植物三萜皂苷生物合成途径及调控机制研究进展

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摘要 三萜皂苷是一类天然存在的结构多样的三萜苷类化合物, 广泛分布于植物中. 三萜皂苷不仅是植物抵御病原微生物和食草动物的防御化合物, 还具有多种药理活性, 被广泛应用在医药、日化、食品、农业等领域. 近年来, 随着科学技术的发展和研究的深入, 植物三萜皂苷生物合成途径的基本框架及调控机制研究已经取得了一定的进展. 植物三萜皂苷种类繁多、结构复杂, 虽然有着共同的前体合成途径, 但后修饰阶段具有高度特异性和多样性. 本文综述了三萜皂苷的生物合成途径、关键酶基因、转录调控以及微生物细胞工程方面的研究进展, 并对今后的发展方向进行了展望, 以期为相关领域的研究提供参考.

关键词 三萜皂苷, 生物合成, 关键酶, 调控机制

三萜皂苷(triterpenoids saponins)是由三萜皂苷元和一个或多个糖基和/或其他化学基团缩合而成的一系列结构多样的天然化合物^[1], 主要分布在五加科、蝶形花科、石竹科、桔梗科、毛茛科、玄参科、葫芦科等植物中^[2]. 植物中三萜皂苷常分布在特定的器官和组织, 如人参(*Panax ginseng*)、桔梗(*Platycodon grandiflorus*)的根, 麦蓝菜(*Vaccaria hispanica*)的种子, 罗汉果(*Siraitia grosvenori*)的果实和积雪草(*Centella asiatica*)的茎、叶等部位均积累着丰富的三萜皂苷. 三萜皂苷元的骨架由30个碳原子构成, 常见的为四环

三萜和五环三萜, 其中最常见的三萜母核类型为达玛烷型(dammarane)和齐墩果烷型(oleanane). 三萜皂苷不仅参与植物的通讯、防御和感觉调控, 还具有抗炎、抗癌、抗菌、抗病毒等多种生物活性^[3,4]. 甘草(*Glycyrrhiza uralensis*)中的甘草酸、罗汉果中的罗汉果苷的甜味分别约为蔗糖的941倍和300倍^[5,6], 可用作天然甜味剂. 此外, 三萜皂苷是一种两性化合物, 在水中可以形成稳定的泡沫^[7], 常被用于制作清洁剂、发泡剂和乳化剂等. 三萜皂苷的这些特性, 使其在医药、食品、日化、农业等领域受到了广泛关注, 这均

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得利于三萜皂苷的结构多样性。然而, 由于三萜皂苷结构复杂、含量低, 使得直接提取、分离, 或通过化学合成等方法间接获取三萜皂苷都相对困难, 限制了其广泛应用^[8]。利用生物技术对三萜皂苷生物合成途径进行研究, 从而提高目标代谢产物的含量, 或利用合成生物学方法生产三萜皂苷, 已成为当前研究热点。现今在三萜皂苷生物合成途径解析、转录调控机制及微生物细胞工程方面取得了一定进展, 进一步阐明三萜皂苷生物合成的分子机制, 将有助于实现其在植物或微生物中的人工调控, 以提高产量。因此, 本文总结了三萜皂苷生物合成途径研究中的新技术、新方法, 综述了三萜皂苷生物合成途径、关键酶基因、转录调控以及三萜皂苷微生物细胞工程方面的最新进展, 并对今后的发展方向进行了展望, 以期对相关领域的研究提供参考。

1 三萜皂苷生物合成途径中的新技术和新方法

近年来, 随着高通量测序技术的发展, 许多植物的基因组和转录组信息被在线解码和发布, 为植物三萜皂苷合成途径的解析提供了许多有价值的信息。多组学关联分析或利用大数据发掘三萜皂苷生物合成相关基因已成为当前研究的热点。研究人员已通过转录组学分析对多种植物的三萜皂苷合成途径进行了初步探究, 如甘草^[9]、人参^[10]、罗汉果^[11]、岗梅(*Ilex asprel-*

la)^[12]、刺五加(*Eleutherococcus senticosus*)^[13]、桔梗^[14]、毛冬青(*Ilex pubescens*)^[15]、皂荚(*Gleditsia sinensis*)^[16]和细风轮菜(*Clinopodium gracile*)^[17]等, 这些研究丰富了三萜皂苷生物合成关键元件的基因库资源。Zhang等人^[18]采用靶向代谢组学和转录组学关联分析对远志(*Polygala tenuifolia*)中的三萜皂苷生物合成相关基因进行了初步筛选。Zhan等人^[19]以鹅掌草(*Anemone flaccida*)为材料, 对其进行转录组学和蛋白组学数据关联分析, 发现了与三萜皂苷生物合成相关的126个CYP450s(cytochrome P450-dependent monooxygenases)和32个UGTs(uridine diphosphate (UDP)-glucosyltransferases)基因。中国农业科学院蔬菜花卉研究所黄三文课题组^[20]开展了黄瓜(*Cucumis sativus*)全基因组关联分析, 发现了调控葫芦素(cucurbitacin)合成途径的转录因子(BI, Bt)和相关的基因簇。Yan等人^[21]对美国国立生物技术信息中心(National Center for Biotechnology Information, NCBI)数据库中的9个人参属EST(Expressed Sequence Tag)文库数据进行组装和数据分析, 筛选出16条UGTs序列进行实验验证, 获得了催化人参皂苷合成的第一个糖基转移酶UGTPg1。多组学、大数据及合成生物学的联合使用为三萜皂苷代谢途径的解析提供了新的突破口(图1), 整合基因共表达分析、基因簇鉴定等方法鉴定三萜皂苷生物合成结构基因、转录因子等, 同时利用酵母表达系统和高效的烟草瞬时表达体系等进行功能分析^[22]。新途径和新基因的发掘不仅有助于三萜皂苷在底盘植物或微生

图1 植物三萜皂苷生物合成途径的多组学数据驱动研究(网络版彩图)

Figure 1 Multiomic data-driven studies of the plant triterpenoids saponins biosynthetic pathway (color online)

物中高效合成, 还能为分子设计育种等方面的研究奠定基础。

2 植物三萜皂苷生物合成途径

2.1 三萜皂苷生物合成途径

植物中的三萜均来自异戊烯基焦磷酸(isopentenyl pyrophosphate, IPP, C5)和二甲基烯丙基焦磷酸(dimethylallyl pyrophosphate, DMAPP, C5)。IPP和DMAPP可以由两条不同的途径合成: 细胞质中的甲羟戊酸(mevalonate, MVA)途径和质体中的2-C-甲基-D-赤藓醇-4-磷酸(2-C-methyl-D-erythritol-4-phosphate, MEP)途径。MVA途径以乙酰辅酶A(acetyl-CoA)为起始底物, 经过六步缩合反应生成IPP; MEP途径则是以丙酮酸和甘油醛3-磷酸为起始底物, 经过七步反应合成IPP^[5](图2B)。其中, MVA途径在三萜皂苷的生物合成中起到主导作用^[1,3,5,23]。IPP和DMAPP在香叶基焦磷酸合成酶(geranylpyrophosphate synthetase, GPS)的作用下经过缩合生成香叶基焦磷酸(geranylpyrophosphate, GPP, C10); GPP在法呢基焦磷酸合酶(farnesyl pyrophosphate synthetase, FPS)的催化下再加入第二个IPP单元生成法呢基焦磷酸(farnesyl pyrophosphate, FPP, C15); 然后两个FPP经鲨烯合成酶(squalene synthase, SS)缩合形成角鲨烯(squalene, C30), 随后在鲨烯氧化酶(squalene epoxidase)的作用下环氧化生成2,3-氧化鲨烯(2,3-oxidosqualene, C30)^[5](图2B)。2,3-氧化鲨烯经环化、羟基化、糖基化等结构修饰可以合成各种三萜皂苷, 介导这些反应的酶有氧化鲨烯环化酶(OSCs)、细胞色素P450(CYP450s)和糖基转移酶(UGTs)等(图2A)。

2.2 三萜皂苷生物合成中的关键酶

植物三萜皂苷种类繁多、结构复杂, 虽然它们有着共同的前体合成途径, 但后修饰具有高度特异性和多样性, 而且相关元件的编码基因分布在整个基因组中, 以网状途径作用产生化合物阵列而非单一化合物, 这大大增加了解析三萜皂苷生物合成途径的难度^[1,3]。植物中三萜皂苷的积累具有器官和组织特异性, 而且生物或非生物胁迫均可诱导植物三萜皂苷的合成。三萜皂苷生物合成途径包含了20余步连续的酶促反应, 其中从乙酰辅酶A到2,3-氧化鲨烯的上、中游代谢途

径已研究得比较清晰(图2B), 该过程中研究得比较多的酶有3-羟基-3-甲基戊二酰辅酶A还原酶(HMGR, EC1.1.1.34)、法呢基焦磷酸合酶(FPS, EC2.5.1.1/EC2.5.1.10)、鲨烯合成酶(SS, EC2.5.1.21)、鲨烯环氧酶(SE, EC1.14.99.7), 这些酶在植物中均以基因家族的形式存在。HMGR是植物MVA途径的第一个限速酶^[24]; FPS, SS和SE均处在三萜皂苷合成途径的分支点上^[25]。目前, 仅有少数几种参与三萜皂苷生物合成的HMGR, FPS, SS和SE基因被克隆和鉴定(表1)。

然而, 三萜皂苷生物合成下游代谢通路从2,3-氧化鲨烯到最后的三萜皂苷形成在不同植物中都有各自的规律(图2A), 接下来本文将对后修饰过程中涉及的OSCs, CYP450s和UGTs这三大酶类进行具体阐述。

(1) 氧化鲨烯环化酶。氧化鲨烯环化酶(oxidosqualene cyclase, OSCs, EC5.4.99.x)是三萜皂苷下游合成阶段的第一个限速酶, 也是三萜皂苷和甾醇生物合成途径的分支点, 引导2,3-氧化鲨烯经质子化、环化、重排和去质子化等完成环化过程生成三萜骨架^[7]。该酶形成了一个超基因家族, 家族成员氨基酸同源性较高, 含有DDTAEA序列(参与底物结合)和QW特征序列(与蛋白质结构稳定性相关)这两个高度保守序列^[35]。理论上, 2,3-氧化鲨烯环化能形成100余种三萜骨架, 其结构多样性可能与相关酶的灵活多变核心部位有关, OSC上一个氨基酸序列的改变都可能导致产物发生改变^[7,36-38]。然而, 迄今发现的OSCs产物仅有数十种。在不同OSCs的作用下, 2,3-氧化鲨烯通过“椅-船-椅”式构象变化, 可以转化为protosteryl阳离子, 导致羊毛甾醇(lanosterol)、环阿屯醇(cycloartenol)、葫芦二烯醇(cucurbitadienol)、帕克醇(parkeol)等物质的形成(图2A), 这些物质又可以进一步衍生为植物甾醇、甾体糖苷生物碱、甾体皂苷等; 然而, 羊毛甾醇、环阿屯醇、葫芦二烯醇等物质在植物中也可以作为三萜的前体^[38,39], 在相应酶的催化作用下分别衍生成羊毛脂烷型(lanostane)、环阿屯烷型(cycloartane)和葫芦烷型(cucurbitane)四环三萜皂苷, 如灵芝酸C(ganoderic acid C)、黄芪苷IV(asteagalosid IV)、罗汉果苷IE(mogroside IE)等。2,3-氧化鲨烯通过“椅-椅-椅”式构象变化, 则导致了各种三萜皂苷元的形成, 该过程中的第一个中间产物是dammaranyl阳离子, dammaranyl阳离子可以直接生成达玛烯二醇(dammarenediol-II); 同时, dammaranyl阳离子可以进一步被转化为tirucallanyl阳离子(生

图 2 三萜皂苷生物合成途径。A: 2,3-氧化鲨烯经氧化鲨烯环化酶(OSCs)、细胞色素P450(CYP450s)和糖基转移酶(UGTs)等催化合成各种三萜皂苷。虚线箭头表示多个步骤。OSC: 氧化鲨烯环化酶; CAS: 环阿屯醇合成酶; LAS: 羊毛甾醇合成酶; CS: 葫芦二烯醇合成酶; DS: 达玛烯二醇合成酶; LuS: 羽扇豆烷合成酶; α AS: α -香树素合成酶; β AS: β -香树素合成酶; parkeol: 帕克醇; lanosterol: 羊毛甾醇; cycloartenol: 环阿屯醇; cucurbitadienol: 葫芦二烯醇; orysatinol: 籼稻醇; Dammarenediol-II: 达玛烯二醇; Lupeol: 羽扇豆醇; β -amyrin: β -香树素; α -amyrin: α -香树素; ganoderic acid C: 灵芝酸C; asteagalosid IV: 黄芪苷IV; mogrol: 罗汉果醇; mogrosin: 罗汉果苷; protopanaxadiol: 原人参二醇; ginsenoside Rh2: 人参皂苷Rh2; betulinic acid: 桦木酸; ursolic acid: 熊果酸; asiatic acid: 积雪草酸; 28-Glc-asiatic acid: 28-葡萄糖基积雪草酸; 11-oxo- β -amyrin: 11-oxo- β -香树素; glycyrrhethinic acid: 甘草次酸; glycyrrhizin: 甘草甜素。B: MVA途径和MEP途径生成IPP和DMAPP, 再由IPP和DMAPP合成三萜皂苷前体2,3-氧化鲨烯的过程; Acetyl-CoA: 乙酰辅酶A; AACT: 乙酰辅酶A酰基转移酶; Acetoacetyl-CoA: 乙酰乙酰辅酶A; HMG-CoA: 3-羟基-3-甲基戊二酰辅酶A; HMGs: HMG-CoA合成酶; HMGR: HMG-CoA还原酶; Mevalonate: 甲羟戊酸; MVK: 甲羟戊酸激酶; Metholvalic-5-phosphate: 5-甲羟戊酸磷酸; PMK: 磷酸甲羟戊酸激酶; Metholvalic-5-diphosphate: 5-甲羟戊酸焦磷酸; MVD: 5-焦磷酸甲羟戊酸脱羧酶; G3P: 甘油醛-3-磷酸; Pyruvate: 丙酮酸; DXP: 1-脱氧D-木酮糖-5-磷酸; DXS: DXP合成酶; DXR: DXP还原异构酶; MEP: 2-C-甲基-D-赤藓醇-4-磷酸; CDP-ME: 4-二磷酸胞苷-2-C-甲基-D-赤藓糖醇; MCT: CDP-ME合成酶; CMK: CDP-ME激酶; CDP-ME2P: CDP-ME-2-磷酸; MDS: 2-C-甲基-D-赤藓糖醇-2,4-环二磷酸合成酶; HMBPP: (E)-4-羟基-3-甲基-2-丁烯基-焦磷酸; HDS: HMBPP合成酶; HDR: HMBPP还原酶; IPP: 异戊烯基焦磷酸; IDI: IPP异构酶; DMAPP: 二甲基烯丙基焦磷酸; GPP: 香叶基焦磷酸; GPS: GPP合成酶; FPP: 法呢基焦磷酸; FPS: FPP合成酶; SS: 鲨烯合成酶; Squalene: 鲨烯; SE: 鲨烯氧化酶; 2,3-oxidosqualene: 2,3-氧化鲨烯(网络版彩图)

Figure 2 Biosynthesis of triterpenoid saponins. A: 2,3-Oxidosqualene is catalyzed by oxidosqualene cyclases (OSCs), cytochrome P450 (CYP450s), and glycosyltransferase (UGTs) to produce various triterpenoid saponins. Dotted arrows indicate multiple steps. OSC: oxidosqualene cyclase; CAS: cycloartenol synthase; LAS: lanosterol synthase; CS: cucurbitadienol synthase; DS: dammarenediol-II synthase; LuS: lupeol synthase; α AS: α -amyrin synthase; and β AS: β -amyrin synthase. B: The MVA pathway and MEP pathway generate IPP and DMAPP, and then IPP and DMAPP synthesize the triterpenoid saponin precursor, 2,3-oxidosqualene. AACT: acetyl-CoA C-acetyltransferase; HMG-CoA: 3-hydroxy-3-methylglutaryl-CoA; HMGs: HMG-CoA synthetase; HMGR: HMG-CoA reductase; MVK: mevalonate kinase; PMK: phosphomevalonate kinase; MVD: metholvalic-5-diphosphate decarboxylase; G3P: glyceraldehyde 3-phosphate; DXP: 1-deoxy-D-xylulose-5-phosphate; DXS: DXP synthetase; DXR: DXP reductoisomerase; CDP-ME: 4-cytidine diphosphate-2-C-methyl-D-erythritol; MCT: CDP-ME synthetase; CMK: CDP-ME kinase; CDP-ME2P: CDP-ME-2-phosphate; MDS: 2-C-methyl-D-erythritol-2,4-cyclic phosphate synthetase; HMBPP: (E)-4-hydroxy-3-methyl-2-butenyl-pyrophosphate; HDS: HMBPP synthetase; HDR: HMBPP reductase; IPP: isopentenyl pyrophosphate; IDI: IPP isomerase; DMAPP: dimethylallyl pyrophosphate; GPP: geranyl pyrophosphate; GPS: GPP synthetase; FPP: farnesyl pyrophosphate; FPS: FPP synthetase; SS: squalene synthase; and SE: squalene epoxidase. (color online)

成甘遂烷型皂苷元, tirucallane)或baccharenyl阳离子; baccharenyl阳离子又能被转化为五环的lupanyl阳离子, 从而形成羽扇豆醇(lupeol); lupanyl阳离子的五元环E环重新打开、扩展成六元环的过程中, 产生了中间环化产物germanicyl阳离子, 该阳离子能进一步生成oleanyl阳离子(生成 β -amyrin)和ursanyl阳离子(生成 α -amyrin)(图2A)^[3,7]。介导这些反应的酶主要有达玛烯二醇合成酶(dammarenediol-II synthase, DS)、羽扇豆醇合成酶(lupeol synthase, LuS)、 α -香树素合成酶(α -amyrin synthase, α AS)和 β -香树素合成酶(β -amyrin

synthase, β AS)等, 分别催化2,3-氧化鲨烯形成达玛烷型、甘遂烷型等四环三萜骨架和齐墩果烷型、乌苏烷型(ursane)、羽扇豆烷型(lupine)等五环三萜类骨架, 这些三萜骨架经过进一步修饰可以生成人参皂苷Rh2(ginsenoside Rh2)、flindissone、甘草甜素(glycyrrhizin)、28-葡萄糖基-积雪草酸(28-Glc-asiatic acid)、桦木酸(betulinic acid)等三萜皂苷元或皂苷。此外, Xue等人^[40]在籼稻(*Oryza sativa Japonica*)中发现了一种新的多功能OSC7(GenBank accession number: ABG22399), 该酶可以使2,3-氧化鲨烯通过“椅-半椅-

表 1 已报道的与三萜皂苷生物合成相关的HMGR, FPS, SS和SE基因^{a)}

Table 1 Overview of HMGR, FPS, SS, and SE related to triterpenoid saponin biosynthesis reported in literatures^{a)}

关键酶	基因	GenBank登记号	植物来源	功能分析方法	参考文献
HMGR	<i>PgHMGR1</i>	KM386694	人参	拟南芥突变体/人参(超表达)	[26]
	<i>PnHMGR</i>	KJ578757	三七	三七细胞株系(超表达)	[27]
	<i>HMGR</i>	JN377411	灵芝	灵芝(超表达)	[28]
FPS	<i>PgFPS</i>	DQ087959	人参	大肠杆菌/积雪草/人参毛状根(超表达)	[29]
SS	<i>PgSSI</i>	AB115496	人参	刺五加合子胚愈伤组织	[30]
	<i>GuSQS1</i>	AM182329	甘草	甘草毛状根	[31]
	<i>PnSS</i>	KC953033	三七	三七细胞株系(超表达)	[27]
SE	<i>NSSQE1</i>	FJ232947	黑种草	酵母	[32]
	<i>PgSQE1</i>	AB122078	人参	人参(RNAi)	[33]
	<i>CpSE2</i>	—	西葫芦	酵母/烟草/西葫芦毛状根	[34]

a) 三七, *Panax notoginseng*; 灵芝, *Ganoderma lucidum*; 黑种草, *Nigella sativa*; 西葫芦, *Cucurbita pepo*; 拟南芥, *Arabidopsis thaliana*; 大肠杆菌, *Escherichia coli*

椅”式构象变化转化为一种新的阳离子orysatinyI, 然后进一步生成一种新的五环三萜鞣稻醇(orysatinol) (图2A)。

研究人员已经从多种植物中克隆得到85条与三萜皂苷生物合成相关的OSCs基因, 并且在酵母、同源或异源植物宿主中进行了功能分析(表2)。进化树分析结果表明, “椅-半椅-椅”式构象变化在系统发育树上介于“椅-船-椅”式构象变化和“椅-椅-椅”式构象变化之间, 常见的不同功能的OSCs(如CS, LuS, DS, β AS和 α AS等)分别聚集在发育树的不同分枝上(图3), 可见具有相同功能的OSC序列相似性较高。

(2) 细胞色素P450. 细胞色素P450依赖性单加氧酶(CYP450s, EC1.14.x.x)是三萜皂苷生物合成途径中的关键酶, 催化三萜骨架进行羟基化、羧基化、醛基化、酮基化和脱氢脱水等结构修饰, 形成三萜皂苷的中间体^[1,3,100]。典型的CYP450s具有A、B、C、D四个特征性结构域, 其中结构域A上的(A/G)GX(D/E)T(T/S)序列是底物和氧分子连接的部位, 结构域D上的PFG(A/S/V)GRRXC(P/A/V)G序列是铁血红素通过共价键连接的部位。

CYP450s约占植物基因组蛋白编码基因的1%, 是参与植物代谢的酶中最大的家族^[101]。根据氨基酸序列同源性和系统发育标准, 将同源性>40%和>55%的CYP450s分别归为同一家族和亚家族。CYP450s的命名规则为: CYP及其后面的数字、字母分别表示细胞

色素P450、家族、亚家族和同工酶, v1、v2表示等位基因, P代表假基因。已知植物中有127个CYP450家族, 其中陆生植物CYP450s被进一步划分为11个家系^[101], 参与植物三萜皂苷生物合成的CYP450s主要来自CYP51, CYP71, CYP72和CYP85家系^[102]。CYP85家系中的CYP716家族酶被认为是双子叶植物三萜生物合成多样性的主要贡献者, 其亚家族CYP716A/-C/-E/-Y成员主要参与双子叶植物中五环三萜骨架的氧化; 而来自CYP51家系中的CYP51H亚家族负责单子叶植物中五环三萜骨架的修饰^[101,103,104]。CYP71家系中的CYP93E、CYP72家系中的CYP72A和CYP85家系中的CYP88D亚家族成员与豆科植物中三萜骨架的氧化密切相关^[105]。此外, CYP71家系(成员: CYP705A, CYP71A, CYP71D, CYP81Q)和CYP85家系(成员: CYP87D, CYP88L, CYP708A, CYP716U, CYP716S)也参与了三萜皂苷的生物合成^[104]。

CYP450s数量庞大、底物特异性强、序列相似度低, 而且表达的产物物理性质十分相似难以分开, 这大大加深了解析其生物转化步骤的难度^[106]。目前, 仅有73个参与三萜皂苷生物合成的CYP450s被成功鉴定(表3)。通过对这些CYP450s基因的系统发育分析, 发现不同家系、家族的CYP450s在发育树上被明显的区分(图4), 不同家系中的CYP450s多数具有相似功能。例如, 在这73个CYP450s中, 大约有30个CYP716A家族成员具有C-28氧化酶活性, 它们中大多数催化 α -amyr-

图 3 OSCs的系统发育树. 基于MEGA-X软件, 采用MUSCLE方法进行OSC氨基酸序列(表2)比对, 然后使用最大似然法进行系统进化树构建, Bootstrap值设置为1000. cuc: 葫芦二烯醇; lan: 羊毛甾醇; par: 帕克醇; ort: 牻牛儿苗醇; orp: orysaspirol; ba: β -香树脂素; aa: α -香树脂素; ach: achilleol B; iarb: 萜醇; lu: 羽扇豆醇; ge: 日耳曼醇; dam: 达玛烯二醇; ta: 蒲公英甾醇; da: δ -香树脂素; tir: 甘遂二烯-7,24-dien-3 β -ol; cam: 山茶酚C; mar: marneral; tal: 牻牛儿苗醇; tale: 21,22-环氧牻牛儿苗醇

Figure 3 Phylogenetic tree of the OSCs. OSC amino acid sequences (Table 2) were aligned using MUSCLE with default parameters as implemented in the program MEGA-X. The tree was constructed using the maximum likelihood method with the bootstrap value set to 1000. cuc: cucurbitadienol; lan: lanosterol; par: parkeol; ort: orysatinol; orp: orysaspirol; ba: β -amyryl; aa: α -amyryl; ach: achilleol B; iarb: isoarborinol; lu: lupeol; ge: germanicol; dam: dammarenediol-II; ta: taraxasterol; da: δ -amyryl; tir: tirucalla-7,24-dien-3 β -ol; cam: camelliol C; mar: marneral; tal: thalianol; tale: thalianol-11,22-epoxide

表 2 已报道的与三萜皂苷生物合成相关的OSCs基因^{a)}

Table 2 Overview of OSCs related to triterpenoid saponin biosynthesis reported in literatures^{a)}

类别	基因	GenBank 登记号	植物来源	产物	功能分析方法	参考文献
DS	<i>PNA</i>	AB265170	人参	dammarenediol-II	酵母	[41]
	<i>DDS</i>	AB122080	人参	dammarenediol-II	酵母/人参(RNAi)	[42]
	<i>CaDDS</i>	AY520818	积雪草	dammarenediol-II	酵母	[43]
	<i>PgDDS</i>	AB122080	人参	dammarenediol-II	烟草	[44]
	<i>PnDS</i>	KC422652	三七	dammarenediol-II	酵母	[45]
	<i>PqDS</i>	KC316048	西洋参	dammarenediol-II	酵母	[46]
LuS	<i>OeOEW</i>	AB025343	油橄榄	lupeol	酵母	[47]
	<i>ToTRW</i>	AB025345	蒲公英	lupeol	酵母	[47]
	<i>BPW</i>	AB055511	白桦	lupeol	酵母	[48]
	<i>GgLUS1</i>	AB116228	光果甘草	lupeol	酵母	[49]
	<i>OSC3</i>	AB181245	百脉根	lupeol	酵母	[50]
	<i>RcLUS</i>	DQ268869	蓖麻	lupeol	酵母	[51]
	<i>BgLUS</i>	AB289586	木榄	lupeol	酵母	[52]
	<i>WsOCS/LS</i>	JQ728552	南非醉茄	lupeol	酵母	[53]
	<i>LUP2</i>	KP784688	欧洲山芥	lupeol	酵母/烟草	[54]
	<i>LsOSC1</i>	MG708182	大花紫薇	lupeol	酵母/烟草	[55]
	<i>TaOSC4</i>	MK547514	阿江榄仁	lupeol	酵母	[56]
	<i>ItOSC6</i>	—	鸢尾	lupeol	酵母	[57]
αAS	<i>PSM</i>	AB034803	豌豆	α-amyrin/β-amyrin	酵母	[58]
	<i>IaAS3079</i>	—	岗梅	α-amyrin/β-amyrin	酵母	[59,60]
	<i>IaAS481</i>	—	岗梅	α-amyrin/β-amyrin	酵母	[59,60]
	<i>BvLUP5G</i>	KP784690	欧洲山芥	α-amyrin/β-amyrin	酵母/烟草	[54]
	<i>LsOSC2</i>	MG708183	大花紫薇	α-amyrin/β-amyrin	酵母/烟草	[55]
	<i>TaOSC2</i>	MK547512	阿江榄仁	α-amyrin/β-amyrin	酵母	[56]
	<i>CrAS</i>	JN991165	长春花	α-amyrin/β-amyrin	酵母	[61]
	<i>EjAS</i>	JX173279	枇杷	α-amyrin/β-amyrin	酵母	[62]
	<i>ObAS2</i>	JQ809437	罗勒	α-amyrin/β-amyrin	酵母	[63]
	<i>OSC9</i>	LC485316	百脉根	α-amyrin	酵母	[64]
	<i>ItOSC2</i>	—	鸢尾	α-amyrin/β-amyrin/δ-amyrin	酵母	[57]
βAS	<i>PNY1</i>	AB009030	人参	β-amyrin	酵母	[65]
	<i>PNY2</i>	AB014057	人参	β-amyrin	酵母	[65]
	<i>PSY</i>	AB034802	豌豆	β-amyrin	酵母	[58]
	<i>GgbAS1</i>	AB037203	光果甘草	β-amyrin	酵母	[66]
	<i>AsbAS1</i>	AJ311789	燕麦	β-amyrin	酵母	[67]
	<i>BPY</i>	AB055512	白桦	β-amyrin	酵母	[48]
	<i>MtAMY1</i>	AF78453	蒺藜苜蓿	β-amyrin	酵母	[68]
	<i>EtAS</i>	AB206469	绿玉树	β-amyrin	酵母	[69]
	<i>LjAMY1/ cOSC1</i>	AB181244	百脉根	β-amyrin	酵母	[50]

(续表2)

类别	基因	GenBank 登记号	植物来源	产物	功能分析方法	参考文献
βAS	<i>BgbAS</i>	AB289585	木榄	β-amyirin	酵母	[52]
	<i>VhBS</i>	DQ915167	麦蓝菜	β-amyirin	酵母	[70]
	<i>NsbAS1</i>	FJ013228	黑种草	β-amyirin	酵母	[71]
	<i>AtBAS</i>	AB374428	拟南芥	β-amyirin	酵母	[72]
	<i>β-AS</i>	HM219225	辽东楸木	β-amyirin	大肠杆菌/酵母	[73]
	<i>SITTS1</i>	HQ266579	番茄	β-amyirin	酵母/番茄(超表达)	[74]
	<i>ObAS1</i>	KF636411	罗勒	β-amyirin	酵母	[63]
	<i>MlbAS</i>	KF425519	非洲灌木	β-amyirin	酵母/非洲灌木 (RNAi)	[75]
	<i>KsBAS</i>	KT150523	刺楸	β-amyirin	酵母	[76]
	<i>EsBAS</i>	KX378998	刺五加	β-amyirin	酵母/烟草	[77]
	<i>LsOSC3</i>	MG708184	大花紫薇	β-amyirin	酵母/烟草	[55]
	<i>LsOSC4</i>	MG708185	大花紫薇	β-amyirin	酵母/烟草	[55]
	<i>TaOSC1</i>	MK547511	阿江榄仁	β-amyirin	酵母	[56]
	<i>CqbAS1</i>	KX343074	藜麦	β-amyirin	酵母	[78]
CS	<i>CPQ</i>	AB116238	西葫芦	cucurbitadienol	酵母	[79]
	<i>CsBi/CsCPQ</i>	KM655855	黄瓜	cucurbitadienol	酵母	[20]
	<i>CcCDS2</i>	KM821405	药西瓜	cucurbitadienol	酵母	[80]
	<i>SgCBQ</i>	HQ128567	罗汉果	cucurbitadienol	酵母	[81]
	<i>CmBi/CmCPQ</i>	XM_008461716	香瓜	cucurbitadienol	酵母	[82]
多功能 OSC	<i>BvLUP5P</i>	KP784689	欧洲山芥	α-amyirin/β-amyirin/lupeol	酵母/烟草	[54]
	<i>KcMS</i>	AB257507	秋茄	α-amyirin/β-amyirin/lupeol	酵母	[83]
	<i>MdOSC1</i>	FJ032006	苹果	α-amyirin/β-amyirin/lupeol	酵母	[62,84,85]
	<i>MdOSC3</i>	FJ032008	苹果	α-amyirin/β-amyirin/lupeol	酵母/烟草	[84,85]
	<i>BfOSC1</i>	LC464978	紫荆	α-amyirin/β-amyirin/lupeol	酵母/烟草	[86]
	<i>AtLUP2</i>	NM_106545	拟南芥	tirucalla-7,24-dien-3β-ol/α-amyirin/ β-amyirin/lupeol/taraxasterol/ ψ-taraxasterol/baurenol	酵母	[87]
	<i>CsOSC2</i>	AB058508	闭鞘姜	β-amyirin/lupeol/germanicol	酵母	[88]
	<i>LjAMY2</i>	AF478455	百脉根	lupeol/β-amyirin/20-hydroxylupeol	酵母/烟草/百脉根 (毛状根)	[89]
	<i>RsM1</i>	AB263203	木榄	germanicol/β-amyirin/lupeol	酵母	[52]
	<i>RsM2</i>	AB263204	木榄	taraxerol/β-amyirin/lupeol	酵母	[52]
	<i>MdOSC4</i>	KT383435	苹果	β-amyirin/lupeol/germanicol	酵母/烟草	[84]
	<i>MdOSC5</i>	KT383436	苹果	β-amyirin/lupeol	酵母/烟草	[84]
	<i>SITTS2</i>	HQ266580	番茄	α-amyirin/β-amyirin/taraxasterol/ δ-amyirin	酵母/番茄(超表达)	[74]
	<i>BfOSC2</i>	LC464979	紫荆	β-amyirin/lupeol/germanicol	酵母/烟草	[86]
	<i>AtBARS1</i>	NM_117625	拟南芥	baruol/β-amyirin	酵母	[90]
	<i>AtCAMS1</i>	NM_148667	拟南芥	β-amyirin/camelliol C/achilleol A	酵母	[91]
	<i>AtLUP1</i>	U49919	拟南芥	β-amyirin/lupeol	酵母	[92]

(续表2)

类别	基因	GenBank 登记号	植物来源	产物	功能分析方法	参考文献
多功能 OSC	<i>AtMRN1</i>	NM_123624	拟南芥	marneral/marnerol/camelliol C/achilleol A/ δ8-polypodatetraenol	酵母	[93]
	<i>AtTHAS1</i>	NM_124175	拟南芥	thalianol, thalianol-21,22-epoxide	酵母	[94]
	<i>AtPEN1</i>	NM_117622	拟南芥	arabidiol, arabidiol-20,21-epoxide	酵母	[93]
	<i>OsOSC6</i>	KC416147	水稻	α-amyrin/β-amyrin	酵母/水稻	[95]
	<i>OsOSC7</i>	ABG22399	水稻	orysatinol/orysapirol	酵母	[40]
	<i>OsPS/OsOSC7</i>	AK066327	水稻	parkeol	酵母	[96]
	<i>OsOSC8</i>	AK070534	水稻	achilleol B/tirucalla-7,24-dien-3β-ol/α-amy- rin/β-amyrin/ψ-taraxasterol/butyrospermol/ dammar-20,24-dien-3β-ol	酵母	[96,97]
其他	<i>OsOSC10</i>	—	水稻	friedelin	酵母	[97]
	<i>OsIAS/ OsOSC11</i>	AK067451	水稻	isoarborinol	酵母/拟南芥 (超表达)	[39]
	<i>OsOSC12/ OsPTSI</i>	—	水稻	poaceatpetol	酵母/水稻	[98]
	<i>AtLASI</i>	NM_114382	拟南芥	lanosterol	酵母	[99]

a) 西洋参, *Panax quinquefolius*; 油橄榄, *Olea europaea*; 蒲公英, *Taraxacum officinale*; 白桦, *Betula platyphylla* var. *japonica*; 光果甘草, *Glycyrrhiza glabra*; 百脉根, *Lotus japonicus*; 蓖麻, *Ricinus communis*; 木榄, *Bruguiera gymnorhiza*; 南非醉茄, *Withania somnifera*; 欧洲山芥, *Barbarea vulgaris*; 大花紫薇, *Lagerstroemia speciosa*; 阿江榄仁, *Terminalia arjuna*; 鸢尾, *Iris tectorum*; 豌豆, *Pisum sativum*; 长春花, *Catharanthus roseus*; 枇杷, *Eriobotrya japonica*; 罗勒, *Ocimum basilicum*; 燕麦, *Avena strigose*; 蒺藜苜蓿, *Medicago truncatula*; 绿玉树, *Euphorbia tirucalli*; 辽东楸木, *Aralia elata*; 番茄, *Solanum lycopersicum*; 药西瓜, *Citrullus colocynthis*; 藜麦, *Chenopodium quinoa*; 香瓜, *Cucumis melo*; 秋茄, *Kandelia candel*; 苹果, *Malus domestica*; 紫荆, *Bauhinia fortificata*; 闭鞘姜, *Costus speciosus*; 水稻, *Oryza saliva*

in/β-amyrin/lupeol骨架C-28位连续三步氧化形成羟基、醛和羧基^[106,118,120,124]。而CYP716E和CYP716S家族成员分别催化三萜C-6、C-2α羟基化, CYP93E家族主要在C-24位催化三萜氧化。此外, CYP450s还具有催化三萜骨架C-3, C-11, C-12, C-16, C-19, C-20, C-21, C-22, C-23, C-25, C-30等位置氧化的作用。

(3) 糖基转移酶。来自糖基转移酶家族I的糖基转移酶(UGTs, EC2.4.1.x), 主要参与植物中天然产物的糖基化, 能将尿嘧啶核苷二磷酸糖上的活性糖基转移到植物中的小分子化合物受体上^[132], 与三萜皂苷生物合成相关的糖基转移酶属于这一类。UGTs催化进行的三萜糖基化通常被认为是三萜皂苷生物合成的最后一步, 是三萜皂苷的多样性和生物活性形成的关键环节^[3]。典型的三萜皂苷含有2~5个糖元, 通常在皂苷元的C-3和/或C-28位置上形成糖链; 也存在C-4, C-16, C-20, C-21, C-22和/或C-23位置糖基化的三萜皂苷^[1,132]。常见的糖元有葡萄糖(glucose, Glc)、半乳糖(galactose, Gal)、葡萄糖醛酸(glucuronic acid, GlcA)、鼠李糖(rhamnose, Rha)、木糖(xylose, Xyl)和阿拉伯糖(arabi-

nose, Ara)等五元糖或六元糖。糖链的数量、组成及其在三萜骨架上的位置, 均影响着三萜皂苷的生物活性、稳定性、溶解性和细胞分隔性^[102]。

UGTs也以基因家族的形式存在于植物体内。根据氨基酸序列的同源性及其催化特征, UGTs被分为不同的家族(同源性>40%)和亚家族(同源性>60%)^[3,133]。尽管序列同源性不高, 但典型的植物次级代谢性UGTs具有相似的结构域, 拥有PSPG(plant secondary product glycosyltransferase)基序, 该基序是UGTs中与底物结合的区域^[2,25]。

随着高通量测序技术的不断发展, 越来越多植物的UGT基因序列被公布, 但仅有少数几种植物中的UGTs基因被克隆和鉴定, 其中有44个参与了三萜皂苷的生物合成, 总结在表4中, 这些UGTs主要来自UGT71, 73, 74, 85, 91和94家族^[2]。对这些UGT基因进行系统发育分析, 结果显示, 不同家族的UGTs处在发育树的不同分枝上(图5), 且具有相似的功能。例如, UGT73家族的成员多数具有C-3或C-28位葡萄糖糖基化活性, 而UGT74s和UGT94s常表现出C-3位葡萄糖糖

表 3 已报道的与三萜皂苷生物合成相关的CYP450s基因^{a)}Table 3 Overview of CYP450s related to triterpenoid saponin biosynthesis reported in literatures^{a)}

编号	基因	GenBank 登录号	植物来源	底物	反应	产物	功能分析方法	参考文献
1	<i>CYP51H10</i>	DQ680852	燕麦	β -amyrin	C12,C13 β 环氧化/ C16 β 羟基化	12,13 β -epoxy-16 β -hydroxy- β -amyrin	酵母	[107]
2	<i>CYP71D353</i>	KF460438	百脉根	dihydro-lupeol/20-hy- droxy-lupeol	C-20羟基化/C-28 氧化	20-hydroxy-lupeol/20-hy- droxy-betulinic acid	烟草/百脉根(毛状 根)	[89]
3	<i>CYP81Q58</i>	KM655862	黄瓜	19-hydroxy-cucurbita- dienol	C-25羟基化/双键 位移	19,25-dihydroxy cucurbita- dienol	酵母/黄瓜	[20]
4	<i>CYP81Q59</i>	—	香瓜	11-carbonyl-20 β -hy- droxy-cucurbitadienol	C-2 β 羟基化	11-carbonyl-2 β ,20 β -dihy- droxy-cucurbitadienol	酵母	[82]
5	<i>LjCYP93E1</i>	LC414182	百脉根	β -amyrin	C-24羟基化	24-hydroxy- β -amyrin	酵母/ <i>Lotus retro- transposon 1</i> (<i>LORE1</i>)-tagged lines	[64]
6	<i>CYP93E1</i>	AF135485	大豆	β -amyrin/24-hydroxy- β -amyrin/sophoradiol	C-24羟基化/C-24 氧化	24-hydroxy- β -amyrin/24- carboxy- β -amyrin/soyasa- pogenol B	酵母	[108]
7	<i>CYP93E2</i>	DQ335790	蒺藜苜蓿	β -amyrin	C-24羟基化	24-hydroxy- β -amyrin	酵母	[106,108]
8	<i>CYP93E3</i>	AB437320	甘草	β -amyrin/24-hydroxy- β -amyrin	C-24羟基化/C-24 氧化	24-hydroxy- β -amyrin/24- carboxy- β -amyrin	酵母	[108,109]
9	<i>CYP93E4</i>	KF906535	花生	β -amyrin/24-hydroxy- β -amyrin	C-24羟基化/C-24 氧化	24-hydroxy- β -amyrin/24- carboxy- β -amyrin	酵母	[108]
10	<i>CYP93E5</i>	KF906536	鹰嘴豆	β -amyrin/24-hydroxy- β -amyrin	C-24羟基化/C-24 氧化	24-hydroxy- β -amyrin/24- carboxy- β -amyrin	酵母	[108]
11	<i>CYP93E6</i>	KF906537	光果甘草	β -amyrin/24-hydroxy- β -amyrin	C-24羟基化/C-24 氧化	24-hydroxy- β -amyrin/24- carboxy- β -amyrin	酵母	[108]
12	<i>CYP93E7</i>	KF906538	小扁豆	β -amyrin	C-24羟基化	24-hydroxy- β -amyrin	酵母	[108]
13	<i>CYP93E8</i>	KF906539	豌豆	β -amyrin/24-hydroxy- β -amyrin	C-24羟基化/C-24 氧化	24-hydroxy- β -amyrin/24- carboxy- β -amyrin	酵母	[108]
14	<i>CYP93E9</i>	KF906540	菜豆	β -amyrin/24-hydroxy- β -amyrin	C-24羟基化/C-24 氧化	24-hydroxy- β -amyrin/24- carboxy- β -amyrin	酵母	[108]
15	<i>CYP72A67</i>	DQ335780	蒺藜苜蓿	oleanolic acid/hedera- genin/ gypsogenic acid/ gypsogenin	C-2 β 羟基化	2 β -hydroxy-oleanolic acid/ bayogenin/medicagenic acid/2 β , 3 β -dihydroxyolean- 12-en-23-oxo-28-oic acid	酵母/蒺藜苜蓿(突 变株系)	[110]
16	<i>CYP72A61v2</i>	AB558145	蒺藜苜蓿	24-hydroxy- β -amyrin	C-22 β 羟基化	soyasapogenol B	酵母	[111]
17	<i>CYP72A69</i>	LC143440	大豆	soyasapogenol B	C-21 β 羟基化	soyasapogenol A	酵母/大肠杆菌	[112]
18	<i>CYP72A475</i>	MH539812	燕麦	12,13 β -epoxy, 16 β -hy- droxy- β -amyrin	C-21 β 羟基化	12,13 β -Epoxy, 16 β ,21 β -di- hydroxy- β -amyrin	烟草	[113]
19	<i>CYP72A63</i>	AB558146	蒺藜苜蓿	β -amyrin	C-30氧化	11-deoxoglycyrrhetinic acid	酵母	[114]
20	<i>CYP72A154</i>	AB558153	甘草	β -amyrin/11-oxo- β - amyrin	C-30氧化	30-hydroxy- β -amyrin/gly- cyrrhetinic acid	酵母	[114]
21	<i>CYP72A68v2</i>	DQ335782	蒺藜苜蓿	oleanolic acid	C-23氧化	gypsogenic acid	酵母	[111]
22	<i>CYP72A397</i>	KT150517	刺楸	oleanolic acid	C-23羟基化	hederagenin	酵母	[76]
23	<i>CYP72A552</i>	—	欧洲山芥	oleanolic acid	C-23羟基化	hederagenin	酵母/烟草	[115]
24	<i>CYP714E19</i>	KT004520	积雪草	oleanolic acid/ursolic acid	C-23羟基化	hederagenin/23-hydroxy ur- solic acid	酵母	[116]
25	<i>CYP716A1</i>	NM_123002	拟南芥	α -amyrin/ β -amyrin/lu- peol	C-28氧化	ursolic acid/oleanolic acid/ betulinic acid	酵母	[117]
26	<i>CYP716A12</i>	DQ335781	蒺藜苜蓿	α -amyrin/ β -amyrin/lu- peol	C-28氧化	ursolic acid/oleanolic acid/ betulinic acid	酵母	[106,118]

(续表3)

编号	基因	GenBank 登录号	植物来源	底物	反应	产物	功能分析方法	参考文献
27	<i>CYP716A15</i>	AB619802	葡萄	α -amyrin/ β -amyrin/lupeol	C-28氧化	ursolic acid/oleanolic acid/betulinic acid	酵母	[106]
28	<i>CYP716A51</i>	AB706297	百脉根	α -amyrin/ β -amyrin/lupeol	C-28氧化	ursolic acid/oleanolic acid/betulinic acid	酵母/百脉根毛状根(CRISPR/Cas9)	[64]
29	<i>CYP716A154/CYP716AL1</i>	JN565975	长春花	α -amyrin/ β -amyrin/lupeol	C-28氧化	ursolic acid/oleanolic acid/betulinic acid	酵母	[119]
30	<i>CYP716A175</i>	XM_008392874	苹果	α -amyrin/ β -amyrin/lupeol	C-28氧化	ursolic acid/oleanolic acid/betulinic acid	酵母	[84]
31	<i>CYP716A265</i>	MG708187	大花紫薇	α -amyrin/ β -amyrin/lupeol	C-28氧化	ursolic acid/oleanolic acid/betulinic acid	酵母/烟草	[55]
32	<i>CYP716A266</i>	MG708188	大花紫薇	α -amyrin/ β -amyrin/lupeol	C-28氧化	ursolic acid/oleanolic acid/betulinic acid	酵母/烟草	[55]
33	<i>CYP716A2</i>	LC106013	拟南芥	α -amyrin/ β -amyrin/lupeol	C-16/C-22 α -C-28羟基化	22 α -hydroxy- α -amyrin/22 α -hydroxy- β -amyrin/16-hydroxy- β -amyrin/betulin	酵母	[117]
34	<i>CYP716A179</i>	LC157867	甘草	α -amyrin/ β -amyrin/lupeol	C-28氧化/C-22 α 羟基化	ursolic acid/oleanolic acid/betulinic acid	酵母	[120]
35	<i>CYP716A259</i>	LC325360	白骨壤	α -amyrin/ β -amyrin/lupeol	C-28氧化	ursolic acid/oleanolic acid/betulinic acid	酵母	[121]
36	<i>CYP716A140</i>	KU878853	桔梗	β -amyrin/16 β -hydroxy- β -amyrin/12,13 α -epoxy- β -amyrin	C-28氧化	oleanolic acid/16 β -hydroxy-oleanolic acid/12,13 α -epoxy-oleanolic acid	酵母	[103]
37	<i>CYP716A14v2</i>	KF309251	黄花蒿	α -amyrin/ β -amyrin/ δ -amyrin	C-3氧化	α -amyrone/ β -amyrone/ δ -amyrone	酵母/烟草/黄花蒿(RNAi)	[122]
38	<i>CYP716A44</i>	AK329870	番茄	α -amyrin/ β -amyrin	C-28氧化	ursolic acid/oleanolic acid	酵母	[123]
39	<i>CYP716A46</i>	XM_004243858	番茄	α -amyrin/ β -amyrin	C-28氧化	ursolic acid/oleanolic acid	酵母	[123]
40	<i>CYP716A83</i>	KU878849	积雪草	α -amyrin/ β -amyrin	C-28氧化	ursolic acid/oleanolic acid	酵母	[103]
41	<i>CYP716A86</i>	KU878848	积雪草	α -amyrin/ β -amyrin	C-28氧化	ursolic acid/oleanolic acid	酵母	[103]
42	<i>CYP716A252</i>	JQ958967	罗勒	α -amyrin/ β -amyrin	C-28氧化	ursolic acid/oleanolic acid	酵母/罗勒(VIGS)	[124]
43	<i>CYP716A253</i>	JQ958968	罗勒	α -amyrin/ β -amyrin	C-28氧化	ursolic acid/oleanolic acid	酵母/罗勒(VIGS)	[124]
44	<i>CYP716A80</i>	KP795926	欧洲山芥	β -amyrin/lupeol	C-28氧化	oleanolic acid/betulinic acid	酵母/烟草	[54]
45	<i>CYP716A81</i>	KP795925	欧洲山芥	β -amyrin/lupeol	C-28氧化	oleanolic acid/betulinic acid	酵母/烟草	[54]
46	<i>CYP716A141</i>	LC209200/ KU8878855	桔梗	β -amyrin/oleanolic acid	C-28氧化/C-16 β 羟基化	oleanolic acid/16 β -hydroxy-oleanolic acid	酵母	[103,105]
47	<i>CYP716A180</i>	KJ452328	白桦	lupeol	C-28氧化	betulinic acid	酵母	[125]
48	<i>CYP716A17</i>	AB619803	葡萄	β -amyrin	C-28氧化	oleanolic acid	酵母	[106]
49	<i>CYP716A52v2</i>	JX036032	人参	β -amyrin	C-28氧化	oleanolic acid	酵母	[126]
50	<i>CYP716A75</i>	KF318733	非洲灌木	β -amyrin	C-28氧化	oleanolic acid	酵母/非洲灌木/蒺藜苜蓿	[75]
51	<i>CYP716A78</i>	KX343075	藜麦	β -amyrin	C-28氧化	oleanolic acid	酵母	[78]
52	<i>CYP716A79</i>	KX343076	藜麦	β -amyrin	C-28氧化	oleanolic acid	酵母	[78]
53	<i>CYP716A94</i>	KT150521	刺楸	β -amyrin	C-28氧化	oleanolic acid	酵母	[76]
54	<i>CYP716A110</i>	KU878864	洛杉矶糙斗菜	β -amyrin	C-28氧化	oleanolic acid	酵母	[103]
55	<i>CYP716A140v2</i>	LC209199	桔梗	β -amyrin	C-28氧化	oleanolic acid	酵母	[105]
56	<i>CYP716A244</i>	KX354739	刺五加	β -amyrin	C-28氧化	oleanolic acid	酵母/烟草	[77]
57	<i>CYP716A254</i>	-	鹅掌草	β -amyrin	C-28氧化	oleanolic acid	酵母	[127]

(续表3)

编号	基因	GenBank 登录号	植物来源	底物	反应	产物	功能分析方法	参考文献
58	<i>CYP716A111</i>	KY047600	洛杉矶耧斗菜	β -amyrin	C-16 β 羟基化	16 β -hydroxy- β -amyrin	酵母	[103]
59	<i>CYP716C11</i>	KU878852	积雪草	ursolic acid/oleanolic acid/6 β -hydroxy-oleanolic acid	C-2 α 羟基化	corosolic acid /maslinic acid/6 β -hydroxy-maslinic acid	酵母	[103]
60	<i>CYP716C53</i>	LC325362	白骨壤	oleanolic acid/ursolic acid	C-2 α 羟基化	maslinic acid/corosolic acid	酵母	[121]
61	<i>CYP716C55</i>	MG708191	大花紫薇	ursolic acid/oleanolic acid	C-2 α 羟基化	corosolic acid/maslinic acid	酵母/烟草	[55]
62	<i>CYP716E26</i>	XM_004241773	番茄	α -amyrin/ β -amyrin	C-6 β 羟基化	6 β -hydroxy- α -amyrin/daturadiol	酵母	[123]
63	<i>CYP716E41</i>	KU878851	积雪草	ursolic acid/oleanolic acid/maslinic acid	C-6 β 羟基化	6 β -hydroxy-ursolic acid/6 β -hydroxy-oleanolic acid/6 β -hydroxy-maslinic acid	酵母	[103]
64	<i>CYP716Slv2/CYP716A53v2</i>	JX036031	人参	protopanaxadiol	C-6羟基化	protopanaxatriol	酵母/人参	[119,128]
65	<i>CYP716S5</i>	KU878856	桔梗	β -amyrin/oleanolic acid	C-12,13 α 环氧化	12,13 α -epoxy- β -amyrin/12,13 α -epoxy-oleanolic acid	酵母	[103]
66	<i>CYP716U1/CYP716A47</i>	JN604536	人参	dammarenediol-II	C-12羟基化	protopanaxadiol	酵母/人参	[129]
67	<i>CYP716Y1</i>	KC963423	柴胡	α -amyrin/ β -amyrin	C-16 α 羟基化	16 α -hydroxy- α -amyrin/16 α -hydroxy- β -amyrin	酵母	[100,103]
68	<i>CYP87D16</i>	KF318735	非洲灌木	β -amyrin	C-16 α 羟基化	16 α -hydroxy- β -amyrin	酵母/非洲灌木/蒺藜苜蓿	[75]
69	<i>CYP87D18</i>	HQ128570	罗汉果	11 α -hydroxy-cucurbitadienol/24,25-epoxycucurbitadienol/24,25-dihydroxy-cucurbitadienol	C-11羟基化/氧化	11-oxo-cucurbitadienol/11-oxo-24,25-epoxycucurbitadienol/mogrol	酵母	[6,130]
70	<i>CYP87D20</i>	KM655862	黄瓜	cucurbitadienol/11-oxo-cucurbitadienol	C-11氧化/C-20 β 羟基化	11-oxo-cucurbitadienol/11-carbonyl-20 β -hydroxy-cucurbitadienol	酵母	[82]
71	<i>CYP88D6</i>	AB433179	甘草	β -amyrin	C-11氧化	11-oxo- β -amyrin	酵母	[109]
72	<i>CYP88L2</i>	NP_001295866	黄瓜	cucurbitadienol	C-19羟基化	19-hydroxy-cucurbitadienol	酵母/黄瓜(RNAi)	[20,82]
73	<i>CYP88A13</i>	MN200611	蒺藜苜蓿	medicagenic acid	C-16 α 羟基化	zanhic acid	酵母/烟草/蒺藜苜蓿根毛	[131]

a) 大豆, *Glycine max*; 花生, *Arachis hypogaea*; 鹰嘴豆, *Cicer arietinum*; 小扁豆, *Lens culinaris*; 菜豆, *Phaseolus vulgaris*; 葡萄, *Vitis vinifera*; 白骨壤, *Avicennia marina*; 黄花蒿, *Artemisia annua*; 洛杉矶耧斗菜, *Aquilegia coerulea*; 鹅掌草, *Anemone flaccida*; 柴胡, *Bupleurum falcatum*

基化作用. 来自不同家族的UGTs可能具有相同的区域选择性, 不同的UGTs在不同底物的同一位置可以有相同的功能^[2]. 然而, 植物UGTs具有很大程度的底物混乱性, 所以仅通过序列分析和体外生化研究来预测UGTs的生物功能仍不够准确^[3,134]. Ribeiro等人^[131]还发现, 已鉴定的皂苷生物合成酶基因在蒺藜苜蓿基因组上常常表现为重复簇状排列, 该特征在糖基转移酶编码基因中尤为明显, 这或许是皂苷代谢进化的驱动力之一.

3 三萜皂苷生物合成的调控

植物中, 三萜皂苷的生物合成受基因调控的影响. 通过增加基因拷贝数或沉默竞争代谢通路基因, 可人为改变三萜生物合成途径基因的表达, 提高三萜皂苷化合物的含量^[8]. 研究表明, 通过HMGR, FPS, SS和SE基因的过表达或RNAi(RNA interference)可实现人参、三七等植物中三萜皂苷生物合成的调控^[27,33,154,155]. 此外, 茉莉酸衍生物(如茉莉酸甲酯,

表 4 已报道的与三萜皂苷生物合成相关的UGTs基因^{a)}Table 4 Overview of UGTs related to triterpenoid saponin biosynthesis reported in literatures^{a)}

编号	基因	GenBank 登录号	植物来源	底物	反应	产物	功能分析 方法	参考文献
1	<i>UGT71G1</i>	AY747627	蒺藜苜蓿	medicagenic acid, UDP-Glc	糖基化	medicagenic acid-Glc	大肠杆菌	[134]
2	<i>UGT73AD1</i>	KP195716	积雪草	asiatic acid/madecassic acid, UDP-Glc	C-28 β 糖基化	28 β -Glc-asiatic acid/28 β - Glc-madecassic acid	大肠杆菌	[135]
3	<i>UGT73AE1</i>	KJ956788	红花	glycyrrhetic acid, UDP-Glc	O/S/N-糖基化	glycyrrhizin	大肠杆菌	[136]
4	<i>UGT73AH1</i>	MF471454	积雪草	asiatic acid, UDP-Glc	C-28糖基化	28-Glc-asiatic acid	大肠杆菌	[137]
5	<i>UGT73AM3</i>	—	黄瓜	cucurbitacin C, UDP-Glc	C-3糖基化	3-O- β -D-Glc- cucurbitacin C	大肠杆菌/ 黄瓜	[138]
6	<i>UGT73C10</i>	JQ291613	欧洲山芥	β -amyrin/oleanolic acid/ betulinic acid/hedera- genin/3-Glc-oleanolic acid, UDP-Glc	C-3/C-28糖基化	3-Glc- β -amyrin/3-Glc-olea- nolic acid/3-Glc-betulinic acid/28-Glc-betulinic acid/3- Glc-hederagenin/3-Glc-28- Glc-oleanolic acid	大肠杆菌	[139]
7	<i>UGT73C11</i>	JQ291614	欧洲山芥	β -amyrin/oleanolic acid/ betulinic acid/hedera- genin, UDP-Glc	C-3/C-28糖基化	3-Glc- β -amyrin/3-Glc-olea- nolic acid/3-Glc-betulinic acid/3-Glc-hederagenin/28- Glc-betulinic acid	大肠杆菌/ 烟草	[139,140]
8	<i>UGT73C12</i>	JQ291615	欧洲山芥	β -amyrin/oleanolic acid/ betulinic acid/hedera- genin/3-Glc-oleanolic acid/3-Glc-hederagenin, UDP-Glc	C-3/C-28糖基化	3-Glc- β -amyrin/3-Glc-olea- nolic acid/3-Glc-betulinic acid/28-Glc-betulinic acid/3- Glc-hederagenin/3-Glc-28- Glc-oleanolic acid/3-Glc-28- Glc-hederagenin/3-Glc-28- Glc-betulinic acid	大肠杆菌	[139]
9	<i>UGT73C13</i>	JQ291616	欧洲山芥	β -amyrin/oleanolic acid/ betulinic acid/hedera- genin/3-Glc-oleanolic acid/3-Glc-hederagenin, UDP-Glc	C-3/C-28糖基化	3-Glc- β -amyrin/3-Glc-olea- nolic acid/3-Glc-betulinic acid/28-Glc-betulinic acid/3- Glc-hederagenin/3-Glc-28- Glc-oleanolic acid/3-Glc-28- Glc-hederagenin/3-Glc-28- Glc-betulinic acid	大肠杆菌/ 烟草	[139]
10	<i>UGT73C22</i>	MF448363	欧洲山芥	oleanolic acid/hedera- genin, UDP-Glc	C-3/C-28糖基化	3-Glc-oleanolic acid/3-Glc- 28-Glc-oleanolic acid/3-Glc- 28-Glc-hederagenin/28-Glc- hederagenin/28-Glc-oleano- lic acid	大肠杆菌/ 烟草	[139]
11	<i>UGT73C23</i>	MF448369	欧洲山芥	oleanolic acid/hedera- genin, UDP-Glc	C-3/C-28糖基化	3-Glc-oleanolic acid/3-Glc- 28-Glc-oleanolic acid/3-Glc- hederagenin/3-Glc-28-Glc- Hederagenin/3-glc-23-glc- hederagenin	大肠杆菌/ 烟草	[140]
12	<i>UGT73C25</i>	MF448366	欧洲山芥	oleanolic acid/hedera- genin, UDP-Glc	C-3/C-23/C-28糖 基化	3-Glc-oleanolic acid/3-Glc- 28-Glc-oleanolic acid/3-Glc- hederagenin/3-glc-23-glc-he- deragenin	大肠杆菌/ 烟草	[140]
13	<i>UGT73C21</i>	MF448360	欧洲山芥	oleanolic acid/hedera- genin, UDP-Glc	C-3糖基化	3-Glc-oleanolic acid/3-Glc- hederagenin	大肠杆菌/ 烟草	[140]
14	<i>UGT73C26</i>	MF448353	欧洲山芥	oleanolic acid/hedera- genin, UDP-Glc	C-3糖基化	3-Glc-oleanolic acid/3-Glc- hederagenin	大肠杆菌/ 烟草	[140]

(续表4)

编号	基因	GenBank 登录号	植物来源	底物	反应	产物	功能分析 方法	参考文献
15	<i>UGT73C27</i>	MF448357	欧洲山芥	oleanolic acid/ hederagenin, UDP-Glc	C-3糖基化	3-Glc-oleanolic acid/3- Glc-hederagenin	大肠杆菌/ 烟草	[140]
16	<i>UGT73F18</i>	MN200615	蒺藜苜蓿	medicagenic acid	C-3糖基化	3-Glc-medicagenic acid	酵母/烟草	[131]
17	<i>UGT73F19</i>	MN200616	蒺藜苜蓿	medicagenic acid/ gypsogenic acid	C-3糖基化	3-Glc-medicagenic acid/ 3-Glc-gypsogenic acid	酵母/烟草	[131]
18	<i>PjOAGT</i>	MH819287	竹节参	oleanolic acid, UDP-GlcA	C-3糖基化	oleanolic acid 3-O- glucuronide	大肠杆菌	[141]
19	<i>PzOAGT1</i>	MH819284	姜状三七	oleanolic acid, UDP-GlcA	C-3糖基化	oleanolic acid 3-O- glucuronide	大肠杆菌	[141]
20	<i>PzOAGT2</i>	MH819285	姜状三七	oleanolic acid, UDP-GlcA	C-3糖基化	oleanolic acid 3-O- glucuronide	大肠杆菌	[141]
21	<i>PzOAGT3</i>	MH819286	姜状三七	oleanolic acid, UDP-GlcA	C-3糖基化	oleanolic acid 3-O- glucuronide	大肠杆菌	[141]
22	<i>GuUGAT</i>	KT759000	甘草	glycyrrhetic acid/3- GlcA-glycyrrhetic acid, UDP-GlcA	C-3糖基化	3-GlcA-glycyrrhetic acid/ glycyrrhizin	大肠杆菌	[142]
23	<i>UGT73F2</i>	AB628089	大豆	saponin A0- α , UDP-Glc	C-22糖基化	saponin Ab	大肠杆菌/ 定点突变分 析/转基因 互补试验	[143]
24	<i>UGT73F3</i>	FJ477891	蒺藜苜蓿	hederagenin/soyasapo- genol A/soyasapogenol B/bayogenin/medica- genic acid, UDP-Glc	C-28/C-3糖基化	hederagenin-C28-O-Glc/ soyasapogenol A-Glc /soya- sapogenol B-Glc/ bayogenin- C28-O-Glc/ medicagenic acid-C28-O-Glc	大肠杆菌/ 蒺藜苜蓿 (基因功能 缺失分析)	[144]
25	<i>UGT73F4</i>	AB628091	大豆	saponin A0- α , UDP-Xyl	C-22糖基化	saponin Aa	大肠杆菌/ 定点突变分 析/转基因 互补试验	[143]
26	<i>UGT73B4</i>	NM_001367432	大豆	soyasapogenol B/soya- saponin I, UDP-Glc/ UDP-Gal	C-22糖基化	22-Glc-soyasapogenol B, 22-Gal-soyasapogenol B, 22-Glc-soyasaponin I, 22- Gal-soyasaponin I	大肠杆菌	[145]
27	<i>UGT73F17</i>	MH049688	甘草	glycyrrhizin, UDP-Glc	C-30/C-29糖基化	licorice-saponin A3	大肠杆菌	[146]
28	<i>UGT73K1</i>	AY747626	蒺藜苜蓿	hederagenin/soyasapo- genol B/soyasapogenol E, UDP-Glc	糖基化	hederagenin-Glc, soyasapogenol B-Glc, soyasapogenol E-Glc	大肠杆菌	[134]
29	<i>UGT74AE2</i>	JX898529	人参	protopanaxadiol/ginse- noside compound K, UDP-Glc	C-3糖基化	ginsenoside Rh2/ginsenoside F2	酵母	[147]
30	<i>UGT74M1</i>	DQ915168	麦蓝菜	gypsogenic acid/betuli- nic acid/hederagenin/ gypsogenin/echinocystic acid/16 α -hydroxygypso- genic acid/quillaic acid, UDP-Glc	C-28糖基化	28-Glc-gypsogenic acid/28- Glc-betulinic acid/28-Glc- hederagenin/28-Glc-gypso- genin/28-Glc-echinocystic acid/28-Glc-16 α -hydroxy- gypsogenic acid/28-Glc- quillaic acid	大肠杆菌	[70]
31	<i>UGT94Q2</i>	JX898530	人参	ginsenoside Rh2/ginse- noside F2, UDP-Glc	C-3糖基化	ginsenoside Rg3/ ginsenoside Rd	酵母/西洋 参/人参 (RNAi)	[147,148]

(续表4)

编号	基因	GenBank 登录号	植物来源	底物	反应	产物	功能分析 方法	参考文献
32	<i>UGT71A27/UGTPg1</i>	KM491309	人参	dammarenediol-II/protopanaxadiol/protopanaxatriol/ginsenoside Rh2/ginsenoside Rg3, UDP-Glc	C-20糖基化	20S-O-β-(D-glucosyl)-dammarenediol II(DMG)/ginsenoside compound K/ginsenoside F1/ginsenoside F2/ginsenoside Rd	酵母	[21]
33	<i>UGTPg100</i>	KP795113	人参	protopanaxatriol, UDP-Glc	C-6糖基化	ginsenoside Rh1	酵母	[149]
34	<i>UGTPg101</i>	KP795114	人参	protopanaxatriol/ginsenoside F1, UDP-Glc	C-20/C6糖基化	ginsenoside F1/ginsenoside Rg1	酵母	[149]
35	<i>UGTPg45</i>	KM401918	人参	protopanaxadiol/ginsenoside compound K, UDP-Glc	C-3糖基化	ginsenoside Rh2/ginsenoside F2	酵母/大肠杆菌	[150]
36	<i>UGTPg29</i>	KM401911	人参	ginsenoside Rh2/ginsenoside F2, UDP-Glc	C-3糖基化	ginsenoside Rg3/ginsenoside Rd	酵母/大肠杆菌	[150]
37	<i>Pq3-O-UGT2 (UGT94)</i>	KR106207	西洋参	ginsenoside Rh2/ginsenoside F2, UDP-Glc	C-3糖基化	ginsenoside Rg3/ginsenoside Rd	酵母/西洋参毛状根(RNAi)	[148]
38	<i>Pq3-O-UGT1 (UGT74)</i>	KiR028477	西洋参	protopanaxadiol, UDP-Glc	C-3糖基化	ginsenoside Rh2	酵母/西洋参毛状根(超表达/RNAi)	[151]
39	<i>UGT73P2/GmSGT2</i>	AB473730	大豆	soyasapogenol B/3-O-glucuronide, UDP-Gal	C-3糖基化	soyasaponin III	大肠杆菌	[152]
40	<i>UGT91H4/GmSGT3</i>	AB473731	大豆	soyasaponin III, UDP-Rha	C-3糖基化	soyasaponin I	大肠杆菌	[152]
41	<i>UGRdGT</i>	—	三七	ginsenoside Rd, UDP-Glc	糖基化	ginsenoside Rb1	体外生物转化	[153]
42	<i>UGT74AC1</i>	HQ259620	罗汉果	mogrol, UDP-Glc	C-3糖基化	mogroside IE	大肠杆菌	[81]
43	<i>UGT720-269-1</i>	—	罗汉果	mogrol/mogroside I-A1, UDP-Glc	C-3/C-24糖基化	mogroside I-A1/mogroside IIE	酵母/大肠杆菌	[6]
44	<i>UGT94-289-3</i>	—	罗汉果	mogroside IIE/mogroside IIIx/mogroside IV-A/siamenoside, UDP-Glc	C-3/C-24糖基化	mogroside IIIx/mogroside IV-A/siamenoside/mogroside V	酵母/大肠杆菌	[6]

a) Glc: glucose, 葡萄糖; GlcA: glucuronic acid, 葡萄糖醛酸; Gal: galactose, 半乳糖; Xyl: xylose, 木糖; Rha: rhamnose, 鼠李糖. 红花, *Carthamus tinctorius*; 竹节参, *Panax japonicus* var. *bipinnatifidus*; 姜状三七, *Panax zingiberensis*

methyl jasmonate, MeJA)的外源处理能影响植物中三萜皂苷的积累。在人参、积雪草、三七、西洋参、藜麦等植物中, MeJA处理能提高植物体内、离体组织或细胞内FPS, SS, SE, OSCs, CYP450s及UGTs等酶基因的表达活性, 从而 影响其皂苷的积累^[2,29,33,42,78,119,129,151,156~158], 但不同酶对MeJA的响应存在差异。三萜皂苷的生物合成除了受相关酶基因调控外, 还受到转录因子和microRNA(miRNA)等在转录或转录后水平上的调控。因此, 下文将对植物三萜皂苷生物合成中已报道的转录因子和miRNA进行总结。

3.1 转录因子

转录因子(transcription factors, TFs)是一类DNA结合蛋白, 可以通过自身或与其他蛋白的相互作用激活或抑制基因转录^[159]。TFs在植物次生代谢产物生物合成过程中可以同时控制多个关键基因的表达。目前, 已报道调控植物三萜生物合成的TFs类型有bHLH(basic helix-loop-helix)、AP2/ERF(APETALA2/ethylene-responsive factor)、bZIP(basic region/leucine zipper motif)、WRKY等四种(图6)。Mertens等人^[160]从莢藜苜

图5 UGTs的系统发育树。基于MEGA-X软件, 采用MUSCLE方法进行UGT氨基酸序列(表4)比对, 然后使用最大似然法进行系统进化树构建, Bootstrap值设置为1000

Figure 5 Phylogenetic tree of the UGTs. UGT amino acid sequences (Table 4) were aligned using MUSCLE with default parameters as implemented in the program MEGA-X. The tree was constructed using the maximum likelihood method with the bootstrap value set to 1000

藜中鉴定出两种同源的碱性螺旋-环-螺旋(helix-loop-helix)家族的MeJA诱导转录因子(bHLH TFs), TSAR1 (triterpene saponin biosynthesis activating regulator 1)和TSAR2。其中, TSAR1可以促进非溶血性大豆皂苷的生物合成, 激活*bAS*, *CYP93E2*, *CYP72A61v2*等相关酶基因的表达, TSAR2可促进溶血性大豆皂苷的合成, 并激活*bAS*, *CYP716A12*和*CYP72A68v2*等相关酶基因的表达。而后, 该研究团队基于蒺藜苜蓿种子发育的转录组数据, 通过基因共表达分析, 鉴定出另一个转录因子TSAR3, 该转录因子可以激活*bAS*, *CYP716A12*, *CYP72A67*, *CYP72A68*, *CYP88A13*和*UGT73F3*等相关酶基因的表达, 促进蒺藜苜蓿中溶血性皂苷的合成^[131]。甘草中的bHLH3可激活*bAS*, *CYP93E3*和

*CYP72A566*的表达, 且系统发育分析表明, bHLH3与TSAR2(53%)的氨基酸序列相似性高于TSAR1(43%)^[161]。Jarvis等人^[162]发现了藜麦中的两个bHLH转录因子TSARL1和TSARL2, 它们可以增加藜麦体内三萜生物合成途径中基因的表达。此外, 研究学者们也在黄瓜^[20]、三七^[163]、人参^[164]、白桦^[165]等植物中发现了调控三萜生物合成的bHLH转录因子。近年来, 研究还发现, bHLH转录因子与植物三萜类化合物生物合成相关基因簇的表达调控有关, 例如, TSAR1和TSAR2参与HMGR1和MKB1(MAKIBISHI1, RING membrane anchor-like E3 ubiquitin ligase)所在基因簇的表达调控, 促进蒺藜苜蓿三萜皂苷生物合成^[160]; 而黄瓜中的两个组织特异性bHLH转录因子Bl(bitter leaf)和Bt(bitter

图 6 已报道的与三萜皂苷生物合成相关的转录因子。bHLH, 碱性螺旋-环-螺旋转录因子; AP2/ERF, APETALA2/乙烯反应因子; bZIP, 碱性亮氨酸拉链转录因子; WRKY, WRKY 转录因子。向上的箭头表示正调控; 向下的箭头表示负调控(网络版彩图)

Figure 6 Transcription factors related to triterpenoid saponin biosynthesis reported in literatures. bHLH: basic helix-loop-helix transcription factor; AP2/ERF: APETALA2/ethylene-responsive factor; bZIP: basic region/leucine zipper motif transcription factor; and WRKY: WRKY transcription factor. The upward arrows indicate positive regulation and the downward arrows indicate negative regulation (color online)

fruit)可以对葫芦素合成相关基因簇进行调控^[20]。除了bHLH家族外,也发现了AP2/ERF, bZIP, WRKY家族的转录因子参与植物三萜皂苷生物合成调控的情况,例如,三七中的AP2/ERF转录因子ERF1,在三七皂苷生物合成中起正调控因子作用^[159];而北柴胡(*Bupleurum chinense*)中的bZIP转录因子bZIP134,起着负调控柴胡皂苷生物合成的作用^[166]。南非醉茄中的WRKY转录因子WRKY1对其体内三萜化合物withanolides的生物合成起着积极的调控作用^[167]。Sun等人^[168]报道,西洋参中的WRKY1可以调控拟南芥中的FPPS, SQS和SQE等三萜合成途径基因,但并未研究其对三萜类代谢产物的影响。现今,关于三萜皂苷生物合成相关转录因子的研究仍较少,加强此方面的研究不仅有助于了解植物三萜皂苷代谢途径的调控机制,还可以将其作为调控工具应用到相关代谢工程,助力植物三萜皂苷基础研究和人工合成的发展。

3.2 miRNA

miRNA是一类内源性非编码小RNA,在植物基因表达调控中发挥着重要作用^[169]。近年来,研究发现miRNA是许多植物中调节次级代谢物产生的主要生物因子^[170]。因此,为了高效获取植物中的三萜皂苷,除了确定其合成和代谢途径的关键酶基因和转录因子,研究miRNA在转录后水平对关键酶基因和相关转录因子的调控作用也是至关重要的。随着高通量测序技术和生物信息学的快速发展,越来越多植物的miRNA被预测出来。Mathiyalagan等人^[171]研究表明,miR854c可能靶向调控高丽参人参皂苷生物合成中的FPS基因,而miR1439b和miR1439h可能靶向调控 β AS基因。Wei等人^[172]预测三七中的miR5021, miR5163, miR5293和novel_miR_27可能靶向调控萜类主链生物合成途径中的基因。Fan等人^[173]结合转录组数据对苍耳(*Xanthium strumarium*)中miRNA及其靶基因进行了鉴定与分析,结果表明,miR5255和miR5491分别靶向调控SE和 β AS,进而对萜类生物合成进行调控。李华云等人^[174]、陈艳琳等人^[175]分别对长白山人参和甘草中miRNA的靶基因进行了预测和相应的生物信息学分析。Kajal和Singh^[176]报道吊兰(*Chlorophytum borivilianum*)中的miR9662, miR894, miR172和miR166可能参与了其体内三萜皂苷生物合成的调控。这些工作为miRNA在三萜皂苷生物合成中的调控机制研究提供

了数据资源,然而,三萜皂苷生物合成相关的miRNA研究仍处于起步阶段,迄今为止报道的相关miRNA仍较少,且多数仅进行了功能预测,这些miRNA作用的靶基因及其调控植物三萜皂苷代谢的分子机制仍有待进一步研究。

4 微生物细胞工厂中三萜皂苷的合成

合成生物学是涉及分子生物学、微生物学、生物工程、计算科学以及材料科学等多个领域的具有巨大潜力的综合性学科,以生命科学理论为指导,旨在通过参考化合物的生物合成途径,人工设计、构建具有某种特定功能或全新的生物系统^[177,178]。目前,合成生物学已被广泛应用于药物生产、疫苗开发、再生医学等方面^[179]。随着合成生物学的发展和植物三萜皂苷生物合成途径的不断解析,利用合成生物学理论和技术生产结构复杂、稀有的生物活性三萜皂苷已取得了一定成果。

通过合成生物学技术在微生物中设计代谢途径和模块组成的生物元件,利用微生物的发酵工程生产次生代谢产物,已成为现今获得稀有次生代谢产物的一种最先进、最快捷的方式。与植物相比,微生物具有生长快、节约土地、大规模发酵等优点。其中,酵母是最常用微生物,尤其在三萜皂苷的合成上拥有独特的优势,这是由于酵母细胞膜上可以积累大量的三萜类化合物麦角固醇或合成途径的中间体。近年来,随着合成生物学的发展,一些三萜皂苷和苷元已在微生物细胞工厂成功合成(表5)。在微生物细胞工厂中从头合成人参皂苷是当前研究的热点,目前,已在酿酒酵母(*Saccharomyces cerevisiae*)中成功合成了三种苷元(原人参二醇, protopanaxadiol, PPD; 原人参三醇, protopanaxatriol, PPT; 齐墩果酸, oleanolic acid, OA)、两种非天然人参皂苷(compound K, CK; 3 β ,12 β -Di-O-Glc-PPD)和四种天然人参皂苷(Rh2, Rg3, RF1和Rh1)。Han等人^[129]将人参达玛烯二醇合成酶(PgDS)、人参CYP716A47和拟南芥CYP450还原酶1(*AtCPR1*)基因导入酿酒酵母中共表达,首次在微生物中合成了人参皂苷苷元PPD。随后,该研究团队又在酵母工程菌中相继合成了PPT和OA^[119,126],但是这三种人参皂苷元在酵母中的产量均较低。为了提高产量,Dai等人^[180]通过提高关键基因的表达活性和进行相关编码基因密码子优化等措施,使PPD产量增加了

表 5 几种重要三萜类化合物的异源合成水平

Table 5 Heterologous synthesis of several important triterpenoids

三萜皂苷或中间体	产量	参考文献
原人参二醇(PPD)	1.2 g/L	[180]
原人参三醇(PPT)	15.9 mg/L	[181]
人参皂苷CK(ginsenoside CK)	1.4 mg/L	[21]
人参皂苷Rg3	1.3 mg/L	[147]
人参皂苷RF1	42.1 mg/L	[149]
人参皂苷Rh1	92.8 mg/L	[149]
人参皂苷Rh2	300 mg/L	[182]
3 β ,12 β -Di-O-Glc-PPD	9.05 mg/L	[183]
甘草次酸(GA)	18.9 mg/L	[184]
齐墩果酸	606.9 mg/L	[185]
山楂酸	384 mg/L	[186,187]
科罗素酸	141 mg/L	[186,187]

262倍;在此基础上,再通过双相发酵工艺优化,最终使PPD产量提高到1.2 g/L. 研究还发现,把OA, PPD和PPT的功能模块导入同一底盘细胞,可同时生产这三种人参皂苷元,这便是第一代基于多组分概念的“人参酵母”细胞工厂^[181]. 另外,相关研究团队还报道了细胞工厂生产三萜10 g/L级别的方案,可以使酵母工程菌生产的达玛烯二醇产量高达15 g/L^[188]. 中国科学院上海植物生理生态研究所与中国科学院上海药物研究所合作,对人参皂苷CK, Rh2, Rg3, RF1和Rh1生物合成途径中的关键糖基转移酶和P450还原酶PgCPR1进行了详细研究,通过酵母细胞工厂的构建和优化,实现了利用单糖发酵生产这些稀有参皂苷的工艺^[21,149,150]. 此外,科研工作者也开展了酿酒酵母生产甘草次酸(glycyrrhetic acid, GA)^[184]、柴胡皂苷元^[100]和罗汉果苷V^[6]等方面的研究. Wang等人^[189]将密码子优化的CYP88D6和CYP72A154,与 β -AS和来自拟南芥的细胞色素P450还原酶(NADPH-cytochrome P450)基因引入酿酒酵母中,成功合成了GA (14 μ g/L);进一步研究发现,甘草细胞色素b5(Gu-CYB5)的引入,可使GA产量提高8倍;采用组合发酵策略,GA产量可提高近630倍,达8.78 mg/L;通过使用不同植物源的细胞色素P450还原酶和增加Uni25647的拷贝数,可使GA产量增加到(18.9 $\pm$ 2.0) mg/L^[184]. 中国科学院天津工业生物技术研究所团队还报道了一个涵盖差异转录组分析、代谢物分析、候选基因在底盘细胞中的表征、化合物鉴定和植物

组织培养的“即插即用”生物合成途径快速解析平台,并通过该平台开发出了生产山楂酸(384 mg/L)和科罗素酸(141 mg/L)的酿酒酵母细胞工厂^[186,187].

虽然三萜皂苷在微生物细胞工厂中的合成已取得了一定的进展,但仍面临着许多挑战,例如,大多数三萜皂苷的生物合成途径尚不清楚,一些关键酶很难在微生物宿主中表达,某些三萜皂苷对微生物细胞具有毒性,工程细胞异源合成效率低,人工智能等自动化技术使用程度较低等. 针对这些问题,研究学者们也陆续提出了许多解决方案,比如使用底物混杂酶^[182],将蛋白质工程^[190]、形态学工程^[191]等技术应用其中,进行代谢流量动态调节和区域化设置^[5,100]等. 随着生物学技术和人工智能技术的发展,三萜皂苷的人工合成将迎来新的时代.

5 展望

三萜皂苷是植物中一类重要的次级代谢产物,在植物和外界环境的相互作用中起着重要作用,且具有多种应用价值,与人类的生活和发展息息相关. 随着现代生物技术的发展,三萜皂苷生物合成途径不断被解析,关键酶基因、转录调控、生物工程方面的研究也取得了一定进展. 相关研究主要集中在三萜皂苷生物合成后修饰阶段关键酶(OSC, CYP450和UGT)基因的发掘,并在微生物(酵母)和/或异源宿主(如模式植物:烟草)中进行功能分析,但其中大多数在植物体内

的功能仍处于未知状态; 而且关键酶的具体催化过程、结构修饰和转录调控机制(转录因子、miRNA等)方面的研究依然较少, 人们对三萜皂苷生物合成途径的认识仍不够系统和全面; 再加上三萜皂苷种类繁多, 在植物中的生物合成途径冗长而复杂, 使得三萜皂苷生物合成途径全面解析、植物体内三萜皂苷代谢调控和采用合成生物学及体外培养等方法来生产三萜皂苷仍存在困难。

随着分子生物学、生物信息学、代谢组学、蛋白组学和合成生物学等学科的发展及其在各个领域的应用和渗透, 以大数据为驱动, 开展多层次的研究, 将加速三萜皂苷的基础研究和相关产业发展。未来, 或可加强以下四方面研究。

(1) 利用多组学和大数据进行三萜皂苷生物合成途径功能基因的发掘和代谢调控网络的研究, 构建从基因到代谢的调控网络, 并将功能基因组学技术(如VIGS, RNAi和CRISPR/Cas9等)应用到非模式植物中, 探讨其生化功能, 明确三萜皂苷合成的调控机制, 进一步解析植物三萜皂苷的生物合成途径。

(2) 进行三萜皂苷在植物体内合成部位及其转运机制研究。研究三萜皂苷合成部位, 并构建相关元件特异性表达的启动子, 使其只在合成部位表达, 实现三萜皂苷的定向合成; 或通过沉默竞争途径关键酶基因的表达, 来增强三萜皂苷的生物合成; 开展三萜皂苷相关转运蛋白及其转运机制的研究。

(3) 开展三萜皂苷合成生物学研究。将基因工程技术和生物工程技术结合, 在微生物中构建三萜皂苷代谢途径, 通过微生物生产大量有价值的三萜皂苷。可以通过提高前体物质的供给、增加相关酶基因的效率, 或抑制竞争代谢途径, 改善培养条件等来提高目标代谢物的生产。同时, 加强人工智能等自动化技术在微生物细胞工厂方面的应用。

(4) 通过体外组织培养来生产三萜皂苷。采用植物组织培养、细胞培养、体细胞胚胎发生和根培养等方法来生产三萜皂苷, 并将细胞工程技术、基因工程等技术应用其中, 获得高效合成三萜皂苷、遗传性质稳定、培养特性优良的基因工程植物细胞系, 以实现三萜皂苷的大规模生产。

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Advances in understanding of the biosynthetic pathway and regulatory mechanism of triterpenoid saponins in plants

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Triterpenoid saponins are a type of triterpenoid glycoside. Widely distributed in plants, triterpenoid saponins play a role in resisting pathogenic microorganisms and herbivores, but they also have a variety of pharmacological activities that have been widely adopted in medicine, common chemicals, food, and agriculture, to name a few. In recent years, with the development of science and technology and the deepening of research, the basic framework and regulatory mechanisms of triterpenoid saponin biosynthesis in plants have become better understood. Plant triterpenoid saponins have multiple types of complex structures and, although they share a common precursor synthesis pathway, their post-modification stages are highly specific and diverse. In this paper, the biosynthetic pathway, key enzyme genes, transcriptional regulation, and microbial cell engineering of triterpenoid saponins were reviewed, and the future development direction of triterpenoid saponins, which could provide theoretical reference for related research in the abovementioned fields, was prospected.

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