

质体3-磷酸甘油醛脱氢酶基因 *GAPCp1* 过表达 对草莓果实成熟与代谢产物的影响

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摘要:【目的】分析探讨质体3-磷酸甘油醛脱氢酶基因(plastid glyceraldehyde-3-phosphate dehydrogenase, *GAPCp1*)对草莓果实成熟及其代谢产物的影响。【方法】以‘红颜’草莓果实为材料, 通过分子克隆获得 $FaGAPCp1$ 基因全长序列, 随后进行过表达载体构建、瞬时侵染草莓果实, 并对其进行代谢组学分析。【结果】与对照组相比, 过表达 $FaGAPCp1$ 抑制草莓果实成熟的同时显著下调了部分氨基酸及其衍生物、花青素、有机酸及其衍生物、黄酮类物质的含量, 同时显著上调了原花青素与维生素中核黄素的含量。【结论】*GAPCp1*通过影响草莓果实的代谢进程抑制草莓果实的成熟。

关键词:草莓; *FaGAPCp1*; 成熟; 代谢产物; 过表达

中图分类号:S668.4 文献标志码:A 文章编号:1009-9980(2020)10-1487-12

Effect of overexpression of plastid glyceraldehyde-3-phosphate dehydrogenase1 on fruit ripening and metabolites in strawberry

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Abstract:【Objective】Plastid glyceraldehyde-3-phosphate dehydrogenase (GAPCp) is a key enzyme in glycolysis. Besides its catalytic function, GAPCp participates in the regulation of plant stress response, growth and development. However, its role in strawberry fruit ripening has been rarely mentioned. Through overexpression of *FaGAPCp1* gene in the fruits, combined with metabonomics, the effect of *FaGAPCp1* gene on fruit ripening and metabonolites of strawberry was studied. 【Methods】The strawberry (*Fragaria × ananassa* ‘Benihoppe’) plants were grown in greenhouse in Chengdu, China. Fruits at de-greening (DG, 18 d after anthesis) stage were chosen for RNA extraction and *FaGAPCp1* transient gene expression. Total RNA was extracted using a modified CTAB protocol. Approximately 1 μg of total RNA was reverse-transcribed to cDNA cloning using a SMART™ RACE cDNA Synthesis Kit (TaKaRa), and primers were designed for *FaGAPCp1* cloning. For transient overexpression, the cDNA fragments of *FaGAPCp1* were inserted into the vector *Eco*R I -*Xba* I -cut pCAMBIA1301, and the recombinant plasmid was transformed into *Agrobacterium tumefaciens* strain GV3101 by the freeze-thaw method. A 5 mL culture of a single *Agrobacterium* colony was inoculated on LB medium (containing 20 μg · mL⁻¹ Rif, 40 μg · mL⁻¹ Gen and 50 μg · mL⁻¹ Kan) and cultured overnight at 28 °C. The product was then transferred to 50 mL LB medium (containing 20 μg · mL⁻¹ Rif, 40 μg · mL⁻¹ Gen and 50 μg · mL⁻¹ Kan) and cultured at 28 °C. After the culture medium was turbid, the cells were collected by centrifugation (5 000 × g, 5 min, 20 °C), and then resuspended in infiltration buffer (containing 10 mmol · L⁻¹ MgCl₂, 10 mmol · L⁻¹ MES, 200 mmol · L⁻¹ acetosyringone), the OD₆₀₀ of cells reached 1.0-2.0. The sterilized 1 mL syringe was used to inject bacterial liquid into the fruits of DG stage. Ten similar-

收稿日期:2020-04-15 接受日期:2020-06-30

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sized fruits were used for the infiltration experiment. The fruits injected with empty vector were used as control. Each fruit was a biological repeat. The fruits were collected on the 3rd day after transformation, and the phenotype was photographed. Then, the fruits were quickly frozen with liquid nitrogen. The expression of *FaGAPCp1* gene was detected by real-time PCR using synthesized cDNA as template and *FaActin* as the reference gene. Next, metabolite profiling was performed using a widely targeted metabolome method by Wuhan Metware Biotechnology Co., Ltd. (Wuhan, China). The freeze-dried *FaGAPCp1* overexpressing fruits were crushed using a mixer mill (MM 400, Retsch) with a zirconia bead for 1.5 min at 30 Hz. 100 mg powder was weighted and extracted overnight at 4 °C with 1.0 mL 70% aqueous methanol. After centrifugation at 10 000×g for 10 min, the extracts were absorbed and filtered and then were analyzed using an LC-electrospray ionization (ESI)-MS/MS system. The metabolites were quantified using the multiple reactions monitoring (MRM) method. The results were obtained from Wuhan Metware Biotechnology Co., Ltd. (Wuhan, China) and the metabolites related to fruit ripening were visualized as heat maps by Tbtools (1.6.1) software. 【Results】 The qRT-PCR results showed that the *FaGAPCp1* had higher expression in the *FaGAPCp1* overexpression fruits compared with that of the control. In addition, overexpressed *FaGAPCp1* delayed the coloring of strawberry fruits on the 3 d after *Agrobacterium* injection. Furthermore, metabonomics analysis showed that 108 differential metabolites were detected in the *FaGAPCp1* overexpressed fruits compared with those of the control. 50 up-regulated differential metabolites mainly belonged to nucleotide and their derivates, proanthocyanidins, vitamins and hydroxycinnamoyl derivatives, and 58 down-regulated differential metabolites contained amino acids and their derivatives, anthocyanins, flavonoids, organic acids and their derivatives. Among the 12 detected amino acids and their derivatives, except for the relative contents of L-(+)-Lysine and D-Alanyl-D-Alanine, the relative contents of S-(5'-Adenosyl)-L-methionine and other amino acids were significantly decreased in the *FaGAPCp1*-overexpression fruits. The relative contents of 9 anthocyanins in the *FaGAPCp1*-overexpression fruits were significantly decreased, including the main anthocyanins of strawberry fruits, that is, cyanidin 3-*O*-glucoside, pelargonidin 3-*O*-beta-*D*-glucoside and pelargonidin 3-*O*-malonylhexside. The relative content of procyanidin A3, which was closely related to anthocyanin, was significantly increased in the *FaGAPCp1*-overexpression fruits. Meanwhile, 9 organic acids and their derivatives were detected, except for trans-Muconic acid, the relative contents of 7 organic acids such as 2-isopropylmalate, 3-hydroxybutyrate, 2-hydroxyisocaproic acid and rosmarinic acid were significantly decreased compared with those of the control. Among the vitamins, the relative contents of riboflavin and nicotinamide-N-oxide were up-regulated, while the relative content of methyl nicotinate was down-regulated. Except for p-coumaraldehyde and cinnamic acid, the relative contents of 5 hydroxycinnamoyl derivatives were significantly up-regulated in the *FaGAPCp1*-overexpression fruits. Furthermore, 23 flavonoids were detected, including 7 flavonoids, 5 flavonols, 4 flavone C-glycosides, 4 flavanones and 3 isoflavones. Flavonols accounted for a large proportion of the detected flavonoids, among them the relative contents of methylquercetin *O*-hexoside, isorhamnetin *O*-hexoside and isorhamnetin 5-*O*-hexoside were significantly increased compared with those of the control. The relative contents of flavone, flavone C-glycosides, flavanone and isoflavone were significantly decreased in the *FaGAPCp1*-overexpression fruits. 【Conclusion】 The *GAPCp1* was a negative regulator for fruit ripening in strawberry, which is regulated by affecting the metabolic process of fruit ripening.

Key words: Strawberry; *FaGAPCp1*; Ripening; Metabonomics; Overexpression

甘油醛-3-磷酸脱氢酶(glyceraldehyde-3-phosphate dehydrogenase,GAPDH)是所有原核和真核生物糖酵解代谢途径中的关键酶。根据其细胞学定位的不同可分为GAPC、GAPCp和GAPA/B三类同工型。在很长一段时间内,GAPDH都被认为是一个组成型基因,在基因表达分析中作为内参基因^[1]。然而,随着研究的深入却发现细胞质GAPDH(cytosolic glyceraldehydes-3-phosphate dehydrogenase,GAPC)有许多新的功能,能积极响应各种生物与非生物胁迫,如盐、干旱、热、冷、厌氧、辐射以及病原菌侵染等^[2-7]。质体GAPDH(plastid glyceraldehydes-3-phosphate dehydrogenase,GAPCp)也被相继报道参与植物初生根和小孢子发育^[8],且拟南芥GAPCp双突变体(*gapcp1gapcp2*)会抑制植株地上部分的生长^[9]。由此说明,在糖酵解中GAPDH是一个多功能蛋白,与植物发育以及环境适应密切相关^[10]。

果实发育和成熟调控机制研究一直是果树学科的一个重点和热点问题。草莓不仅是重要的果树树种,而且是研究非跃变型果实发育和成熟分子基础的典型材料之一。近年研究发现,脱落酸(abscisic acid,ABA)和蔗糖在调控草莓果实发育和成熟进程中具有重要作用^[11-12]。田间喷施ABA和蔗糖能有效缩短草莓果实发育和成熟时间4~12 d^[13],因此,外源喷施ABA和蔗糖是一种有效缩短草莓果实生长期,提高其冬季产量的潜在方法。此外,课题组前期研究发现,ABA和蔗糖促进草莓果实成熟的同时还抑制FaGAPCp1的表达^[13],推测FaGAPCp1可能参与草莓果实成熟过程,但其在果实成熟中的作用尚不明确。鉴于此,笔者拟通过基因瞬时表达技术以及代谢组学的方法探讨FaGAPCp1对草莓果实成熟的影响,以期探索质体GAPDH的新功能,为草莓果实成熟的调控提供更丰富的理论基础。

1 材料和方法

1.1 材料

试验材料为纵径3~3.5 cm、大小相对一致并处于浅绿期(约花后18 d)的‘红颜’草莓果实(*Fragaria × ananassa* ‘Bebihoppe’)。2018年11月中旬取自四川省崇州市草莓种植基地大棚,用于农杆菌瞬时转化。

PrimeSTAR Max DNA Polymerase、SYBR染料、DL 2000 DNA marker、DL 15000 DNA marker、Prime-

Script II第一链cDNA合成试剂盒购于大连宝生物公司。pEASY-Blunt Cloning Kit DNA连接试剂盒购于全式金公司。胶回收试剂盒购于欧米茄公司。2×Taq PCRMaster MIX、Marker III购于天根生化科技(北京)有限公司。*Eco*R I、*Xba* I核酸内切酶和T₄DNA连接酶购于NEB公司。卡那霉素(Kan)、利福平(Rif)、庆大霉素(Gen)、乙酰丁香酮(AS)购于Sigma生物公司。

pEASY-Blunt Cloning Simple Vector、Trans1-T₁ Phage Resistant感受态细胞购于北京全式金生物技术公司;过表达载体(pCAMBIA1301-35S-Nos)和农杆菌GV3101为实验室保存。

1.2 方法

1.2.1 载体构建 参照Chen等^[14]的方法提取草莓总RNA,并通过RT-PCR技术扩增获得‘红颜’草莓FaGAPCp1序列,基因序列全长为1 318 bp。RT-PCR使用的克隆引物为FaGAPCp1-F(5'-ATTGT-GAGGTGCCGTTGT-3')和FaGAPCp1-R(5'-AG-ATCTGATCCTTGCGTAC-3')。将克隆序列送去上海生工生物技术有限公司测定。测序成功后,在引物的两端加上特异性酶切位点及保护碱基,合成含酶切位点的引物:O-FaGAPCp1-F(5'-CCG-GAATTCCCATGGCCAAGATCAAGATTGGC-3')和O-FaGAPCp1-R(5'-GCTCTAGACCT-CAGAGCCTCAATCAATTAACTGTGG-3'),对测序成功的FaGAPCp1片段进行扩增。随后使用*Eco*R I和*Xba* I限制性内切酶对序列和过表达载体进行双酶切并使用DNA连接试剂盒连接目的基因和过表达载体。将连接产物全部转入大肠杆菌感受态。倒置过夜培养后,挑取单菌落扩大培养并进行菌液PCR检测。对检测成功的阳性克隆菌液进行质粒提取、鉴定和测序。

1.2.2 农杆菌瞬时转化草莓 参照Chen等^[14]的方法对草莓果实进行预处理。运用冻融法分别将测序成功的重组过表达载体35S::FaGAPCp1和过表达空载pCAMBIA1301-35S-Nos转入农杆菌GV3101中并于28℃下避光培养2 d。挑取单菌落于含20 μg·mL⁻¹ Rif、40 μg·mL⁻¹ Gen和50 μg·mL⁻¹ Kan的LB液体培养基中于28℃下进行扩大培养,随后进行菌液PCR检测。将检测无误的35S::FaGAPCp1和pCAMBIA1301-35S-Nos农杆菌分别加入50 mL LB液体培养基(含20 mg·L⁻¹ Rif、50 mg·L⁻¹ Kan、40

$\text{mg} \cdot \text{L}^{-1}$ Gen)中 28°C $200 \text{ r} \cdot \text{min}^{-1}$ 培养至 OD_{600} 为 $0.8\sim1.0$; 室温 $4\,000\times g$ 离心 15 min , 弃上清液; 用浸染液 ($0.5 \text{ mol} \cdot \text{L}^{-1}$ MES pH 5.6, $1 \text{ mol} \cdot \text{L}^{-1}$ MgCl_2) 洗菌, 室温 $4\,000\times g$ 离心 15 min , 弃上清液; 各加入 50 mL 浸染液 ($0.5 \text{ mol} \cdot \text{L}^{-1}$ MES pH 5.6, $1 \text{ mol} \cdot \text{L}^{-1}$ MgCl_2 , $200 \mu\text{mol} \cdot \text{L}^{-1}$ As), 25°C $50 \text{ r} \cdot \text{min}^{-1}$ 黑暗条件下培养 $3\sim4 \text{ h}$; 室温 $4\,000 \text{ g}$ 培养离心 15 min , 加入浸染液 ($0.5 \text{ mol} \cdot \text{L}^{-1}$ MES pH 5.6, $1 \text{ mol} \cdot \text{L}^{-1}$ MgCl_2 , $200 \mu\text{mol} \cdot \text{L}^{-1}$ As), 调节 $35S::FaGAPCp1$ 和 pCAMBIA1301-35S-Nos 的 OD_{600} 值至 $0.8\sim1.0$ 。 $35S::FaGAPCp1$ 和 pCAMBIA1301-35S-Nos 侵染液便可直接使用, 用 1 mL 无菌注射器吸取 $300\sim500 \mu\text{L}$ 侵染液, 从浅绿期的离体草莓果蒂处缓慢注射, 对照组与过表达组各注射 10 个果实。每个果实为 1 个生物学重复。注射完后擦干果实表面的侵染液并放于培养瓶中; 将培养瓶置于人工气候箱, 黑暗条件培养 12 h 后, 转换为白天 $23^\circ\text{C}/16 \text{ h}$ 、夜间 $18^\circ\text{C}/8 \text{ h}$ 、相对湿度 $70\%\sim90\%$ 的条件下培养。期间拍照记录果实表型变化, 果实于第 5 天采样, 拍照液氮冷冻后, 于 -80°C 保存备用。

1.2.3 草莓果实过表达 $FaGAPCp1$ 基因表达效率的检测 用改良 CTAB 方法^[15] 分别提取转化 5 d 后对照组和过表达组各 10 个草莓果实的总 RNA, 并反转录成 cDNA。以合成的 cDNA 为模板, 采用实时荧光定量 PCR 检测 $FaGAPCp1$ 基因表达量变化。荧光定量 PCR 引物为 q- $FaGAPCp1$ -F ($5' \text{- GGATA-CACCGATGAAGAT-3'}$) 和 q- $FaGAPCp1$ -R ($5' \text{- GT-GGAACTAAGTGCTAAC-3'}$)。

1.2.4 代谢产物分析测定项目及方法 在检测草莓果实 $FaGAPCp1$ 基因过表达效率后, 分别将农杆菌注射 5 d 后的对照与过表达 $FaGAPCp1$ 草莓果实随机分成含有 3 、 3 和 4 个草莓果实的 3 组重复。使用

带有氧化锆珠的混合机 (mm400, retsch) 在 30 Hz 下将冷冻干燥的草莓果实样品粉碎 1.5 min 。在 4°C 下, 用 1.0 mL 的 70% 甲醇水溶液提取 100 mg 粉末。 $10\,000\times g$ 离心 10 min 后, 在 LC-MS 分析之前, 将提取物用 $250 \text{ mg} \cdot 3 \text{ mL}^{-1}$ 石墨化碳黑 SPE 小柱吸收并过滤。

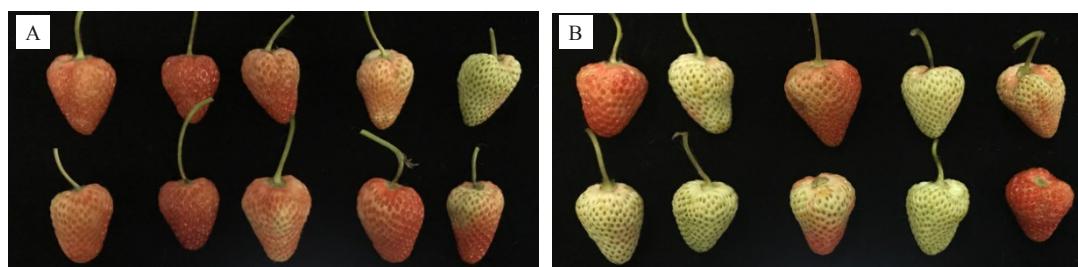
代谢物分析是由武汉美特华生物技术有限公司 (中国武汉) 采用广泛针对性的代谢组分法进行的。使用 LC-ESI-MS/MS 系统的方法提取冻干样品。代谢产物的定量是使用多重反应监测 (MRM) 方法进行的。代谢组数据返回后, 对不同质控样本 (Quality Control, QC) 质谱检测分析的总离子流图 (Total Ion Current Chromatograms, TIC) 进行重叠展示分析, 以判断代谢物提取和检测的重复性^[16]。分析多反应监测模式 (Multiple Reaction Monitoring, MRM) 代谢物检测多峰图, 并对其进行矫正, 以确保定性定量结果的准确性和可靠性^[17]。

1.2.5 代谢产物数据可视化 根据实验目的与武汉美特华生物技术有限公司 (中国武汉) 返回的非靶向代谢组学结果, 对差异代谢物进行筛选、分析。将各类差异代谢物的含量值导入 TBtools (1.6.1) 软件制作热图。

2 结果与分析

2.1 过表达 $FaGAPCp1$ 的果实表型及表达量变化

农杆菌注射 5 d 后对草莓果实表型进行观察发现, 对照组和过表达组的草莓果实色泽呈现显著差异, 且对照组果实上色数量与面积远高于 $FaGAPCp1$ 过表达组 (图 1)。随后分别提取对照组和过表达组果实的 RNA, 并选取 $FaActin$ 作为内参基因进行 qRT-PCR。结果 (图 2) 表明, 过表达组的 $FaGAP-$

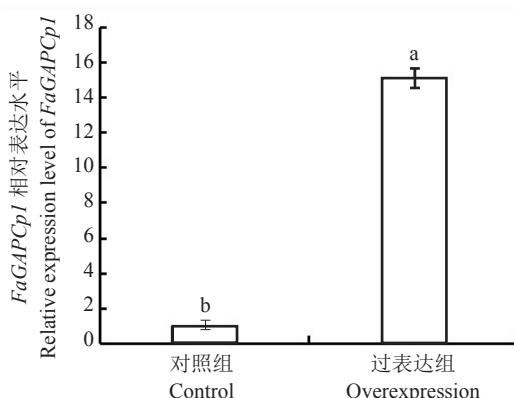


A. 对照组; B. $FaGAPCp1$ 过表达组。

A. Control; B. $FaGAPCp1$ overexpression.

图 1 农杆菌注射 5 d 后草莓果实表型

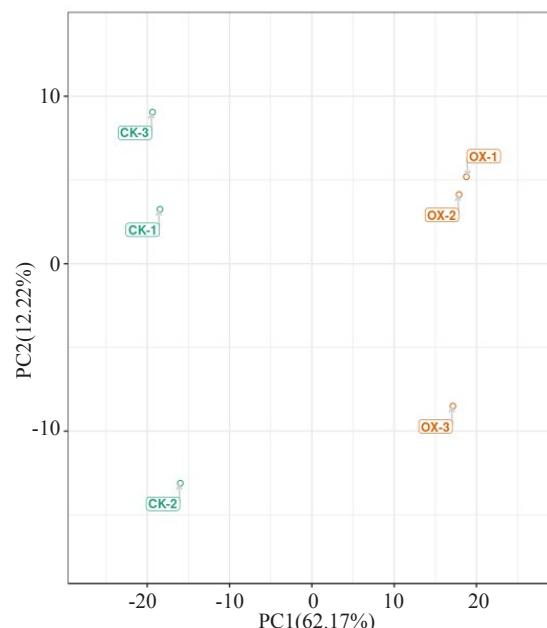
Fig. 1 Phenotype of strawberry fruit on the 5 d after Agrobacterium injection

图2 *FaGAPCp1*的相对表达水平Fig. 2 Relative expression level of *FaGAPCp1*

Cp1 转录水平较对照组提高了 15.13 倍,说明 *FaGAPCp1* 在草莓果实中已成功被过表达。

2.2 统计分析

基于 HPLC-HRMS 数据对过表达草莓果实和对照组草莓果实进行主成分分析(Principal Component Analysis, PCA),发现两组草莓呈现显著差异(图3)。两主成分分数体现了 74.39% 的总变异,对照图3 中两组草莓果实的相对位置发现,两者在



X 轴表示第一主成分, Y 轴表示第二主成分。OX 表示 *FaGAPCp1* 过表达组样本(OX-1、OX-2、OX-3),CK 表示对照组样本(CK-1、CK-2、CK-3)。下同。

The X axis represents the first principal component, and the Y axis represents the second principal component. OX (OX-1, OX-2, OX-3) and CK (CK-1, CK-2, CK-3) indicate samples of *FaGAPCp1*-overexpression group and control group respectively. The same below.

图3 各组样品与质控品质谱数据的 PCA 得分图

Fig. 3 PCA score of mass spectrum data of control and overexpression samples

PC1 轴上相距甚远,两组样本代谢组分离趋势明显,且 PC1 对该差异的贡献率为 62.17%,说明两组草莓果实的代谢化合物存在显著差异,各组内的差异也在可接受范围内,后续的分析具有可行性。

为了更加清楚、直观地说明对照组与过表达组草莓果实代谢物的差异,将代谢产物数据归一化后进行聚类热图制作。由图4可知,对照组的 3 个样本(CK-1、CK-2、CK-3)代谢模式相似,过表达组的 3 个样本(OX-1、OX-2、OX-3)代谢模式相似,说明对照组和过表达组各自的样本重复性较好。两组样本的代谢物含量总体呈现相反的变化趋势,例如在过表达组中含量上调(红色)的代谢物在对照组表现为含量下调(绿色),即过表达组与对照组的代谢产物具有显著差异。

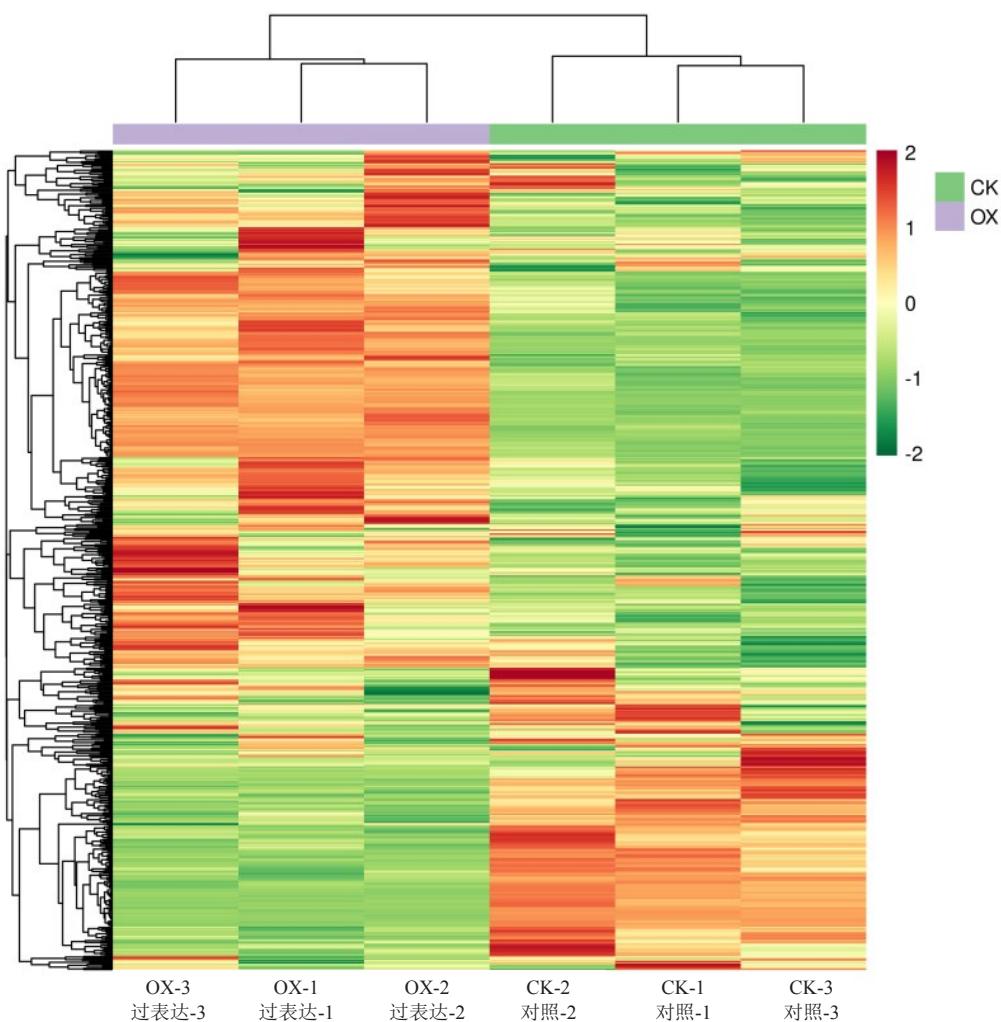
2.3 差异代谢物筛选分析

火山图(Volcano Plot)通常用来展示具有差异的代谢物。火山图中的每个点表示一种代谢物,横坐标表示某代谢物在两样品中定量差异倍数的对数值;纵坐标表示重要性投影(Variable Importance in Project, VIP)。横坐标绝对值越大,说明物质含量在两样品间的含量倍数差异越大;纵坐标值越大,表明含量差异越显著,筛选得到的差异代谢物越可靠。由图5可知,与对照组相比,在过表达 *FaGAPCp1* 组中含量下调的差异代谢物(绿色)比含量上调(红色)的差异代谢物数量更多且其差异更为显著。

2.4 差异代谢物分析

通过代谢组分析,从对照与过表达 *FaGAPCp1* 草莓果实中共筛选出 108 种具有显著性差异的代谢物(表1),其中有 50 种代谢物显著上调,主要为核苷酸及其衍生物、原花青素、维生素类和羟基肉桂酰衍生物等;有 58 种代谢物显著性下调,主要为氨基酸及其衍生物、花青素、黄酮类物质和有机酸及其衍生物。为具体探究与草莓果实成熟相关代谢物的含量变化,通过对数据归一化处理后,将各类代谢物分别制作热图(图6),为后续分析提供清晰与直观的图像。

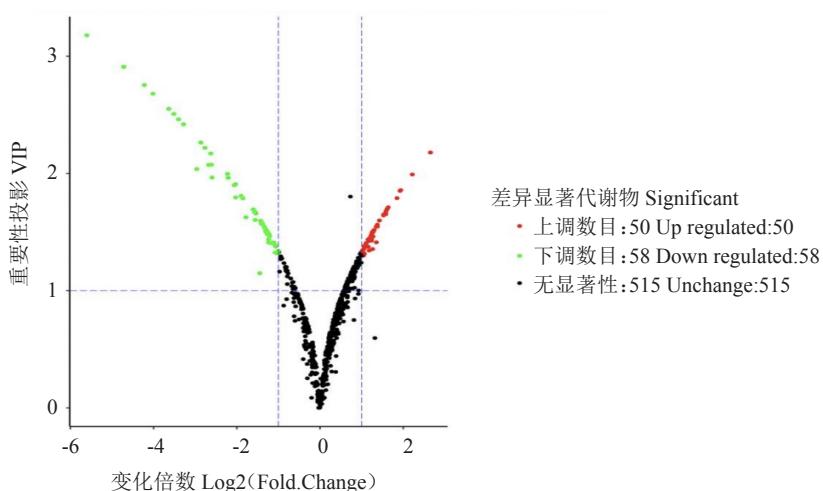
氨基酸是草莓果实成熟的初生代谢物,在本实验中,有 12 种氨基酸及其衍生物被检测到。除 L-(+)-赖氨酸和 D-丙氨酸-D-丙氨酸含量较对照分别显著上调 2.1 和 2.36 倍外,其余氨基酸,如 S-腺苷蛋氨酸、L-犬尿氨酸、L-色氨酸、L-酵母氨酸、5-羟基色氨酸、L-(-)-酪氨酸等含量均较对照显著下调,其中



红色表示高含量,绿色表示低含量。颜色从红到绿,表示 $\log_{10}(FPKM+1)$ 值从大到小。

Red color indicates high relative content and green color indicates low relative content. Color from red to green indicates the value of $\log_{10}(FPKM + 1)$ is from high to low.

图 4 样品总体聚类分析
Fig. 4 The heat map of all metabolite



绿色的点代表含量下调的差异代谢物,红色的点代表含量上调的差异代谢物,黑色的点代表检测到但差异不显著的代谢物。

The green dot and the red dot in the volcano plot represent the differential metabolite which content is declined or increased compared with the control, respectively, and the black dot represents the metabolite which content is not significant difference compared with the control .

图 5 差异代谢火山图
Fig. 5 Differential metabolism volcano plot

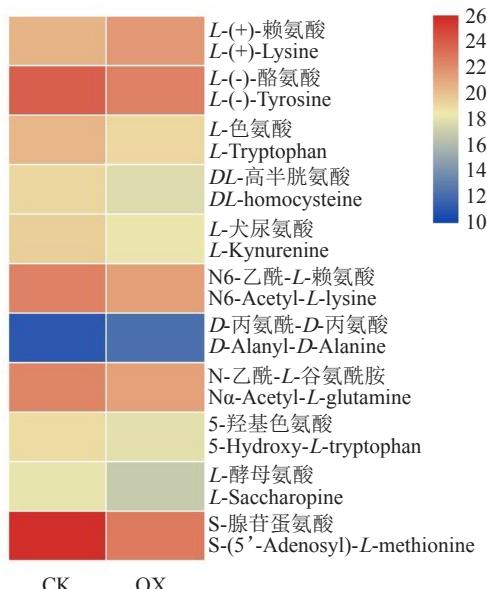
表1 显著性差异的代谢物
Table 1 Metabolites of significant difference

类别 Category	物质名称 Compound name	含量变化 Content change	变化倍数 Fold change
氨基酸及氨基酸衍生物 Amino acids and their derivatives	L-犬尿氨酸 L-Kynurenine	下调 Down	0.43
	L-(+)-赖氨酸 L-(+)-Lysine	上调 Up	2.10
	L(-)-酪氨酸 L(-)-Tyrosine	下调 Down	0.41
	L-色氨酸 L-Tryptophan	下调 Down	0.40
	DL-高半胱氨酸 DL-homocysteine	下调 Down	0.38
	左旋肌肽 L-Carnosine	下调 Down	0.37
	N6-乙酰-L-赖氨酸 N6-Acetyl-L-lysine	下调 Down	0.43
	D-丙氨酸-D-丙氨酸 D-Alanyl-D-Alanine	上调 Up	2.36
	N-乙酰-L-谷氨酰胺 N-Acetyl-L-glutamine	下调 Down	0.47
	5-羟基色氨酸 5-Hydroxy-L-tryptophan	下调 Down	0.49
维生素类 Vitamins	L-酵母氨酸 L-Saccharopine	下调 Down	0.43
	S-腺苷蛋氨酸 S-(5'-Adenosyl)-L-methionine	下调 Down	0.13
	烟酸甲酯 Nicotinic acid methyl ester	下调 Down	0.14
	核黄素 Riboflavin	上调 Up	3.06
	N-氧化烟酰胺 Nicotinamide-N-oxide	上调 Up	2.56
花青素及原花青素 Anthocyanins and procyanins	矢车菊属 3-O-丙二酰己糖苷 Cyanidin 3-O-malonylhexoside	下调 Down	0.16
	天竺葵色素 3-O-丙二酰己糖苷 Pelargonidin 3-O-malonylhexoside	下调 Down	0.05
	原花青素 A3 Procyanidin A3	上调 Up	4.66
	矢车菊素 O-丁香酸 Cyanidin O-syringic acid	下调 Down	0.09
	矢车菊素 O-乙酰基己糖苷 Cyanidin O-acetylhexoside	下调 Down	0.08
	天竺葵素 O-乙酰基己糖苷 Pelargonidin O-acetylhexoside	下调 Down	0.04
	矢车菊素 3-O-葡萄糖苷 Cyanidin 3-O-glucoside (Kuromanin)	下调 Down	0.10
	矢车菊素 3-O-芸香糖苷 Cyanidin 3-O-rutinoside (Keracyanin)	下调 Down	0.06
	花青素苷 Cyanidin 3,5-O-diglucoside (Cyanin)	下调 Down	0.34
	天竺葵素 3-O-葡萄糖苷 Pelargonidin 3-O-beta-D-glucoside	下调 Down	0.09
黄酮 Flavone	羟甲基黄酮 5-O-己糖苷 Selgin 5-O-hexoside	下调 Down	0.27
	五羟黄酮 O-丙二酰己糖苷 Tricetin O-malonylhexoside	下调 Down	0.22
	白杨素 O-丙二酰己糖苷 Chrysanthemum O-malonylhexoside	下调 Down	0.33
	金圣草黄素 O-己糖基-O-戊糖苷 Chrysoeriol O-hexosyl-O-pentoside	上调 Up	2.11
	木犀草素 O-己糖基-O-葡萄糖酸 Luteolin O-hexosyl-O-gluconic acid	下调 Down	0.21
黄酮醇 Flavonol	木犀草素 O-琥珀酸-O-己糖苷 Luteolin O-eudesmic acid-O-hexoside	上调 Up	2.04
	橘皮素 Tangeretin	上调 Up	2.09
	甲基槲皮素 O-己糖苷 MethylQuercetin O-hexoside	上调 Up	2.38
	异鼠李素 O-己糖苷 Isorhamnetin O-hexoside	上调 Up	2.01
	异鼠李素 5-O-己糖苷 Isorhamnetin 5-O-hexoside	上调 Up	2.46
黄酮碳糖苷 Flavone C-glycosides	槲皮素 O-乙酰基己糖苷 Quercetin O-acetylhexoside	下调 Down	0.33
	二氢杨梅素 Dihydromyricetin	下调 Down	0.44
	橙皮素 C-己糖基-O-己糖基-O-己糖苷	下调 Down	0.02
	Hesperetin C-hexosyl-O-hexosyl-O-hexoside		
	圣草酚 C-己糖基-O-己糖苷 Eriodictiol C-hexosyl-O-hexoside	下调 Down	0.15
黄烷酮 Flavanone	8-C-己糖苷-木犀草素 O-己糖苷 8-C-hexosyl-luteolin O-hexoside	上调 Up	2.25
	圣草酚 C-己糖 Eriodictyol C-hexoside	下调 Down	0.38
	柚皮素 O-丙二酰己糖苷 Naringenin O-malonylhexoside	下调 Down	0.38
	橙皮素 O-丙二酰基己糖苷 Hesperetin O-malonylhexoside	上调 Up	2.40
	根皮素 Phloretin	下调 Down	0.37
	阿福豆素 Afzelechin (3,5,7,4'-Tetrahydroxyflavan)	上调 Up	2.42

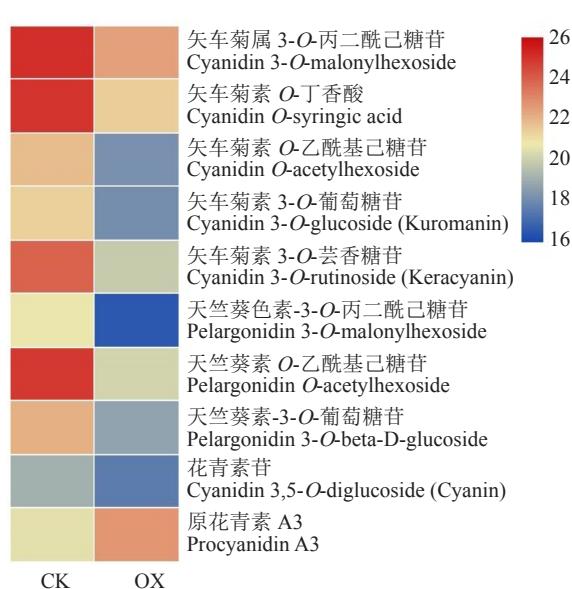
表1(续) Table 1(continued)

类别 Category	物质名称 Compound name	含量变化 Content change	变化倍数 Fold change
异黄酮 Isoflavone	大豆苷 Daidzein 7-O-glucoside (Daidzin)	下调 Down	0.14
	黄豆黄素 Glycitein	上调 Up	2.23
	鹰嘴豆素 7-O-葡萄糖苷(印度黄檀苷) Sissotrin	下调 Down	0.28
有机酸及其衍生物 Organic acids and their derivatives	2-异丙基苹果酸 2-Isopropylmalate	下调 Down	0.34
	迷迭香酸 Rosmarinic acid	下调 Down	0.42
	3-羟基丁酸 3-Hydroxybutyrate	下调 Down	0.34
	2-羟基-4-甲基戊酸 2-Hydroxyisocaproic acid	下调 Down	0.24
	对-羟基苯乙酸 p-Hydroxyphenyl acetic acid	下调 Down	0.43
	反式乌头酸 <i>Trans-citridic</i> acid	下调 Down	0.24
	2-(甲酰氨基)苯甲酸 2-(formylamino) benzoic acid	下调 Down	0.50
	反,反-黏康酸 <i>Trans, trans</i> -Muconic acid	上调 Up	2.15
	5-羟基己酸 5-hydroxyhexanoic acid	下调 Down	0.41
	咖啡醛 Caffeic aldehyde	上调 Up	2.60
羟基肉桂酰衍生物 Hydroxycinnamyl derivatives	芥子酸吡喃葡萄糖苷 1-O-beta-D-Glucopyranosyl sinapate	上调 Up	3.80
	肉桂酸 Cinnamic acid	下调 Down	0.38
	氢化肉桂酸 Hydrocinnamic acid	上调 Up	2.88
	芥子醇 Sinapyl alcohol	上调 Up	6.29
	芥子醛 Sinapinaldehyde	上调 Up	2.24
	香豆醛 p-Coumaraldehyde	下调 Down	0.24

A

氨基酸及其衍生物
Amino acids and their derivatives

B

花青素及原花青素
Anthocyanins and procyanins

红色表示高含量,蓝色表示低含量。颜色从红到蓝,表示 $\log_{10}(FPKM+1)$ 值从大到小。图中各代谢物对应中文名称见表 1。

Red color indicates high relative content and blue color indicates low relative content. Color from red to blue indicates the value of $\log_{10}(FPKM + 1)$ is from high to low. Check Table 1 for the Chinese names of each metabolite.

图 6 *FaGAPCp1* 过表达草莓果实与成熟相关代谢物的聚类热图

Fig. 6 The cluster heat map of ripening related metabolites in *FaGAPCp1* overexpression strawberry fruit

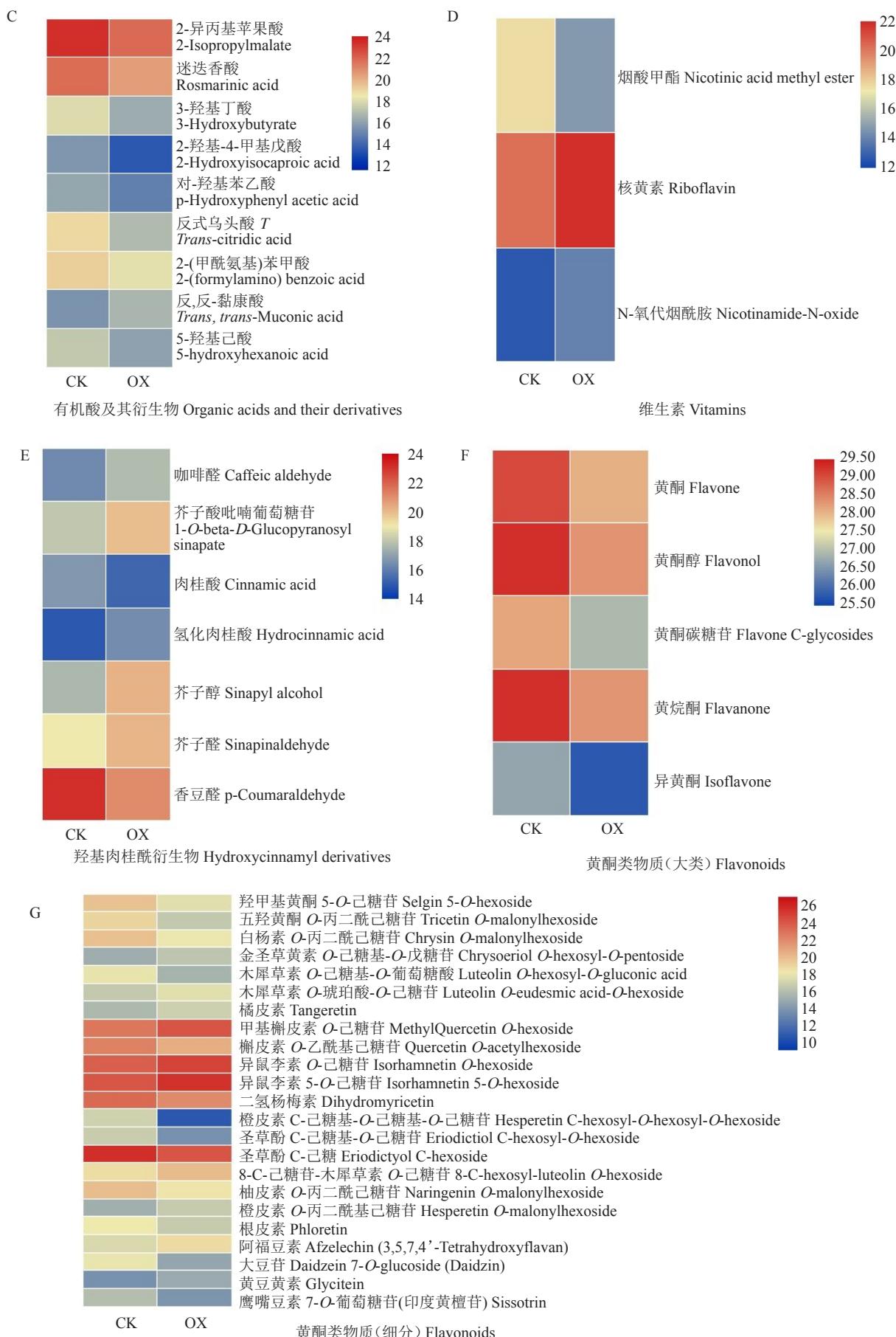


图6(续) Fig. 6 (Continued)

S-腺苷蛋氨酸含量最高且下降程度最大,为对照的0.13倍(图6-A)。

随着草莓果实成熟,花青素含量逐渐增加,而原花青素含量逐渐减少。研究发现,在过表达*FaGAPCp1*果实中,花青素类的矢车菊素O-丙二酰己糖苷(含量最高)、矢车菊素O-丁香酸、矢车菊素3-O-葡萄糖苷等物质的含量均出现显著下调。其中天竺葵素O-乙酰基己糖含量显著下调至对照组的0.44倍(图6-B)。与此同时,原花青素A3的含量较对照显著升高了4.66倍(图6-B)。此外,9种有机酸及其衍生物中,除反,反-黏康酸外,其余物质例如2-异丙基苹果酸、3-羟基丁酸、反式乌头酸和5-羟基己酸等的含量均较对照显著下调(图6-C)。维生素类物质中,核黄素(含量最高)和N-氧化烟酰胺的含量上调,烟酸甲酯含量下调(图6-D)。在羟基肉桂酰衍生物中,除肉桂酸和香豆醛(含量最高)含量较对照分别显著下调0.38倍与0.24倍外,咖啡醛、芥子酸吡喃葡萄糖苷、氢化肉桂酸、芥子醇和芥子醛5种物质含量均为显著上调,其中芥子醇含量上调最为显著,是对照的6.29倍(图6-E)。

黄酮类化合物是草莓果实发育和成熟过程中主要的次生代谢产物。在过表达*FaGAPCp1*草莓果实中,共检测到羟甲基黄酮5-O-己糖苷、甲基槲皮素O-己糖苷、8-C-己糖苷-木犀草素、柚皮素O-丙二酰己糖苷等23种黄酮类化合物含量出现了显著变化(图6-G)。在上述黄酮类化合物中,黄酮醇占比最高,分别占对照组和过表达组黄酮类化合物总相对含量的82.7%和85.3%(图6-F),黄酮醇中甲基槲皮素O-己糖苷、异鼠李素O-己糖苷和异鼠李素5-O-己糖苷的相对含量较对照均显著升高,且异鼠李素5-O-己糖苷相对含量升高最为明显,是对照的2.46倍。除黄酮醇外,黄酮、黄酮碳糖苷、黄烷酮和异黄酮的含量均较对照显著降低,从而导致草莓果实黄酮类化合物相对含量在过表达组较对照呈现显著下调。

3 讨 论

草莓是一种非呼吸跃变型果实,在其果实成熟过程中会伴随着营养组分、风味口感、气味、质地以及色泽的变化,这些变化均与代谢物的组成和含量变化密切相关。对于GAPDH功能多样性的研究大多集中在GAPC上,目前研究认为GAPC是一个衔接胁迫信号与代谢改变的信息枢纽^[10],而对于GAP-

Cp的研究较少。*GAPCp*在植物地下部分作为糖酵解途径的关键酶,为丝氨酸生物合成提供3-磷酸甘油酸^[18]。在本次实验中,过表达*FaGAPCp1*能显著抑制草莓果实的上色。花青素是草莓果实成熟的一个重要指标。随着草莓果实的成熟,花青素含量逐渐增加,而原花青素含量逐渐减少^[19-20],因此从表观现象初步认为过表达*FaGAPCp1*抑制了草莓果实成熟。进一步通过代谢组分析发现,*FaGAPCp1*的过表达导致草莓果实花青素的相对含量显著下调。草莓果实花青素种类主要是矢车菊素-3-葡萄糖苷、天竺葵素-3-葡萄糖苷、天竺葵素-3-芸香糖苷和天竺葵素-丙二酰葡萄糖苷^[20-21]。以上4种花青素均在*FaGAPCp1*过表达和对照果实中检测到,且相对含量较对照显著下调。与此同时,过表达*FaGAPCp1*导致草莓果实原花青素A3的相对含量较对照显著上调。其原因可能与花青素含量下调有关。Yang等^[22]在探究红皮‘新红星’梨和其绿皮突变体花青素积累分子机制的过程中发现,无色花色素还原酶(*leucoanthocyanidin reductase*, LAR)和花青素还原酶(*anthocyanidin reductase*, ANR)促进原花青素积累的同时,抑制了花青素的积累,推测原花青素与花青素的含量有着相互制约的关系。

在植物的次生代谢产物中,黄酮类化合物常以糖苷类化合物的形态存在于植物体中,并对草莓果实的颜色以及风味物质产生影响^[23]。黄酮类化合物对植物的抗氧化、抗胁迫和繁衍有着重要作用,也作为信号分子参与各类生物代谢途径^[24-25]。植物中的黄酮类化合物可以分为黄酮类、黄酮醇类、二氢黄酮醇类、黄烷醇类、黄烷二醇类、花色素类及原花色素类等^[23]。黄酮类化合物含量在草莓果实绿熟期最高,随着草莓果实成熟而逐渐减少^[19]。在草莓果实中存在的黄酮类化合物主要是黄酮醇中的槲皮素、山奈酚、芦丁以及槲皮素和山奈酚的衍生物等^[26]。在本实验中,黄酮醇在黄酮类化合物中占比最高,且其中的槲皮素衍生物甲基槲皮素O-己糖苷的相对含量在过表达*FaGAPCp1*果实中较对照显著上调,槲皮素O-乙酰基己糖苷的相对含量显著下调,黄酮和黄烷酮的相对含量显著下调,且下调幅度高于甲基槲皮素O-己糖苷相对含量的增加幅度,从而导致过表达*FaGAPCp1*草莓果实黄酮类化合物相对含量的降低。由此说明,*FaGAPCp1*主要是通过影响槲皮素O-乙酰基己糖苷、黄酮和黄烷酮的相对含量变

化来影响草莓果实黄酮类化合物的相对含量变化。

氨基酸及其衍生物与有机酸是草莓果实的重要组分,也是许多风味物质的合成原料。草莓果实中的主要氨基酸种类为天冬氨酸、谷氨酸、丙氨酸和亮氨酸等^[27],主要含有的有机酸为柠檬酸和苹果酸^[28],且氨基酸和有机酸及其两者衍生物的含量均随着草莓果实成熟而减少。在本实验中,氨基酸及其衍生物以及有机酸的总相对含量较对照减少。在拟南芥gapcp双突变体(gapcp1和gapcp2)中,游离氨基酸的含量相比野生型显著增多^[29]。在过表达AtGAPCp的拟南芥中,氨基酸含量较对照减少^[8]。而有机酸的下降推测可能是由于FaGAPCp1过表达使糖酵解加速,为其下游的三羧酸循环提供了充足底物,进而促进有机酸参与三羧酸循环从而被消耗。

4 结 论

FaGAPCp1参与了草莓果实成熟过程,主要通过影响氨基酸及其衍生物、花青素、原花青素、黄酮类物质和有机酸及其衍生物等物质的代谢来影响草莓果实的成熟进程。

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欢迎订阅2021年《河北果树》

《河北果树》是河北省果树学会主办的果树专业技术期刊,中国核心期刊(遴选)数据库、中国学术期刊综合评价数据库统计源期刊,中国期刊全文数据库、中文科技期刊数据库收录期刊,河北省优秀科技期刊。主要刊登落叶果树的品种资源、栽培管理、病虫防治、储藏加工等方面的新成果、新技术、新知识和新信息,开设栏目有专题论述、试验研究、经验交流、百花园、工作历、广告与信息。本刊特色是通俗易懂、科学实用、技术先进、内容丰富、信息量大、可读性强、发行面广。读者对象为果树科研和推广人员、农林院校师生、各级涉农领导和广大果农。本刊国内外公开发行,季刊,每

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