glucose dehydratase, and the second domain corresponds to UDP glucose 4-keto-6-deoxyglucose epimerase/reductase. The former domain is the exclusive one catalyzing maysin or apimaysin biosynthesis [33]. Its gene expression pattern in the tissues is similar to the *sm1* profile [2].

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