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甲基化儿茶素合成途径研究进展

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摘 要:自 EGCG3"Me、ECG3"Me 在 1982 年首次从茶鲜叶中被发现以来,人们陆续发现了多种甲基化儿茶素。研究发现甲基化儿茶素具有许多生物学活性,某些活性甚至比 EGCG 还要显著,具有广泛的应用前景。本文对国内外有关甲基化儿茶素合成的研究作了综述,重点介绍几种甲基化儿茶素的化学合成方法以及生物合成途径目前取得的进展,以期能为甲基化儿茶素的研究提供帮助。

关键词:儿茶素;甲基化 EGCG;生物合成;化学合成

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Progress on Synthesis of Methylated Catechins

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Abstract:Since EGCG3"Me,ECG3"Me were discretedfirstly in tea leaves in 1982,a variety of methylated catechins had be found. Studisshowedthat methylated catechins had many biological activities,even more significant than EGCG in some aspect,which has broad application. This paper reviewedstudies about the synthesis of methylated catechins,includedseveral chemical synthesis and progress on biosynthesis pathway in tea leaf,in order to provide new insights and help into catechins and methylated catechins using Biotechnology.

Key words:catechin;methylated EGCG;biosynthesis;chemical synthesis

儿茶素是茶叶中最重要的生理活性物质,约占多酚类 70%,茶叶干重的 12% ~24%,属黄烷醇类,其基本结构是 α-苯基苯并吡喃,主要由表没食子儿茶素没食子酸酯(EGCG)、表没食子儿茶素(EGC)、表儿茶素没食子酸酯(ECG)、表儿茶素(EC)、儿茶素(C)等组成[1]。甲基化儿茶素是儿茶素苯环上的酚羟基被甲氧基取代而形成的一系列甲基化衍生物,自 Saijo R 等[2]在 1982 年首次从绿茶中提取出 ECG3"Me、EGCG3"Me 以来,目前被发现和鉴定的甲基化儿茶素有 27 种[2-11],其结构如图 1。

研究发现甲基化儿茶素具有显著的抗过敏、抗氧化、抗癌症、抗肥胖等生物活性[12-17],因而日益受到科研工作者的关注。然而,调查发现甲基化儿茶素只存在于部分茶树中,且只有很少品种茶叶中含量超过 1%[18-21],这极大限制了其开发应用。目前合成甲基化儿茶素的化学合成的方法有很多,而儿茶素和甲基化儿茶素生物合成途径仍存争议,但也取得了明显进展。

1 化学合成

在化学修饰方面,可分为甲基供体的修饰和保护基团的修饰。

1.1 重氮甲烷合成法

Yanase E 等[22]用重氮甲烷与 EGCG 的甲醇溶液在 25 ℃条件下,以及重氮甲烷、EGCG 甲醇溶液与酯溶液混合在-50 ℃条件下反应共合成了 11 种甲基化 EGCG 产物,分别是(17、18、19、20、21、22、24、30、31、32、34)。同时得出了 EGCG 上的酚羟基在重氮甲烷中的反应活性是 4" >3" >3′ >4′的结论。

1.2 硫酸二甲酯合成法

Utenova B 等[23]将硫酸二甲酯加入到 EGCG、碳酸钾、丙酮的混合溶液中,在充氮气条件下回流搅拌 6h,反应产物过滤后再真空浓缩,最后用已烷: 乙酸乙酯: 丙酮(6:3:1)冲洗色谱柱得到八甲基化产物(34)。

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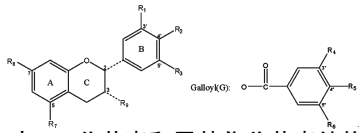


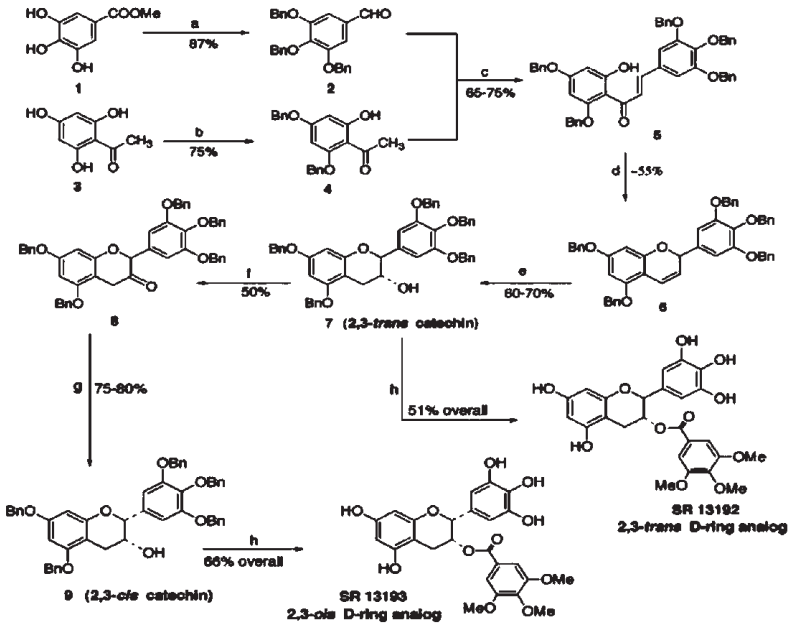
表 1 儿茶素和甲基化儿茶素结构式

Table 1 The structure of catechins and methylated catechins

编号 No.	名称 Name	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9
1	EGCG	OH	OH	OH	OH	OH	OH	OH	OH	G
2	ECG	OH	OH	H	OH	OH	OH	OH	OH	G
3	EGC	OH	OH	OH	–	–	–	OH	OH	OH
4	EC	OH	OH	H	–	–	–	OH	OH	OH
5	GC	OH	OH	OH	–	–	–	OH	OH	OH
6	(+)C	OH	OH	H	–	–	–	OH	OH	OH
7	GCG	OH	OH	OH	OH	OH	OH	OH	OH	G
8	ECG3"Me	OH	OH	H	OCH_3	OH	OH	OH	OH	G
9	ECG4"Me	OH	OH	H	OH	OCH_3	OH	OH	OH	G
10	ECG4′Me	OH	OCH_3	H	OH	OH	OH	OH	OH	G
11	ECG3′,4"diMe	OCH_3	OH	H	OH	OCH_3	OH	OH	OH	G
12	ECG4′,4"diMe	OH	OCH_3	H	OH	OCH_3	OH	OH	OH	G
13	ECG3",4"diMe	OH	OH	H	OCH_3	OCH_3	OH	OH	OH	G
14	EGC4′Me	OH	OCH_3	OH	–	–	–	OH	OH	OH
15	GCG3"Me	OH	OH	OH	OCH_3	OH	OH	OH	OH	G
16	GCG4"Me	OH	OH	OH	OH	OCH_3	OH	OH	OH	G
17	EGCG3′Me	OCH_3	OH	OH	OH	OH	OH	OH	OH	G
18	EGCG4′Me	OH	OCH_3	OH	OH	OH	OH	OH	OH	G
19	EGCG3"Me	OH	OH	OH	OCH_3	OH	OH	OH	OH	G
20	EGCG4"Me	OH	OH	OH	OH	OCH_3	OH	OH	OH	G
21	EGCG3",4"diMe	OH	OH	OH	OCH_3	OCH_3	OH	OH	OH	G
22	EGCG3′,4"diMe	OCH_3	OH	OH	OH	OCH_3	OH	OH	OH	G
23	EGCG3′,3"diMe	OCH_3	OH	OH	OCH_3	OH	OH	OH	OH	G
24	EGCG3",5"diMe	OH	OH	OH	OCH_3	OH	OCH_3	OH	OH	G
25	EGCG4′,4"diMe	OH	OCH_3	OH	OH	OCH_3	OH	OH	OH	G
26	EGCG4′,3",4"triMe	OH	OCH_3	OH	OCH_3	OCH_3	OH	OH	OH	G
27	EGCG3′,3",5"triMe	OCH_3	OH	OH	OCH_3	OH	OCH_3	OH	OH	G
28	EGCG4′,3",5"triMe	OH	OCH_3	OH	OCH_3	OH	OCH_3	OH	OH	G
29	EGCG3",4",5"triMe	OH	OH	OH	OCH_3	OCH_3	OCH_3	OH	OH	G
30	EGCG3′,4′,5′,3",4",5"sixMe	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OH	OH	G
31	EGCG7,3′,4′,5′,3",4",5"hepMe	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OH	OCH_3	G
32	EGCG5,3′,4′,5′,3",4",5"hepMe	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OH	G
33	EGCG5,7,3′,4′,3",4",5"hepMe	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OH	OCH_3	OCH_3	G
34	EGCG5,7,3′,4′,5′,3",4",5"octMe	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	G

1.3　碘甲烷合成法

Meng XF 等[24]将 EGCG、碘甲烷、碳酸钾在丙酮中混合,水浴中超声 3 h,获得了 3 种甲基化产物,单甲基、双甲基、三甲基化 EGCG 各 1 种,分别是(20、25、26)。吕海鹏等[10]以碘甲烷作为甲基供体对 EGCG 进行修饰,利用分离制备型液相得到 5 种甲基化 EGCG 产物,其结构式如图 1 中(20、25、32、33、34)所示。



a,I,BnBr,K2CO3,DMF. Ⅱ,LAH,THF. Ⅲ,PCC,CH_2Cl_2. b,2 equivof BnBr,K_2CO_3,HMPA. c,piperidine,EtOH,reflux. d,$NaBH_4$,THF/EtOH. e,I,BH_3-THF. Ⅱ,H_2O_2,NaOH. f,Dess-Martin periodinane,CH_2Cl_2. g,L-Selectride,THF,room temp. h,I,3,4,5-trimethoxybenzoylchloride,DMAP,Et3N. Ⅱ,H2/Pd/C,dioxane.

图 2　EGCG3",4",5"-triMe 的合成路线

Fig. 2　The synthesis of EGCG3",4",5"-triMe

1.4　苄基保护合成法

Zaveri 等[7]首先利用苄基基团对 EGCG 上的 5、7、3′、4′、5′位点进行保护,再将间羟基苯甲醛进行甲基化,最后连接,然后将苄基水解变成 EGCG3",4",5"-triMe,其合成路线如图 2 所示。Wan SB 等[25]则利用苄基保护基团合成了 3 种甲基化 ECG(8、9、13),6 种甲基化 EGCG(18、19、20、21、25、26),其中 8、25、26 的合成路线如图 3 所示。Lai 等[26]利用苄基保护基团合成了 EGCG4"Me。

1.5　硝基苯磺酰基保护合成法

Aihara 等[27]通过硝基苯磺酰基合成了 4 甲基化 EGCG(18、19、20、23),其合成策略是利用硝基苯磺酰基对 EGC 上的羟基进行保护或选择性甲基化,再将没食子酸进行选择性甲基化或保护,最后进行连接,再将硝基苯磺酰基水解,得到目标物质,其中 EGCG3"Me、EGCG4"Me 的合成途径如图 4 所示。

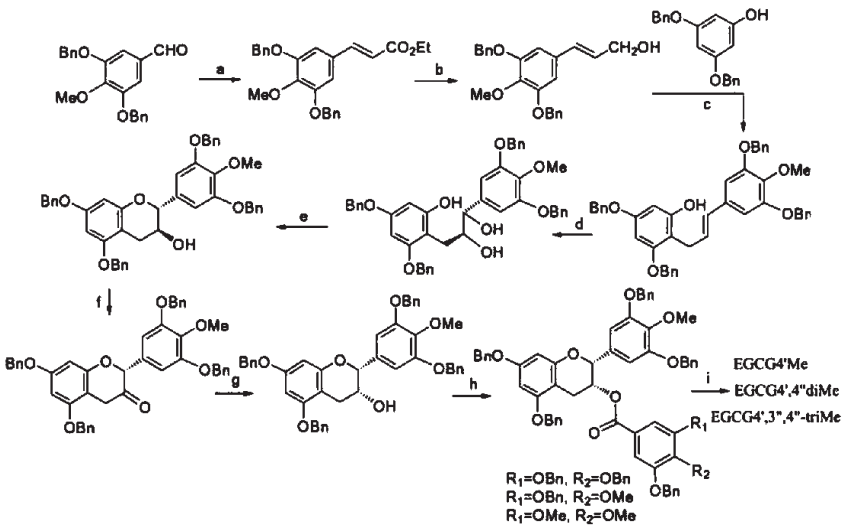
前三种方法虽然简便,但是反应产物具有随机性,后两种方法虽然反应复杂,但可对产物生成进行有效控制,目的性强。

2　生物合成

植物类黄酮合成的大致步骤已基本探明,如图 5。同位素示踪实验证明,类黄酮分子中的 A 环是由三个乙酸分子头尾相接而成,而 B 环与 C 环上的碳原子则来自苯丙氨酸,苯丙氨酸经苯丙酸盐途径形成查尔酮后,再进入各种不同的类黄酮合成途径。在茶树上的一些研究也证明,茶树中类黄酮物质的合成途径与大多数植物基本一致[28,29]。

2.1　生物合成相关酶类

儿茶素的化学结构主体是由 A,B,C,D 四个环组成,其中 A,B,C 三环组成了 2-苯基苯并吡喃的基本结构,即类黄酮物质的基本结构,再连上 D 环即形成酯型儿茶素,最后加上甲基就变成了甲基化儿茶素。这些途径中可能涉及到的酶类有 PAL(苯丙氨酸脱氨酶)、C4H(肉桂酸羧化酶)、4CL(4-香豆酰



(a) Triethyl phosphonoacetate/NaH/THF,0 ℃ - rt;(b) DIBAL/THF,78 ℃ - rt;(c) $H_2SO_4/SiO_2/CH_2Cl_2$,rt;(d) (i) TBSCl/imidazole/DMF, rt;(ii) D-mix α/$CH_3SO_2NH_2/H_2O$/t-BuOH/CH_2Cl_2,0 ℃;(iii) TBAF/THF,rt;(e) (i) $CH(OEt)_3$/PPTS/CH_2Cl_2,60 ℃;(ii) K_2CO_3/ eOH/ ME,rt;(f) Dess - Martin priodinane/CH_2Cl_2,rt;(g) L-Selectride/THF,78 ℃;(h) acid chloride/DMAP/CH_2Cl_2,rt;(i) H_2/$Pd(OH)_2$/MeOH/ HF.

图 3 EGCG4′Me、EGCG4′,4" Me、EGCG4′,3",4" Me 的合成路线

Fig. 3 The synthesis of EGCG3",4",5"-triMe

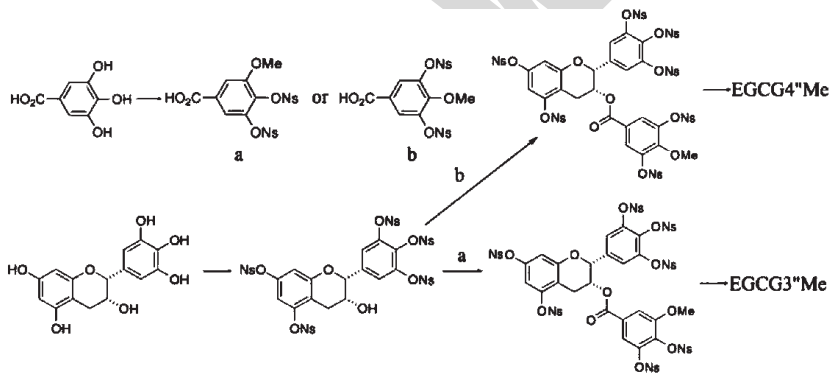


图 4 EGCG3" Me、EGCG4" Me 的合成路线

Fig. 4 The synthesis of EGCG3" Me and EGCG4" Me

CoA 连接酶)、CHS(查尔酮合成酶)、CHI(查尔酮异构酶)、F3H(黄烷酮 3-羟化酶)、二氢黄酮醇 3′羟化酶(F3′H)、二氢黄酮醇 3′5′羟化酶(F3′5′H)、二氢黄烷醇-4-还原酶(DFR)、无色花色素还原酶(LAR)、花青素合成酶(ANS)、花青素还原酶(ANR)、依赖 UDPG 的没食子酰葡萄糖苷转移酶(UGGT)、儿茶素没食子酰基转移酶(ECGT)、儿茶素-O-甲基转移酶(CsOMT)等[8,30-32]。

2.2 类黄酮物质生物合成

儿茶素合成途径中的第一个中间产物是查尔酮,由 CHS 催化丙二酸单酰辅酶 A 的三个乙酸基和对羟苯丙烯酰辅酶 A 的一个乙酸基缩合而成,是类黄酮合成途径中研究的最清楚的一个酶,在茶树中也已早被克隆和鉴定[33,34]。CHI 催化查尔酮异构化为黄烷酮柑橘素,柑橘素是类黄酮合成途径中第一个稳定的中间产物,它与查尔酮都是重要的中间产物,由此形成其它的类黄酮物质[35,36]。马春雷等克隆了茶树中的 CHI 基因,并与番茄的 CHI 基因对比分析,同源性高达 82%[37]。单育等发现 CHS、CHI 基因的表达与儿茶素含量变化趋势一致,这也间接证明了这两个基因参与了儿茶素的合成[38]。

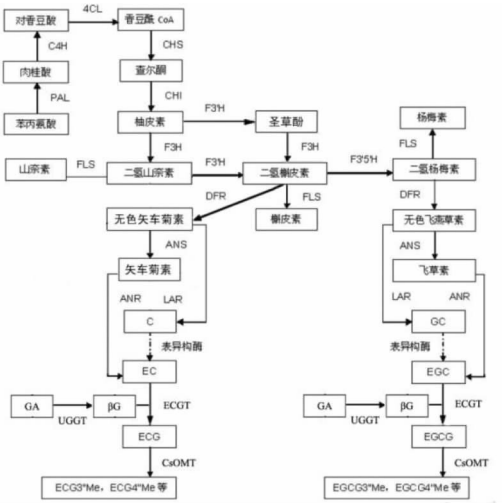


图 5　茶叶中儿茶素及甲基化儿茶素合成途径

Fig. 5　The synthesis of catechins and methylated catechins in tea leaf

黄烷酮在 F3H 的催化下,C 环 3 位羟化而形成二氢黄酮醇,后者是另外两种 B 环羟化酶,即二氢黄酮醇 3′羟化酶(F3′H)和二氢黄酮醇 3′5′羟化酶(F3′5′H)的底物,由 F3H,F3′H 和 F3′5′H 三种羟化酶所催化反应的产物是合成花色素苷、儿茶素类物质的直接前体[32,39]。陈啸天等[40]克隆了 F3′5′H 基因,体外表达发现该酶对柚皮素的活性很低,同时证实茶树粗酶中确实存在 F3′5′H 酶的活性,但是没检测出对二氢山奈酚的活性。Punyasiri 等通过体外酶活发现 F3H 的最适底物为柚皮素[31]。胡晓婧等[41]克隆了茶树中的 F3H 基因,发现该酶可将反应底物柚皮素和圣草酚分别转化为二氢山奈素(DHK)和二氢槲皮素(DHQ)。这些结果说明了 F3′5′H 催化 B 环 5′ 羟基化反应最适底物可能处于二氢黄酮水平上。Singh 等[42]发现 F3H 基因的表达与儿茶素的富集具有明显的相关性。Wei 等[43]通过转录组测序从 10.99 万条基因序列中发现了 4 个影响该儿茶素组成比率的关键基因,即 CsF3′5′H,Cs F3′H,CsF3′H2 和 CsF3′H3,这些基因表达量的比率与相应儿茶素组分比率极显著正相关,同时两者的相关性在 13 个茶树品种中也得到了进一步的验证。以上结果均证明 F3H,F3′H 和 F3′5′H 三种羟化酶参与了茶树儿茶素生物合成。

2.3　儿茶素生物合成

二氢黄酮醇等在二氢黄烷醇-4-还原酶(DFR)下可还原为相应的黄烷-3,4-二醇(无色花色素),无色花色素是花色素苷、儿茶素等许多类黄酮物质合成途径中的最后一个中间产物,可在 ANS 催化下形成花青素[44,45]。Stafford 等利用体外酶学手段,发现在 DFR 和 LAR 催化下,二氢黄酮醇形成无色花青素,然后形成反式儿茶素 C 和 GC[46,47]。Xie 等[48,49]发现儿茶素 EC 和 EGC 来自于花色素的还原,并不存在儿茶素 C 和 GC 差向异构形成 EC 和 EGC 的过程,这一发现为儿茶素的合成提出了一条新途径。Punyasiri 等[31]通过体外酶学反应,发现在 NADPH 的协助下,茶叶中的 ANR 可催化矢车菊色素(Cyanidin)、飞燕草色素(Delphindin)转变成 EC 和 EGC,由此他推测出一条儿茶素生物合成的新途径,即经过 CHS、CHI、FHT 和 DFR 形成的 3,4-黄烷二醇,后在 ANS 和 ANR 的作用下可产生 EC 和 EGC,若在 LAR 催化下则产生 C 和 GC。马春雷等发现 LAR 和 DFR 这两个基因的表达量随着不同茶树品种儿茶素含量的增加而增加,这与 Punyasiri 等以及张宪林等关于相关酶活性的研究结果较为相符,这证实了 LAR 与 DFR 的协同作用与总儿茶素含量和茶多酚含量紧密相关这一结论[31,37,50]。

由于茶树体内没食子酸含量很低,而儿茶素酯化需要大量没食子酸,说明儿茶素酯化反应进行非常迅速[51,52],暗示编码该酶的基因转录水平高或者酶的催化效率高。Gross 等[53,54]从在研究橡木树叶中的 UDP-glucose: vanillate 1-*O*-glucosyl transferase 酶时发现,1-*O*-没食子酰-*β*-葡糖糖(βG)是单宁合成有效的酰基供体,同样也是其受体。刘亚军等[30]从茶叶中分离得到了 1-*O*-没食子酰-*β*-葡糖糖(*β*G),并证实儿茶素的没食子酰基化过程却与水解单宁合成途径具有相似性,即 *β*G 是它们合成的酰基供体,

并涉及到两个新酶:尿苷二磷酸葡萄糖:没食子酰基-1-*O*-β-D-葡萄糖转移酶(UGGT)和表儿茶素:1-*O*-没食子酰基-β-D-葡萄糖 *O*-没食子酰基转移酶(ECGT),而 ECGT 酶目前也已从茶叶中分离纯化[55]。

2.4 甲基化儿茶素生物合成

甲基化儿茶素是儿茶素的酚羟基被-OCH_3 取代的结果,植物 *O*-甲基转移酶可分为两类,Ⅰ类酶的氨基酸数目在 231-248 之间,以咖啡酰 CoA-*O*-甲基转移酶为代表;Ⅱ类酶的氨基酸数目在 344 ~ 383 之间,以咖啡酸-*O*-甲基转移酶为代表[56]。目前两种类型的酶都在茶树中有克隆出来,然而类型Ⅱ的酶并无儿茶酚-*O*-甲基转移酶活性[57,58]。

Kirita 等[8]首次从茶叶中克隆了一个类型Ⅰ的儿茶酚-*O*-甲基转移酶(CsOMT)基因,并成功合成了 4 种甲基化儿茶素 EGCG3" Me、EGCG4" Me、EGCG3",5"-diMe 、EGCG3′,4",5"-triMe。Zhang 等[59]也从茶树中成功克隆咖啡酰辅酶 A-*O*-甲基转移酶(CCoAOMT)基因,体外酶学实验合成 EGCG4" Me,EGCG3" Me,and EGCG3′Me。目前从从茶叶中直接分离纯化的甲基化儿茶素都为酯型甲基化儿茶素[2,11,60-63],这说明儿茶酚-*O*-甲基转移酶的底物可能只能是酯型儿茶素,具有底物特异性。

3 展望

随着甲基化儿茶素及其生物学活性被不断发现,甲基化儿茶素已经受到了非常大的关注,特别是 EGCG3" Me,EGCG4" Me。茶树中甲基化儿茶素的含量普遍比较低,对于直接从茶叶中大规模提取甲基化儿茶素还存在困难,而化学合成都是利用具有很大毒性或者容易爆炸等危险的试剂,存在很大的安全隐患,不能满足人们对纯度、安全的要求。目前儿茶素及甲基化儿茶素的生物合成途径已被大致摸清,接下来的研究重点应该是生物合成途径中各个酶及其基因的调控机制[64-66],从而为利用生物技术改良茶树品种,定向培育出高甲基化儿茶素或儿茶素品种提供有效手段。

参考文献

1 Fei DM(费冬梅). Study on the enzymatic synthesis of methylated EGCG. Beijing:Chinese Academy of Agricultural Sciences (中国农业科学院),MSc. 2011.

2 Saijo R. Isolation and chemical structures of two new catechins from fresh tea leaf. *Agric Biol Chem*,1982,46:1969-1970.

3 Lambert JD,*et al*. Biotransformation of green tea polyphenols and the biological activities of those metabolites. *Mol Pharm*,2007,4:819-825.

4 Sano M,*et al*. Determination of peroxyl radical scavenging activity of flavonoids and plant extracts using an automatic potentiometric titrator. *J Agric Food Chem*,2003,51:2912-2916.

5 Yano S,*et al*. Relationship between the biological activities of methylated derivatives of (-)-epigallocatechin-3-*O*-gallate (EGCG) and their cell surface binding activities. *J Agric Food Chem*,2007,55:7144-7148.

6 Wu YJ (伍妍俊),*et al*. Present studies and research prospects on the methylated EGCG. *J Tea Sci* (茶叶科学),2010,6:407-413.

7 Zaveri NT. Synthesis of a 3,4,5-trimethoxybenzoyl ester analogue of epigallocatechin-3-gallate (EGCG): a potential route to the natural product green tea catechin,EGCG. *Org Lett*,2001,3:843-846.

8 Kirita M,*et al*. Cloning of a novel O-methyltransferase from *Camellia sinensis* and synthesis of O-methylated EGCG and evaluation of their bioactivity. *J Agric Food Chem*,2010,58:7196-7201.

9 Kirita M,*et al*. Cloning and characterization of a novel O-methyltransferase from *Flammulina velutipes* that catalyzes methylation of pyrocatechol and pyrogallol structures in polyphenols. *Biosci Biotechnol Biochem*,2015,79:1111-1118.

10 Lv HP(吕海鹏),*et al*. Methylation of (-)-Epigallocatechin-3-gallate (EGCG). *Food Sci* (食品科学),2010,15:139-142.

11 Sano M,*et al*. Simultaneous determination of twelve tea catechins by high-performance liquid chromatography with electrochemical detection. *Analyst*,2001,126:816-820.

12 Maeda-Yamamoto M,*et al*. *In vitro* and *in vivo* anti-allergic effects of 'benifuuki' green tea containing O-methylated catechin and ginger extract enhancement. *Cytotechnology*,2007,55(2-3):135-142.

13 Kagaya N,*et al*. Novel function of rare catechin,epigallocatechin-3-(3''-*O*-methyl) gallate,against cold injury in primary rat hepatocytes. *J Biosci Bioeng*,2003,96:559-563.

14 Kawase M,*et al*. Antioxidative activity of (-)-epigallocatechin-3-(3''-*O*-methyl) gallate isolated from fresh tea leaf and preliminary results on its biological activity. *Biosci Biotechnol Biochem*,2000,64:2218-2220.

15 Oritani Y,*et al*. Mechanism of anti-obese effects of 'Benifuuki' green tea. *J Japan Soc Food Sci Technol*,2009,56:412-

418.

16 Kurita I, *et al*. Antihypertensive effect of Benifuuki Tea Containing O-Methylated EGCG. *J Agric Food Chem*, 2010, 58: 1903-1908.

17 Cai S, *et al*. Blockade of the formation of insoluble ubiquitinated protein aggregates by EGCG3" Me in the alloxan-induced diabetic kidney. *PLoS One*, 2013, 8(9): e75687.

18 Maeda-Yamamoto M, *et al*. The change of epigallocatechin-3-*O*-(3-*O*-methyl) gallate content in tea of different varieties, tea seasons of crop and processing method. *J Japan Soc Food Sci Technol*, 2001, 48(1): 64-68.

19 Lv HP(吕海鹏), *et al*. Study on the Content of EGCG3″Me in Different Tea Germplasms and its Changes. *J Tea Sci*(茶叶科学), 2006, 4: 310-314.

20 Tang N(唐娜), *et al*. Study on High Levels of O-methylated EGCG in Hunan Tea Resources. *Farm Prod Proc*(农产品加工), 2010, 5: 36-38.

21 Luo ZF(罗正飞), *et al*. Studies on O-Methylated Epigallocatechin-3-*O*-Gallate in Tea. *J Southwest Agric Univ*(西南大学学报,自科版), 2008, 3: 56-59.

22 Yanase E, *et al*. Synthesis of methyl derivatives of epigallocatechin gallate (EGCg) and their stabilities. *ITE Letters on Batteries, New Technologies & Medicine*, 2005, 6(1): 34-37.

23 Utenova BT, *et al*. Antioxidant activity of O-protected derivatives of (-)-epigallocatechin-3-gallate: inhibition of soybean and rabbit 15-lipoxygenases. *Arkivoc*, 2007, 9: 6-16.

24 Meng X, *et al*. Identification and characterization of methylated and ring-fission metabolites of tea catechins formed in humans, mice, and rats. *Chem Res Toxicol*, 2002, 15: 1042-1050.

25 Wan SB, *et al*. Regiospecific and enantioselective synthesis of methylated metabolites of tea catechins. *Tetrahedron*, 2006, 62: 5897-5904.

26 Lai R, *et al*. The synthesis of methylated epigallocatechin gallate. *Chem Nat Comp*, 2015, 51: 472-475.

27 Aihara Y, *et al*. Regioselective synthesis of methylated epigallocatechin gallate via nitrobenzenesulfonyl (Ns) protecting group. *Bioorg Med Chem Lett*, 2009, 19: 4171-4174.

28 Xia T(夏涛), *et al*. Advances in biosynthesis pathways and regulation of flavonoids and catechins. *Sci Agric Sin*(中国农业科学, 2009, 8: 2899-2908.

29 Takos AM, *et al*. Condensed tannin biosynthesis genes are regulated separately from other flavonoid biosynthesis genes in apple fruit skin. *Plant Sci*, 2006, 170: 487-499.

30 Liu YJ (刘亚军). Study on the synthesis of tea ester catechins and the accumulation of phenolic compounds. Hefei: Anhui Agriculture University(安徽农业大学), MSc. 2011.

31 Punyasiri P, *et al*. Flavonoid biosynthesis in the tea plant Camellia sinensis: properties of enzymes of the prominent epicatechin and catechin pathways. *Arch Biochem Biophys*, 2004, 431(1): 22-30.

32 Winkel-Shirley B. Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. *Plant Physiol*, 2001, 126: 485-493.

33 Herrmann A, *et al*. Two alleles of the single-copy chalcone synthase gene in parsley differ by a transposon-like element. *Molecul General Genetics*, 1988, 212(1): 93-98.

34 Takeuchi A, *et al*. Chalcone synthase from *Camellia sinensis*: isolation of the cDNAs and the organ-specific and sugar-responsive expression of the genes. *Plant Cell Physiol*, 1994, 35: 1011-1018.

35 Ayabe S, Akashi T. Cytochrome P450s in flavonoid metabolism. *Phytochem Rev*, 2006, 5: 271-282.

36 Koes R, *et al*. Flavonoids: a colorful model for the regulation and evolution of biochemical pathways. *Trends Plant Sci*, 2005, 10: 236-242.

37 Ma CL (马春雷). Molecular cloning and expression analysis of chalcone isomerase, flavonol synthase and leucoanthocyantin reducase genes of tea plant(*Camellia sinensis*). Beijing: Chinese Academy of Agricultural Sciences (中国农业科学院), MSc. 2007.

38 Shan Y (单育). The biosynthesis of phenolic compounds in tea plant and the differential expression of related genes. Hefei: Anhui Agriculture University(安徽农业大学), MSc. 2011.

39 Holton TA, Cornish EC. Genetics and biochemistry of anthocyanin biosynthesis. *Plant Cell*, 1995, 7: 1071-1083.

40 Chen XT (陈啸天). Cloning and functional analysis of flavonoid 3′, 5′-hydroxylase enzyme in tea plant (*Camellia sinensis* L.). Hefei: Anhui Agriculture University(安徽农业大学), MSc. 2012.

41 Hu XJ (胡晓婧), *et al*. Cloning and functional analysis of flavanone 3-hydroxylase gene (F3H) in Tea (*Camellia sinensis*). *J Agric Biotechnol*(农业生物技术学报), 2014, 3: 309-316.

42 Singh K, *et al*. An early gene of the flavonoid pathway, flavanone 3-hydroxylase, exhibits a positive relationship with the concentration of catechins in tea (*Camellia sinensis*). *Tree Physiol*, 2008, 28: 1349-1356.

43 Wei K, *et al*. Transcriptome Analysis Reveals Key Flavonoid 3′-Hydroxylase and Flavonoid 3′,5′-Hydroxylase Genes in Affecting the Ratio of Dihydroxylated to Trihydroxylated Catechins in *Camellia sinensis*. *PLOS One*. 2015, 10(e01379259).

44 Gu L(顾林), *et al*. Study on mechanism of biological synthe-

sis and color forming of anthocyanidins and approaches to improve the stability. *Sci Technol Food Ind*(食品工业科技),2007,11:240-244.

45 Furukawa T,*et al*. Coordinate expression of genes involved in catechin biosynthesis in *Polygonum hydropiper* cells. *Plant Cell Report*,2002,21:385-389.

46 Stafford HA,*et al*. Flavan-3-ol biosynthesis The conversion of (+)-dihydromyricetin to Its Flavan-3,4-Diol (Leucodelphinidin) and to (+)-Gallocatechin by reductases extracted from tissue cultures of *Ginkgo biloba* and *Pseudotsuga menziesii*. *Plant Physiol*,1985,78:791-794.

47 Stafford HA,*et al*. Flavan-3-ol biosynthesis the conversion of (+)-dihydroquercetin and flavan-3,4-cis-diol (Leucocyanidin) to (+)-catechin by reductases extracted from cell suspension cultures of Douglas Fir. *Plant Physiol*,1984,76:184-186.

48 Xie DY,*et al*. Role of anthocyanidin reductase,encoded by BANYULS in plant flavonoid biosynthesis. *Science*,2003,299:396-399.

49 Xie DY,*et al*. Anthocyanidin reductases from *Medicago truncatula* and *Arabidopsis thaliana*. *Arch Biochem Biophys*,2004,422(1):91-102.

50 Zhang XL(张宪林), *et al*. Study on the changes of Non-galloylated catechins and relative ezymes in tea shoots. *J Tea Sci*(茶叶科学),2009,5:365-371.

51 Saijo R. Pathway of gallic acid biosynthesis and its esterification with catechins in young tea shoots. *Agric Biol Chem*,1983,47:455-460.

52 Ashihara H,*et al*. Distribution and biosynthesis of flavan-3-ols in *Camellia sinensis* seedlings and expression of genes encoding biosynthetic enzymes. *Phytochemistry*,2010,71:559-566.

53 Gross GG. Partial purification and properties of UDP-glucose:vanillate 1-*O*-glucosyl transferase from oak [*Quercus rubra*] leaves. *Phytochemistry*,1983,22:2179-2182.

54 Niemetz R,*et al*. Enzymology of gallotannin and ellagitannin biosynthesis. *Phytochemistry*,2005,66:2001-2011.

55 Liu Y,*et al*. Purification and characterization of a novel galloyltransferase involved in catechin galloylation in the tea plant (*Camellia sinensis*). *J Biol Chem*,2012,287:44406-44417.

56 Joshi CP,*et al*. Conserved sequence motifs in plant S-adenosyl-L-methionine-dependent methyltransferases. *Plant Mol Biol*,1998,37:663-674.

57 Zhu L,*et al*. Cloning and expression of selenocysteine methyltransferase cDNA from *Camellia sinensis*. *Acta Physiol Plant*,2008,30:167-174.

58 Kato M,*et al*. Caffeine synthase gene from tea leaves. *Nature*,2000,406:956-957.

59 Zhang Y,*et al*. Cloning of a caffeoyl-coenzyme A O-methyltransferase from *Camellia sinensis* and analysis of its catalytic activity. *J Zhejiang Univ-Sci B*,2015,16:103-112.

60 Sano M,*et al*. Novel antiallergic catechin derivatives isolated from oolong tea. *J Agric Food Chem*,1999,47:1906-1910.

61 Sun YL(孙业良),*et al*. Study on the analytical method of EGCG 3″Me by HPLC in Tea. *J Tea Sci*(茶叶科学),2009,29:379-384.

62 Luo ZF(罗正飞),*et al*. Investigation on the isolation and purification of EGCG3" Me by high speed countercurrent chromatography. *J Instru Anal*(分析测试学报),2011,30:813-816.

63 Zhou B(周蓓), *et al*. Isolation of methylated catechins from tea and their analysis by high performance liquid chromatography. *Chin J Anal Chem*(分析化学). 2008,36:494-498.

64 Shi CY,*et al*. Deep sequencing of the *Camellia sinensis* transcriptome revealed candidate genes for major metabolic pathways of tea-specific compounds. *BMC Genomics*,2011,12:131.

65 Liu M,*et al*. Relationship between gene expression and the accumulation of catechin during spring and autumn in tea plants (*Camellia sinensis* L.). *Horticul Res*,2015,2(15011):1-2015.

66 Liu M,*et al*. Changes in the expression of genes related to the biosynthesis of catechins in tea (*Camellia sinensis*L.) under greenhouse conditions. *J Horticul Sci Biotechnol*,2015,90:150-156.

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40 Yu JQ(余建强),*et al*. The inbitory effects of oxysophoridine and oxymartrine on central nervous system in mice. *Ningxia Med J*(宁夏医学杂志),2002,24:13-154.

41 Huang MR(黄美蓉),*et al*. The Effects of oxymatrine inhibiting airway inflammation and airway hyperreactivity in asthmatic rats. *Lishizhen Med Mater Med Res*(时珍国医国药),2007,18:1677-1678.

42 Li SH(李生虎),*et al*. Research of toxicity of alkaloids from *Sophora alopecuroides*. *J Agric Sci*(农业科学研究),2009,30:28-29.

43 Liang L(梁磊),*et al*. Acute toxicity of sophoridine by intraperitonealin jection. *Lishizhen Med Mater Med Res*(时珍国医国药),2011,22:1252-1253.

44 Liang L(梁磊),*et al*. Effect of sophoridine on mice immune organs. *Guangdong Med J*(广东医学),2011,32:301-303.