植物甲基化类黄酮及其 *O*-甲基转移酶研究进展

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摘 要:植物类黄酮是重要的药用成分,其生物学功能与化学结构密切相关。*O*-甲基化修饰可提高类黄酮的稳定性、蛋白亲和力和生物利用度,从而增强其药用活性。*O*-甲基转移酶(*O*-methyltransferase)催化类黄酮合成 *O*-甲基化衍生物,是类黄酮代谢途径中的关键修饰酶。本文综述了植物 *O*-甲基化类黄酮的化学结构、药用功能及其药用价值提高机理;并对植物类黄酮 *O*-甲基转移酶的生物学功能、表达调控与开发潜力等进行了总结展望,以期为植物甲基化类黄酮的进一步研究提供新的思路与途径。

关键词:类黄酮;*O*-甲基化;*O*-甲基转移酶

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Research progress of plant *O*-methoxide flavonoids and *O*-methyltransferases

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Abstract: Plant flavonoids are important medicinal ingredients, whose biological function is closely related to chemical structure, and its stability, protein affinity and bioavailability can be improved by *O*-methylation modification, thereby enhancing its medicinal activity. *O*-Methyltransferase (OMT) catalyzes the synthesis of *O*-methylated derivatives of flavonoids, which is a key modifying enzyme in the flavonoid metabolism. This article reviews the chemical structure, medicinal function, and mechanism of medicinal value enhancement of plant *O*-methylated flavonoids. It is summarized in this article the biological function, expression regulation, and potential of plant flavonoid *O*-methyltransferases, in order to provide new ideas and ways for the further research of plant methylated flavonoids.

Key words: flavonoids; *O*-methylation; *O*-methyltransferases

果蔬、豆类和茶叶中富含类黄酮[1],因其具有抗抑郁[2]、抗炎、抗过敏、抗糖尿病、抗衰老[3]和抗癌[4]等多种药用功能而备受关注[5]。类黄酮结构与其生物功能密切相关,*O*-甲基化类黄酮是含有 OCH_3 基团的类黄酮,其在植物根瘤固氮[6,7]、吸引昆虫传粉[8,9]等生长发育进程及抗虫、抗病[10]、抗杂草[11]等抗逆[12,13]反应中发挥了重要作用。研究证实,*O*-甲基化类黄酮具有更强的抗氧化[14]、抗炎[15]、抗癌[16]等功能,且具有更高的生物利用度[17,18],更具药用价值。类黄酮 *O*-甲基转移酶(FOMT)属于 *O*-甲基转移酶(OMT)亚家族,催化类黄酮合成 *O*-甲基化类黄酮,在植物中广泛存在。本文综述了 *O*-甲基化类黄酮结构与功能,及 OCH_3 基团对其功能的影响;整理归纳了 FOMT 研究现状,并对其进行了生物信息学分析,以期为 *O*-甲基化类黄酮及 FOMT 的进一步的研究与应用开发提供参考。

1 *O*-甲基化类黄酮结构

类黄酮是重要的植物次生代谢产物,在植物中存在超过 10 000 种类黄酮[19],其基本结构为 C6-C3-C4,是一类 2-苯基色原酮类化合物,由两个芳环(A 和 B),通过含氧杂环(环 C)连接形成。其合成起始于丙二酰辅酶 A 和 4-香豆酸辅酶 A[20],与木质

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素具有共同的合成前体,也有一些特殊的类黄酮的合成起始于辅酶 A,如肉桂酸和二羟基香豆酸。类黄酮亚组包括:黄酮、黄酮醇、黄烷酮、黄烷醇、异黄酮、花色素等。修饰反应导致了类黄酮化合物结构与功能的多样性[21],基本的修饰反应包括:酰基化、糖基化、羟基化、异戊烯基化、甲基化等。甲基化是类黄酮重要的修饰反应,包括 *C*-甲基化和 *O*-甲基化两类,*O*-甲基化的类黄酮在植物中广泛存在,而 *C*-甲基化的类黄酮存在于少数植物中,例如无刺藤[22]。*O*-甲基化修饰是一种常见的化学修饰,在类黄酮的不同位点都会发生;类黄酮经不同位点与不同数量的 *O*-甲基化修饰,产生不同的 *O*-甲基化类黄酮,常见的类(异)黄酮如表 1 和 2 所示,其结构骨架分别见图 1 和 2。由表可知,植物类黄酮的 *O*-甲基化修饰在 A 环和 B 环上均会发生,且多发生于 B 环的 3′和 4′号羟基位点和 A 环的 6、7、8 号羟基位点(其中,A 环以 7 号位点修饰居多);而已见报道的植物异黄酮 *O*-甲基化修饰多发生于 B 环的 4′号羟基位点,极少数发生于 A 环的 7 号羟基位点。不同物种的植物类黄酮 *O*-甲基化修饰位点不同,其中,大豆、苜蓿等双子叶植物类黄酮的 *O*-甲基化修饰多发生于 3′和 4′号羟基位点,而水稻等单子叶植物多发生于 3′、5′、7 号羟基位点。

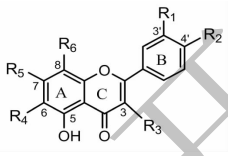

图 1　类黄酮骨架

Fig. 1　Flavonoid skeleton

表 1　常见类黄酮结构

Table 1　Common flavonoid structure

名称 Name	R_1	R_2	R_3	R_4	R_5	R_6	备注 Remarks	酶 Enzyme
Quercetin	OH	OH	OH	H	OH	H	–	–
8-Hydroxy-flavone 7-methyl ether	H	H	H	H	OCH_3	OH	H in C-5	–
Acacetin	**H**	**OCH_3**	H	H	OH	H	–	GmSOMT-2
Apigenin	H	OH	H	H	OH	H	–	–
Apigenin 7-methyl ether	H	OH	H	H	**OCH_3**	H	–	ObFOMT1 ObFOMT2
Chrysoeriol	H	**OCH_3**	H	H	OH	H	–	GmSOMT-9
Eriodictyol	OH	OH	H	H	OH	H	–	CrOMT6
Eriodictyol 3′,4′-dimethyl ether	**OCH_3**	**OCH_3**	H	H	OH	H	–	–
Eupatorin	OH	**OCH_3**	H	**OCH_3**	**OCH_3**	H	–	–
Genkwanin	**OCH_3**	H	H	OH	H	H	–	HvF1-OMT
Homoeriodictyol	**OCH_3**	OH	H	H	OH	H	–	OsROMT-9
Hesperetin	OH	**OCH_3**	H	H	OH	H	–	–
Isorhamnetin	**OCH_3**	OH	OH	H	OH	H	–	GmSOMT-9 MpOMT3
Kaempferol	H	OH	OH	H	OH	H	–	–
Luteolin	OH	OH	H	H	OH	H	–	–
Luteolin 3′-methyl ether	**OCH_3**	OH	H	H	OH	H	–	–
Luteolin 7-methyl ether	OH	OH	H	H	**OCH_3**	H	–	ObFOMT1 ObFOMT2
Myricetin	OH	OH	OH	H	OH	H	OH in C-5′	–
Myricetin 3′,5′-dimethyl ether	**OCH_3**	OH	OH	H	OH	H	**OCH_3** in C-5′	CrOMT2
Naringenin	H	OH	H	H	OH	H	–	–
Nevadensin	H	**OCH_3**	H	**OCH_3**	OH	**OCH_3**	–	ObF8OMT-1
Quercetin 4′-methyl ether	OH	**OCH_3**	OH	H	OH	H	–	GmSOMT-2 MpOMT4

续表 1(Continued Tab. 1)

名称 Name	R_1	R_2	R_3	R_4	R_5	R_6	备注 Remarks	酶 Enzyme
Quercetin 7,3′,4′-trimethyl ether	**OCH_3**	**OCH_3**	OH	H	**OCH_3**	H	–	–
Rhamnetin	OH	OH	OH	H	**OCH_3**	H	–	MpOMT1A
Tricetin	OH	OH	H	H	OH	H	OH in C-5′	–
Tricetin 3′,4′,5′-trimethyl ether	**OCH_3**	**OCH_3**	H	H	OH	H	**OCH_3 in C-5′**	TaOMT2
Tricin	**OCH_3**	OH	H	H	OH	H	**OCH_3 in C-5′**	ABQ58825

注:加粗部分为 *O*-甲基化类黄酮或 OCH_3 基团。
Note: The bold part of the font is *O*-methylated flavonoid or OCH_3 group.

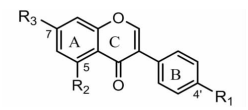

图 2 异黄酮骨架

Fig. 2 Flavonoid skeleton

表 2 常见异黄酮结构

Table 2 Common isoflavonoid structure

名称 Name	R_1	R_2	R_3	备注 Remarks	酶 Enzyme
2,7,4′-Trihydroxyisoflavanone	OH	H	OH	–	–
2,7-**Dihydroxy-4′-methoxyisoflavanone**	**OCH_3**	H	OH	–	LjHI4′OMT MtHI4′OMT
Biochanin A	**OCH_3**	OH	OH	–	GmSOMT-2
Daidzein	OH	H	OH	–	–
Formononetin	**OCH_3**	H	OH	OH in C2	GmSOMT-2 GeHI4′OMT
Genistein	OH	OH	OH	–	–
Genistein 7-methyl ether	OH	H	**OCH_3**	–	–

注:加粗部分为 *O*-甲基化异黄酮或 OCH_3 基团。
Note: The bold part of the font is *O*-methylated isoflavonoid or OCH_3 group.

2 *O*-甲基化修饰对类黄酮功能的影响

2.1 *O*-甲基化类黄酮药用功能

诸多研究表明,*O*-甲基化类黄酮具有多种药用功能,且 *O*-甲基化修饰可提高部分类黄酮的药用活性。C-8 位的 *O*-甲基化修饰可增强类黄酮促胰岛素分泌活性,从而提高抗糖尿病功能[23]。*O*-甲基化修饰还能提高类黄酮抑制糖尿病并发症的能力[24,25],蛋白质糖化形成晚期糖基化终产物 AGEs(advanced glycation end products)[26],引起糖尿病并发症,染料木素可抑制蛋白糖化,而 C-7 位和 C-4′位 *O*-甲基化的染料木素抑制能力更强[27]。肿瘤坏死因子相关凋亡诱导配体(TRAIL)诱导各种肿瘤细胞凋亡,*O*-甲基类黄酮(5,7-dimethoxyflavone、5,7,4′-trimethoxyflavone、3,5,7,3′,4′-pentamethoxyflavone)可通过死亡受体和线粒体途径,增强 TRAIL 诱导的人白血病 MOLT-4 细胞凋亡[28]。刺芒柄花素(C-4′位 *O*-甲基化)可抑制 MCF-7 乳腺癌细胞[29],其还能通过 G0/G1 期阻滞抑制细胞增殖,促进 ES2 和 OV90 卵巢癌细胞的凋亡[30]。5,7,4′-三甲氧基黄酮对 SCC-9 人舌鳞癌细胞抑制效力,是 5,7,4′-三羟基黄酮(芹菜素)的 8 倍,其 IC_{50} 值分别为 5 μM 与 40 μM[31]。*O*-甲基化修饰提高类黄酮的抗氧化活性,提高抗炎能力。异鼠李素是一种 3′-*O*-甲基化槲皮素,能抑制促炎物质分泌,降低活性氧(reactive oxygen species,ROS)含量,具有强抗氧化作用[32]。在应对关节炎时,橙皮素具有镇痛与抗炎的功效[15]。*O*-甲基化类黄酮可影响多药耐药蛋白活性,提高机体防御有毒化合物的能力[33]。

2.2 *O*-甲基化修饰提高类黄酮药用价值的机理

研究表明,*O*-甲基化修饰可提高类黄酮的结构

稳定性、蛋白亲和力、转运力,降低其水溶性,这些性质的改变是其药用价值提升的关键。生物利用度是指制剂中药物被吸收进入人体循环的速度与程度,它反映了化合物在生物体内吸收,分布,代谢和排泄的情况[34]。类黄酮的低生物利用度,使其在临床治疗与化学预防上的应用受到极大的限制[35,36],而 *O*-甲基化能有效提高类黄酮的生物利用度[17]。

O-甲基化类黄酮具有更好的稳定性。类黄酮主要以苷类或游离苷元的形式存在于植物中[37],摄入人体后会进行一系列的代谢反应。首先,类黄酮苷在小肠中水解成苷元[38,39],然后在小肠、大肠或结肠中,与甲基、硫酸盐基团和葡萄糖醛酸发生偶联反应[35],在肝脏完成代谢转化,然后以不同的偶联物进入血液[38]。由于快速的新陈代谢,摄入富含类黄酮的食物后,血液中类黄酮浓度仍低于 10 μM[40]。而 *O*-甲基化修饰可以保护类黄酮免受肝脏代谢的影响,抑制类黄酮与葡萄糖酸和硫酸盐结合,提高其代谢稳定性[17]。

在人体内,*O*-甲基化类黄酮具有更好的转运能力[41],人体内外源化合物的运输主要依赖血清蛋白,*O*-甲基化提高了类黄酮的蛋白亲和力,从而增强转运能力[42]。*O*-甲基化还降低了类黄酮的水溶性,增强疏水性[43],这种疏水相互作用在类黄酮与蛋白结合过程中起到重要作用,从而提高类黄酮的蛋白结合能力。甲基化通过提高黄酮的稳定性与转运能力,提高其肠道吸收量[17,18]与生物利用度,较其他类黄酮具有更强的药用活性,更高的生物利用度也进一步使得 *O*-甲基化类黄酮能够稳定有效地发挥药效。

3　类黄酮 *O*-甲基转移酶(FOMT)研究进展

3.1　FOMT 分类

类黄酮的甲基化修饰反应,由类黄酮甲基转移酶催化完成。根据不同目标附着物,甲基转移酶可以简单地分类为 *O*-甲基转移酶(OMT)、*N*-甲基转移酶(NMT)和 *C*-甲基转移酶(CMT)[44,45]。OMT 在自然界中广泛存在,大多数植物源 OMT 以二苯乙烯和类黄酮等酚类化合物作为底物[43]。FOMT 属于 OMT 亚家族,其保守结构域在 Pfam 数据库(http://pfam.xfam.org/)的索取号为 PF01596。

FOMT 催化 S-腺苷-1-甲硫氨酸(SAM,AdoMet)上的甲基转移到类黄酮上,形成 *O*-甲基衍生物和 S-腺苷-1-高半胱氨酸(SAH)[46]。基于氨基酸序列长度可将植物 OMT 分为两类[47],I 类 OMT 的分子量(约 23 ~ 29 kDa)较低,催化过程需要二价阳离子参与,如:Mg^{2+}、Ca^{2+};这些二价阳离子起稳定结构的作用,还可直接参与底物与酶的结合过程[48]。II 类 OMT 的分子量较大(38 ~ 43 kDa),催化过程不需要阳离子参与,这类 OMT 活性可利用基于组氨酸(His)催化的二元体来增强其酶活性[49],FOMT 大多属于II类OMT[50]。目前,被报道的部分植物类

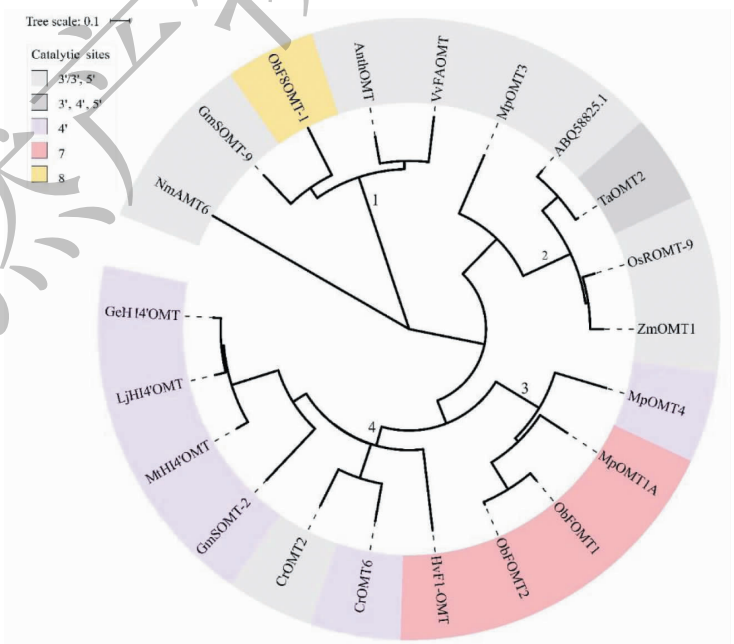

图 3　不同物种 FOMT 进化树分析

Fig. 3　FOMT evolutionary tree analysis of different species

黄酮 *O*-甲基转移酶如表 3 所示。

图 3 进化树分析表明,不同物种的 FOMT 蛋白具有较高同源性。图中不同颜色代表不同的催化位点,分支 1 蛋白来自双子叶植物,分支 2 蛋白来自单子叶禾本科植物,主要催化 3′、4′、5′号位点;分支 3 蛋白来自双子叶唇形科植物,主要催化位点为 C-7 位;除 HvF1-OMT 外,分支 4 蛋白均来自双子叶植物,且大部分属于豆科,催化 C-4′位。总而言之,具有相同催化位点与相同科属的 FOMT 同源性更高。

3.2 FOMT 功能鉴定

在鉴定 FOMT 功能时,通常在原核生物大肠杆菌(*Escherichia coli*)中过表达待鉴定基因,纯化出该基因编码的蛋白,再进行体外酶活实验验证 FOMT 功能。Itoh 等[21]从台湾香檬(*Citrus depressa*)中克隆了 5 个 *FOMT* 基因,其中 *CdFOMT5* 在大肠杆菌中成功表达为可溶性蛋白,体外酶活实验证实 CdFOMT5 可对槲皮素,柚皮素,(-)-表儿茶素和雌马酚的 C-3、C-5、C-6 和 C-7 位进行甲基化修饰。Liu 等[50]从苔藓类植物 *Plagiochasma appendiculatum* 中克隆了 *Pa4′OMT* 基因,在大肠杆菌中成功表达,并检测出 Pa4′OMT 蛋白具有催化芹菜素甲基化的活性。

表 3 植物类黄酮 *O*-甲基转移酶

Table 3 Plant flavonoid *O*-methyltransferase

名称 Name	物种 Species	登录号 Accession No.	长度(aa) Length(aa)	催化位点 Catalytic site	主要产物 Chemical product	参考文献 Ref.
LjHI4′OMT	百脉根 *Lotus japonicus*	AB091686	365	4′	2,7-Dihydroxy-4′-methoxyisoflavanone	51
GmSOMT-2	大豆 *Glycine max*	C6TAY1.1	358	4′	Acacetin、formononetin-biochanin A、quercetin 4′-methyl ether	52
GmSOMT-9	大豆 *Glycine max*	NP_001340280.1	202	3′	Chrysoeriol、isorhamnetin	53
HvF1-OMT	大麦 *Hordeum vulgare*	CAA54616	390	7	Genkwanin	54
–	大麦 *Hordeum vulgare*	ABQ58825	356	3′,5′	Tricin	55
AnthOMT	番茄 *Solanum lycopersicum*	NP_001289828.1	235	3′,5′	Petunidin-3-*O*-glucoside	56
MtHI4′OMT	蒺藜苜蓿 *Medicago truncatula*	Q29U70.1	364	4′	2,7-Dihydroxy-4′-methoxyisoflavanone	57
MpOMT1A	辣薄荷 *Mentha piperita*	AAR09598.1	344	7	Rhamnetin	58
MpOMT3	辣薄荷 *Mentha piperita*	AAR09601.1	364	3′	Isorhamnetin	58
MpOMT4	辣薄荷 *Mentha piperita*	AAR09602.1	343	4′	Quercetin 4′-methyl ether	58
ObF8OMT-1	罗勒 *Ocimum basilicum*	AGQ21571.1	234	8	Nevadensin	59
ObFOMT1	罗勒 *Ocimum basilicum*	AFU50295.1	340	7	Luteolin 7-methyl ether、apigenin 7-methyl ether	60
ObFOMT2	罗勒 *Ocimum basilicum*	AFU50296.1	340	7	Luteolin 7-methyl ether、apigenin 7-methyl ether	60
VvFAOMT	葡萄 *Vitis vinifera*	C7AE94.1	235	3′,5′	Petunidin-3-*O*-glucoside	61,62
OsROMT-9	水稻 *Oryza sativa*	ABB90678	368	3′	Homoeriodictyol、luteolin 3′-methyl ether	63
NmAMT6	喜林草 *Nemophila menziesii*	BBA68560.1	209	3′,5′	Petunidin-3-*O*-glucoside	64
TaOMT2	小麦 *Triticum aestivum*	ABB03907	356	3′,4′,5′	Tricetin 3′,4′,5′-trimethyl ether	65
CrOMT6	长春花 *Catharanthus roseus*	AAR02420.1	359	4′	Eriodictyol 3′,4′-dimethyl ether	66
CrOMT2	长春花 *Catharanthus roseus*	AY343490	348	3′,5′	Myricetin 3′,5′-dimethyl ether	67

3.3 *FOMT* 基因的表达调控

近期研究表明,植物 *FOMT* 的表达,受环境与基因的双重调控。茉莉酸甲酯(MeJA)的可诱导葛根中 *PlOMT*4 基因表达上调,3′-*O*-甲基化异黄酮生物合成量增加[68]。小麦白粉病病原体可诱导大麦 *F*1-*OMT* 基因表达,促进芹菜素 7-*O*-甲基转移酶发挥作用,生成甲基化抗毒素[54];而禾谷镰刀菌侵染大麦后,包括 FOMT 在内的丙烷类生物合成的酶的积累量增加,羟基肉桂酸酰胺和类黄酮含量增加,这使得植物细胞壁增厚,增强抗病能力[69]。在苜蓿中过表达 *IOMT* 基因,发现 IOMT 蛋白可将未受病原菌侵染的苜蓿叶片中的大豆苷元转化为 7-*O*-甲基大豆苷元。此外,使用 $CuCl_2$ 诱导或用茎点霉(*Phoma medicaginis*)感染的 *IOMT* 过表达植株中,4′-*O*-甲基异黄酮(芒柄花素)含量升高[70]。

3.4 *FOMT* 的应用

作为植物天然产物,许多 *O*-甲基化类黄酮在植物体内的含量并不高,而化学合成步骤繁琐,操作不便。因此,许多研究者尝试通过生物合成方法实现 *O*-甲基化类黄酮的大量生产。Lee 等[71]构建了含 3′-OMT(SlOMT3)/7-OMT(OsNOMT)融合蛋白的大肠杆菌,能够将槲皮素、木犀草素、圣草酚、二氢槲皮素,分别转化为甲基鼠李素、毡毛美洲茶素、2-*O*-甲基圣草酚、2-*O*-甲基二氢槲皮素。Berim 等[72]构建了含 FOMT 的酵母菌株,其能够催化类黄酮底物产生柚皮素和木犀草素的 *O*-甲基化衍生物。FOMT 在 *O*-甲基化类黄酮的生物合成过程中起着重要的调控作用。

4 前景展望

长期以来,植物类黄酮的药用价值和生物合成调控备受关注,但类黄酮的生物利用度低,使其在药用开发上受到限制。甲基化修饰可提高类黄酮的代谢稳定性与体内转运能力,提高生物利用度,弥补其在临床应用上的短板。近年来,植物 *O*-甲基化类黄酮及 FOMT 研究成为热点,随着植物基因组学研究的深入,大量植物 *FOMT* 基因被发现,但得到充分功能验证的 *FOMT* 基因还为数不多。而现阶段对于 FOMT 的研究多采用体外酶催化、生化特征分析等手段,仅有少数 *FOMT* 基因进行了体内功能验证,有关 *FOMT* 基因表达调控的研究则更为匮乏。随着 *O*-甲基化类黄酮与 *FOMT* 研究的深入,FOMT 有望用于 *O*-甲基化类黄酮的工厂化生产,为相关医药保健品生产提供高性价比原料;此外,*O*-甲基化类黄酮也是植物体内重要的抗逆代谢物,FOMT 在绿色农药开发、作物抗性育种领域也具有较大的应用潜力。

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