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学术争鸣

Perspective

茶树儿茶素酯化分子机理与研究途径

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摘 要 茶类黄酮的主体成分酯型儿茶素(黄烷−3−醇类)仅专一地、大量存在于茶树(Camellia sinensis)及其近缘种植物中。酯型儿茶素由一种酰基转移酶(未定名)催化没食子酸和简单儿茶素合成。本文中，作者将该酶暂命名为“黄烷−3−醇没食子酰基转移酶”(flavan−3−ol galloyltransferase, GaT)，并提出了儿茶素酯化可能的分子机理及研究途径。比较已知植物酰基转移酶的产物可知，酯型儿茶素与绿原酸的分子结构最相似，推测与二者相对应的酰基转移酶的基因的同源性也最高，因此利用其它植物酯化合成绿原酸的酶的基因序列设计引物，有可能在茶树 EST 文库中克隆出茶树 GaT 基因候选序列，利用 RNAi 瞬间表达和异源表达能够验证其功能。

关键词 茶树; 儿茶素; 酯化; 分子机理

The Molecular Mechanism of Catechins Esterification in *Camellia sinensis* and its Potential Research Method

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Abstract As the main components of tea flavonoids, ester catechins (flavan−3−ols) only exclusively exist in tea plant (*Camellia sinensis*) and its related species with high content. Ester catechins are biosynthesized with non-ester cathchins (simple catechins) and gallic acid and catalyzed by an unknown acyltransferase. In this paper, the authors nominated the enzyme as flavan−3−ol galloyltransferase (GaT) and the potential molecular mechanism and research method of catechins esterification were discussed. Comparing the products of acyltransferases in plants, we should know that the molecular structure of ester catechins is more similar with calleoyl quinic acid (chlorogenic acid), suggested that cloning of *Camellia sinensis* GaT gene (*CsGaT*) will achieved by using primers designed by the DNA sequence of genes which coding acyltransferase catalyzed calleoyl quinic acid biosynthesis, and RNAi transient expression and heterolous expression will characterize the function of *CsGaT*.

Keywords *Camellia sinensis*; Catechins; Esterification; Molecular mechanism

研究背景

儿茶素类(catechins)是茶类黄酮(flavonoids)的主体成分，占绿茶干重的20%~30%，成茶的几乎所有特性如滋味、汤色和香气等都直接或间接与儿茶素有关(Wang et al., 2000)。儿茶素具有抗氧化、抗诱变、抗癌、抗心血管疾病、紫外线辐射保护作用、抗糖尿病、抗菌消炎、减肥和帕金森病的治疗等作用(夏涛和高丽萍, 2009)，属于黄烷−3−醇类化合物，茶树体内主要儿茶素有6种，分别是表没食子儿茶素没食子酸酯[(-)-epigallocatechin−3−gallate, EGCG]、表没食子儿茶素[(-)-epigallocatechin, EGC]、表儿茶素没食子酸酯[(-)-epicatechin−3−gallate, ECG]、没食子儿茶素[(+)-gallocatechin, GC]，儿茶素[(+)-catechin, C]和表儿茶素[(-)-epicatechin, EC]。EGCG和ECG为

酯型儿茶素，其余为非酯型儿茶素(简单儿茶素)。

1酯型儿茶素是茶树的特有类黄酮物质

积累高含量的酯型儿茶素是茶树类黄酮生物合成与代谢的最大特点。简单儿茶素普遍存在于植物体内，是合成原花色素(proanthocyanidin)的前体物质(He et al., 2008; Aron and Kennedy, 2008)，但酯型儿茶素仅专一地、大量存在于山茶科山茶属茶组(Section *Thea*)植物，如茶树[*Camellia sinensis* (L.) O. Kuntze]、普洱茶变种[*C. sinensis* var. *assamica* (Masters) Kitamura]和大理茶(*C. taliensis*)中，茶组的滇缅茶(*C. irrawadiensis*)仅含少量酯型儿茶素，其他山茶属植物仅含简单儿茶素(Nagata and Sakai, 1984; Yagi et al., 2009; Li et al., 2010)。除茶树外，酯型儿茶素仅在葡萄(*Vitis vinifera*)种子中(Guendez et al., 2005)有少量存在。

茶树和普洱茶变种中，酯型儿茶素含量占茶多酚总量的48.7%~91.1% (Li et al., 2010)，研究证实EGCG能够调节多种人类疾病特异目标分子(Nagle et al., 2006)。酯型儿茶素仅存在于茶组少数植物中，说明这些植物体内存在独特的儿茶素酯化反应，并且催化该反应的酶也仅特异地存在这些植物体内。

2儿茶素酯化机理可能的分子机理

植物类黄酮(包括黄烷-3-醇)的生物合成与调节机理已经比较清楚(Winkel-Shirley, 2001; Forkmann and Martens, 2001)。近年茶树类黄酮生物合成研究集中于结构基因的克隆与功能分析(Park et al., 2004; Punyasiri et al., 2004; Lin et al., 2007; Singh et al., 2008; 2009a; 2009b; 马春雷等, 2010)，少量儿茶素积累与结构基因表达的关系研究(Eungwanichayapant and Popluechai, 2009; Ashihara et al., 2010)。

茶树酯型儿茶素的酯化生物合成机理一直为研究者所忽视，甚至催化其反应的酶也尚未命名。仅见的研究表明，茶树嫩梢饲喂没食子酸-G-C^{14}后，能够在EGCG和ECG中检测到放射性，说明酯型儿茶素由没食子酸和简单儿茶素酯化而形成的(Saijo, 1983)。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(Saijo, 1983; Ashihara et al., 2010)，暗示编码该酶的基因转录水平高或者酶的催化效率高。Ashihara等(2010)将催化儿茶素酯化的酶称为黄烷-3醇没食子酸酯合成酶(flavan-3-ol gallate synthase, FGS)，但该名称未表明其反应机理。参照催化其它酯化反应的酶的命名，本文将催化儿茶素酯化的酶暂定名为黄烷-3-醇没食子酰基转移酶(flavan-3-ol galloyltransferase, GaT)，其可能的反应如图1所示。

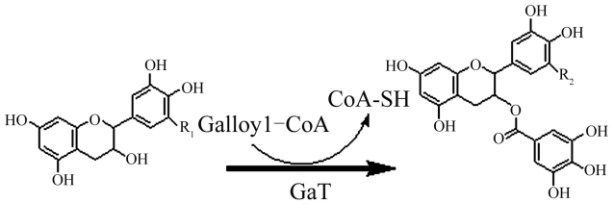

图1 可能的儿茶素酯化反应机理

注: GaT: 没食子酰-CoA:黄烷-3-醇没食子酰基转移酶; EC: R_1=H, (-)-表儿茶素, EGC; R_1=OH, (-)-表没食子儿茶素; ECG: R_2=H, (-)-表儿茶素没食子酸酯; EGCG: R_2=OH, (-)-表没食子儿茶素没食子酸酯

Figure 1 The assumed reaction mechanism of catechins esterfication

Note: GaT: galloyl-coenzyme A:flavan-3-ol galloyltransferase; EC: R_1=H, (-)-epicatechin, EGC; R_1=OH, (-)-epigallocatechin; ECG: R_2=H, (-)-epicatechin gallate; EGCG: R_2=OH, (-)-epigallocatechin gallate

3克隆茶树*GaT*基因可能的途径

如上所述酯型儿茶素仅存在于茶树等少数植物体内，因此很难利用克隆同源基因的方法来克隆茶树*GaT*基因。近年一些植物酰基转移酶基因克隆成功，为克隆茶树*GaT*基因提供了借鉴。

根据儿茶素酯化反应的底物与产物分子结构，可知催化该反应的酶属于植物酰基转移酶的BAHD家族。BAHD酰基转移酶家族利用CoA硫酯，催化形成多种植物次生代谢物。BAHD家族包括4种酰基转移酶，即2个乙酰基转移酶，分别是苯甲醇*O*-乙酰基转移酶(benzylalcohol *O*-acetyltransferase, BEAT)和去乙酰基文多灵4-*O*-乙酰基转移酶(deacetylvindoline 4-*O*-acetyltransferase, DAT),前者催化合成花香物质乙酸苯甲酯和生物碱文多灵(vindoline)。另外2个是苯甲酰/羟基肉桂酰CoA酰基转移酶(benzoyl/hydroxyl-cinnamoyl CoA acyltransferases)，分别是邻氨基苯甲酸*N*-羟基肉桂酰/苯甲酰基转移酶(anthranilate *N*-hydroxycinnamoyl/benzoyltransferase, HCBT)和花青素*O*-羟基肉桂酰转移酶(anthocyanin

O-hydroxycinnamoyltransferase, AHCT)，前者合成一种植物抗毒素anthramides，后者合成酰化的花青素(D'Auria, 2006)。

BAHD酰基转移酶家族的发现和茶树EST文库的构建(Park et al., 2004; 陈亮等, 2005)为茶树黄烷-3醇没食子酸酯合成酶基因的克隆提供了可能。比较已知植物酰基转移酶催化反应的底物和产物，发现酯型儿茶素与咖啡酰奎宁酸(绿原酸)的分子结构最为相似，因此可以根据催化酯化合成绿原酸的酰基转移酶的基因的cDNA序列，在茶树EST文库中BLAST出同源性的高的序列，进而克隆出假定茶树*GaT*基因。

目前已经克隆到催化合成酯化合成绿原酸的酶的基因包括烟草(*Nicotiana tabacum*)和拟南芥羟基肉桂酰基转移酶(hydroxycinnamoyltransferase, NtHCT)基因(Hoffmann et al., 2003; 2004)，烟羟基肉桂酰CoA奎尼酸转移酶(hydroxycinnamoyl CoA transferase, HQT) (Niggeweg et al., 2004)。BAHD家族的催化多样性使得很难仅从其初级结构来预测其功能(D'Auri, 2006)，而根据其他植物催化绿原酸酯化的酶的基因序列，从茶树EST文库中筛选出的同源性高的序列，仅可能是茶树黄烷-3-醇没食子酰基转移酶基因(*CsGaT*)，需要从多个候选基因中进行筛选。

4 RNA干涉是研究茶树基因功能的有效途径

RNA干涉(RNAi, RNA interference)作为一种基因敲除技术广泛应用于许多生物的基因功能分析(Makoto, 2004)，是一种研究未知基因功能的理想方法。植物实现RNAi多依赖于稳定的遗传转化，而茶树的遗传转化非常困难，而且需要非常长的时间(张广辉等, 2010)。

瞬间RNAi表达系统，如农杆菌浸润法(*Agrobacterium* infiltration)，能够有效避免这一困难，是验证候选序列功能的有效方法。农杆菌浸润的瞬间表达系统已经在葡萄(Zottini et al., 2008)、玫瑰(Yasmin and Debener, 2008)等非模式植物上取得成功，并且成功用于基因功能的检测(Shang et al., 2007)。茶树瞬间RNAi表达系统尚缺乏研究，同时茶树体内高含量的多酚类物质对农杆菌侵染与转化有显著的抑制作用(张广辉等, 2006a)，因此，需要建立茶树高效的瞬间RNAi表达系统。

本文作者前期曾建立了发根农杆菌(*Agrobacterium rhizogenesis*)介导的茶树高频发根诱导体系，发现共培养培养基是影响茶树遗传转化的最主要因素(张广辉等, 2006a; Zhang et al., 2007)，并构建了茶树咖啡因合成酶基因的RNAi载体(张广辉等, 2006b)，为建立茶树高效的瞬间RNAi表达系统奠定了基础。利用瞬间RNAi表达系统(农杆菌浸润法或基因枪法)，将茶树黄烷-3醇没食子酰基转移酶(*CsGaT*)基因的候选序列的RNAi载体导入茶树叶片或花中，根据RNAi抑制基因表达效应的传递性可知，RNAi能够抑制*CsGaT*基因的表达，抑制儿茶素的酯化反应，使组织中酯型儿茶素含量降低，而简单儿茶素的积累则不受影响，从而能够确定哪个候选序列基因是茶树的*CsGaT*基因。

5展望

本文提出了一种克隆茶树*CsGaT*基因的可能途径及其功能的验证方法。作者所在课题组正在进行相关研究。需要指出的是，本文仅是基于酯型儿茶素和绿原酸分子结构相似这一特点来推测催化二者合成的酶基因具有同源性。这一假设需要试验来验证。

如上文所述，葡萄种子中存在酯型儿茶素，说明也存在与茶树类似的儿茶素酯化机理。同时已发表大量葡萄EST序列，葡萄遗传转化操作要比茶树容易，因此也可以先克隆葡萄的*GaT*基因，再克隆茶树*GaT*基因，可以避免茶树遗传转化困难的难题。

作者贡献

李家华是本文思路的提出者和主要撰写人；张广辉完成了文献检索和初稿写作；王有国、季鹏章和李竞芸对文章的写作提出宝贵意见。全体作者都阅读并同意最终的文本。

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参考文献

Aron P.M., and Kennedy J.A., 2008, Flavan−3−ols: Nature, occurrence and biological activity, Mol. Nutr. Food Res., 52(1): 79-104

Ashihara H., Deng W.W., Mullen W., and Crozier A., 2010,

Distribution and biosynthesis of flavan−3−ols in *Camellia sinensis* seedlings and expression of genes encoding biosynthetic enzymes, Phytochemistry, 71(5-6): 559-566

Chen L., Zhao L.P., and Gao Q.K., 2005, Sequencing of cDNA clones and analysis of the expressed Sequence tags (ESTs) properties of young tea plant (*Camellia sinensis*) shoots, Nongye Shengwu Jishu Xuebao (Journal of Agricultural Biotechnology), 13(1): 21-25 (陈亮, 赵丽萍, 高其康, 2005, 茶树新梢cDNA克隆测序和表达序列标签(ESTs)特性分析, 农业生物技术学报, 13(1): 21-25)

D'Auri J.C., 2006, Acyltransferases in plants: a good time to be BAHD, Current Opinion in Plant Biology, 9(3): 331-340

Eungwanichayapant P.D., and Popluechai S., 2009, Accumulation of catechins in tea in relation to accumulation of mRNA from genes involved in catechin biosynthesis, Plant Physiology and Biochemistry, 47(2): 94-97

Forkmann G., and Martens S., 2001, Metabolic engineering and application of flavonoids, Current Opinion in Biotechnology, 12(2): 155-160

Guendez R., Kallithraka S., Makris D.P., and Kefalas P., 2005, Determination of low molecular weight polyphenolic constituents in grape (*Vitis vinifera* sp.) seed extracts: Correlation with antiradical activity, Food Chemistry, 89(1): 1-9

He F., Pan Q.H., Shi Y., and Duan C.Q., 2008, Biosynthesis and Genetic Regulation of Proanthocyanidins in Plants, Molecules, 13(10): 2674-2703

Hoffmann L., Besseau S., Geoffroy P., Ritzenthaler C., Meyer D., Lapierre C., Pollet B., and Legrand M., 2004, Silencing of hydroxycinnamoy-coenzyme A shikimate/quinate hydroxylcinnamoyltransferase affects phenylpropanoid biosynthesis, Plant Cell, 16(6): 1446-1465

Hoffmann L., Maury S., Martz F., Geoffroy P., and Legrand M., 2003, Purification, cloning, and properties of an acyltrans-ferase controlling shikimate and quinate ester intermediates in phenylpropanoid metabolism, J. Biol. Chem., 278(1): 95-103

Li J.H., Nesumi A., Shimizu K., Sakata Y., Liang M. Z., He Q.Y., Zhou H.J., and Hashimoto F., 2010, Chemosystematics of tea trees based on tea leaf polyphenols as phenetic markers, Phytochemistry, 71(11-12): 1342-1349

Lin G.Z., Lian Y.J., Ryu J.H., Sung M.K., Park J.S., Park H.J., Park B.K., Shin J.S., Lee M.S., and Cheon C.I., 2007, Expression and purification of His-tagged flavonol synthase of *Camellia sinensis* from *Escherichia coli*, Protein Expression and Purification, 55(2): 287-292

Ma C.Y., Qiao X.Y., and Chen L., 2010, Cloning and expression analysis of leucoanthocyantin reducase gene of tea plant (*Camellia sinensis*), Chaye Kexue (Journal of Tea Science), 30(1): 27-36 (马春雷, 乔小燕, 陈亮, 2010, 茶树无色花色素还原酶基因克隆及表达分析, 茶叶科学, 30(1): 27-36)

Makoto K., 2004, RNA interference in crop plants, Current Opinion in Biotechnology, 15(2): 139-143

Nagata T., and Sakai S., 1984, Difference in caffeine, flavanols and amino acids contents in leaves of cultivated species of *Camellia*, Japan. J. Breed., 34: 459-467

Nagle D.G., Ferreira D., and Zhou Y.D., 2006, Epigallocatechin−3−gallate (EGCG): Chemical and biomedical perspectives, Phytochemistry, 67(17): 1849-1855

Niggeweg R., Michael A.J., and Martin C., 2004, Engineering plants with increased levels of the antioxidant chlorogenic acid, Nat. Biotechnol., 22: 746-754

Park J.S., Kim J.B., Hahn B.S., and Kim K.H., Ha S.H., Kim J.B., and Kim Y.H., 2004, EST analysis of genes involved in secondary metabolism in *Camellia sinensis* (tea), using suppression subtractive hybridization, Plant Science, 166(4): 953-961

Punyasiri P.A.N., Abeysinghe I.S.B., Kumar V., Treutter D., Duy D., Gosch C., Martens S., Forkmann G., and Fischer T.C., 2004, Flavonoid biosynthesis in the tea plant *Camellia sinensis*: properties of enzymes of the prominent epicatechin and catechin pathways, Archives of Biochemistry and Biophysics, 431(1): 22-30

Saijo R., 1983, Pathway of gallic acid biosynthesis and its esterification with catechins in young tea shoots, Agric. Biol. Chem., 47(3): 455-460

Shang Y.J., Schwinn K.E., Bennett M.J., Hunter D.A., Waugh T.L, Pathirana N.N., Brummell D.A., Jameson P.E., and Davies K.M., 2007, Methods for transient assay of gene function in floral tissues, Plant Methods, 3: 1-12

Singh K., Kumar S., Rani A., Gulati A., and Ahuja P.S., 2009a, Phenylalanine ammonialyase (*PAL*) and cinnamate 4−hydroxylase (*C4H*) and catechins (flavan−3−ols) accumulation in tea, Funct. Integr. Genomics, 9(1): 125-134

Singh K., Kumar S., Yadav S.K., and Ahuja P.S., 2009b, Characterization of dihydroflavonol 4−reductase cDNA in tea [*Camellia sinensis* (L.) O. Kuntze], Plant Biotechnol. Rep., 3(1): 95-101

Singh K., Rani A., Kumar S., Sood P., Mahajan M., Yadav S.K., Singh B. and Ahuja P.S., 2008, An early gene of the flavonoid pathway, flavanone 3−hydroxylase, exhibits a

positive relationship with the concentration of catechins in tea (*Camellia sinensis*), Tree Physiology, 28(9): 1349-1356

Wang H., Provan G.J., and Helliwell K., 2000, Tea flavonoids; their functions, utilisation and analysis, Trends Food Sci. Technol., 11: 152-60

Winkel-Shirley B., and Flavonoid B., 2001, A colorful model for genetics, biochemistry, cell biology, and biotechnology, Plant Physiology, 126(2): 485-493

Xia T., and Gao L.P., 2009, Advances in biosynthesis pathways and regulation of flavonoids and catechins, Zhongguo Nongye Kexue (Scientia Agricultura Sinica), 42(8): 2899-2908 (夏涛, 高丽萍, 2009, 类黄酮及茶儿茶素生物合成途径及其调控研究进展, 中国农业科学, 42(8): 2899-2908)

Yagi K., Goto K., and Nanjo F., 2009, Identification of a major polyphenol and polyphenolic composition in leaves of *Camellia irrawadiensis*, Chem. Pharm. Bull., 57(11): 1284-1288

Yasmin A., and Debener T., 2010, Transient gene expression in rose petals via *Agrobacterium* infiltration, Plant Cell Tiss. Organ Cult., 102(2): 245-250

Zhang G.H., Liang Y.R., and Lu J.L., 2006a, *Agrobacterium rhizogenes*-mediated high frequency hairy root induction and genetic transformation in tea plant, Chaye Kexue (Journal of Tea Science), 26(1): 1-10 (张广辉, 梁月荣, 陆建良, 2006a, 发根农杆菌介导的茶树发根高频诱导与遗传转化, 茶叶科学, 26(1): 1-10)

Zhang G.H., Liang Y.R., Lu J.L., and Dong J.J., 2006b, Construction of tea caffeine synthase gene RNAi vector, Chaye Kexue (Journal of Tea Science), 26(4): 243-248 (张广辉, 梁月荣, 陆建良, 董俊杰, 2006b, 茶树咖啡因合成酶基因RNA干涉表达载体构建, 茶叶科学, 26(4): 243-248)

Zhang G.H., Liang Y.R., Lu J.L., Borthakur D., Dong J.J., and Zheng X.Q., 2007, Induction of hairy roots by *Agrobacterium rhizogenes* in relation to L-theanine production in *Camellia sinensis*, Journal of Horticultural Science & Biotechnology, 82(4): 636-640

Zhang G.H., Lv C.Y., and Duan H.X., Plant regeneration and genetic transformation in *Camellia sinensis*, Fenzi Zhiwu Yuzhong (Molecular Plant Breeding), 8(2): 293-297 (张广辉, 吕才有, 段红星, 2010, 茶树离体植株再生与遗传转化, 分子植物育种, 8(2): 345-349)

Zottini M., Barizza E., Costa A., Formentin E., Ruberti C., Carimi F., and Schiavo L.F., 2008, Agroinfiltration of grapevine leaves for fast transient assays of gene expression and for long-term production of stable transformed cells, Plant Cell Rep., 27(5): 845-853