

柑橘果实柠檬酸积累调控基因研究进展

卢晓鹏^{1,2}, 李菲菲^{1,2,3}, 谢深喜^{1,2*}

(¹湖南农业大学园艺园林学院,长沙 410128; ²国家柑橘改良中心长沙分中心,长沙 410128; ³湖南省农业科学院园艺研究所,长沙 410125)

摘要: 有机酸是果实主要的品质组分物质之一,直接影响柑橘果实的口感和风味。多数柑橘果实的柠檬酸占总酸含量的70%以上,是主要的有机酸类型。介绍了柑橘果实柠檬酸的代谢途径和国内外柑橘果实柠檬酸研究涉及的果实类型及有机酸特点,综述了参与调控柑橘果实柠檬酸积累的相关基因,指出了柠檬酸合成相关基因CS在不同报道中的功能差异,整理了目前报道的柠檬酸降解相关的*Aco*、*IDH*、*ACL*、*GAD*、*GS*基因以及贮藏相关的*PH*、*AHA*基因在影响柑橘果实柠檬酸含量中的作用;特别指出了近年来报道的调控柑橘果实柠檬酸积累的相关转录因子研究。柑橘果实柠檬酸积累是一个复杂的过程,受多种因素影响,与其他果实性状研究相比其研究还处于起步阶段。为此,笔者对调控柑橘果实柠檬酸积累的重要基因研究进行了综述,分析了研究现状和存在的不足并展望了今后的研究方向,旨在为柑橘果实柠檬酸代谢研究提供参考。

关键词: 柑橘果实;柠檬酸;基因

中图分类号: S666

文献标志码: A

文章编号: 1009-9980(2018)01-0118-10

Citrate accumulation in citrus fruit: a molecular perspective

LU Xiaopeng^{1,2}, LI Feifei^{1,2,3}, XIE Shenxi^{1,2*}

(¹College of Horticulture and Landscape, Hunan Agricultural University, Changsha 410128, Hunan, China; ²National Centre for Citrus Improvement, Changsha 410128, Hunan, China; ³Institute of Horticulture, Hunan Academy of Agricultural Science, Changsha 410125, Hunan, China)

Abstract: Organic acid is one of the major components in fruit quality, affecting the flavor of citrus fruit directly. Citrate, accounting for more than 70% of total organic acid, is the dominant acid type in most citrus fruits. This paper reviewed the studies on genes involved in citrate accumulation of citrus fruit in past two decades. Citrate is an intermediate of tricarboxylic acid (TCA) cycle which is an important pathway supplying power for organism in both plant and animal. In fruit, citrate was believed to derive from two sources. First, fruit biosynthesizes carbohydrates and confers them into citrate by itself through TCA cycle because fruit skin has both chlorophyll and photosynthesis. Second, citrate biosynthesized in roots or leaves was transported into fruit. Both hypothesis had experimental supports in citrus. Previous studies on citrate accumulation included diverse citrus species, containing lemon, sweet orange, navel orange, Satsuma mandarin and ponkan. Natural citrate difference between citrus cultivars was focused firstly as well as some citrus mutants characterizing specific citrate content. Certainly, citrate differences in fruit induced by treatment or by different developing stage were also chosen for investigation. So far, it is commonly accepted that citrate increased sharply in citrus fruit at early developing stage and then decreased constantly in the following stages. The final citrate content in fruit was determined by many factors, but biosynthesis, degradation, utilization and storage metabolism were focused generally. First, the effect of biosynthesis on

收稿日期: 2017-06-30 接受日期: 2017-11-09

基金项目: 国家自然科学基金(31401824);湖南省教育厅资助科研项目(17B127);国家现代农业(柑橘)产业技术体系(CARS-027);湖南省自然科学基金(2017JJ3168)

作者简介: 卢晓鹏,男,博士,研究方向为果实品质形成与调控。Tel: 0731-84618171, E-mail: puninglu@126.com

*通信作者 Author for correspondence. Tel: 0731-84618171, E-mail: shenxixie@163.com

citrate accumulation. Citrate synthase (CS) is the first step for citrate biosynthesis, so the expression of its encoded genes were paid more attention. Some studies through applying CS inhibitor on Satsuma mandarin and tangelo proved that CS activity affect citrate level in citrus fruit; and similar role of CS was obtained by the study in navel orange. However, many reports believed that there was no clear relationship between CS genes expression and citrate accumulation. This result was supported by the CS studies on pumelo, sweet lemon, sour lemon and sweet orange. Therefore, there was no clear conclusion on CS regulating citrate accumulation in citrus fruit. Second, degrading steps influence citrate accumulation in fruit. Unlike the role of biosynthesis step, the genes contributing to citrate degradation, such as *Aco* and *IDH* genes, worked well in fruit. That has been proved in different citrus species, including sweet lemon, sour lemon, mandarin, Satsuma mandarin, sweet orange and navel orange. Third, many utilizing citrate metabolisms affect citrate content in fruit. Some studies believed there were other pathways contributing to citrate degradation in citrus fruit except TCA cycle. Glutamate decarboxylase (GAD) catalyzes the biosynthesis of γ -aminobutyrate (GABA) which needs citrate as a precursor. It has been suggested that *GAD* genes expression influenced fruit citrate accumulation through GABA shunt. The expression of *GAD* genes influencing citrate utilization so as to affect its final level was found in both citrate degradation during fruit development and citrate decrease caused by environment factor. The *GS* genes are involved in biosynthesis of glutamine and other amino acids which also need citrate as precursor. Studies indicated expression of *GS* genes was correlated with citrate degradation in citrus fruit. ATP-citrate lyase (*ACL*) catalyzes the conversion of citrate to oxaloacetate and acetyl-CoA in cell cytosol. Fourth, storage function of vacuole is important for citrate accumulation in citrus fruit. It has also been implied that expression of *ACL* influenced fruit citrate level during fruit ripening as well as citrate changes under drought stress or ABA treatment. H^+ -ATPase encoded by *PH* and *AHA* genes supplied the H^+ gradient for citrate transporting to vacuole. The expression of these genes was also associated with citrate accumulation in the citrus fruit. Above genes worked in different way so as to affect final citrate level in citrus fruit, but these were not enough to reveal the detail mechanism of citrate accumulation since it is a complex procedure and affected by many factors. Recently, some transcription factors were assessed to regulate citrate accumulation in citrus fruit. Some transcription factors, like ERF, MYB, bHLH, were believed to participate in citrate accumulation. Transient overexpression of *CrMYB73* and *CitERF13* suggested that they interacted with *VAH* genes so as to regulate citrate accumulation in vacuole. Yeast assay exposed some bHLH transcription factors interact with the promoter of *CsAco1*, implying their role in citrate degradation. Herein, we summarized the studies on genes involved in citrate accumulation in citrus fruit and analyzed their different roles in citrate biosynthesis, degradation, utilization and storage. Overall, the regulating network of citrate metabolism and the major gene controlling citrate level in citrus fruit were not revealed clearly. Studies on transcription factors which were involved in citrate metabolism mean a novel and further research direction in this field. This review demonstrated the improvements and shortages of citrate study in citrus fruit and suggested the future research priorities. We hope to provide reference for further study and improvement on citrate metabolism of citrus fruit.

Key words: *Citrus* fruit; Citrate; Gene

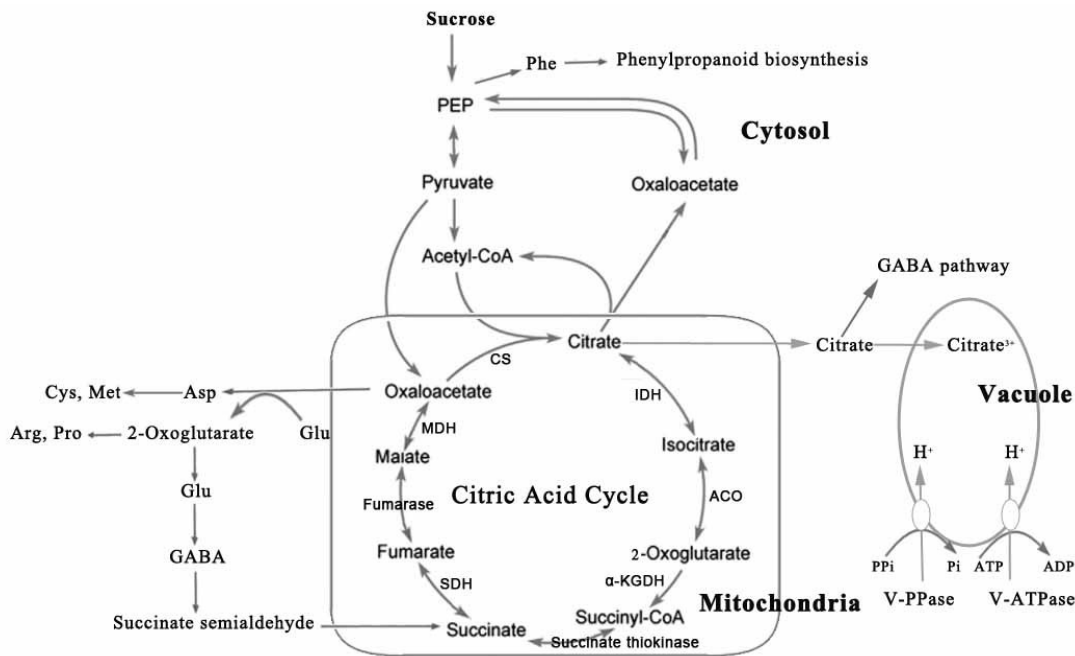
有机酸是柑橘果实内在品质的重要组成部分之一,直接影响柑橘果实口感及消费者的认可度。柠檬酸

是柑橘果实主要的有机酸类型,不同类型柑橘果实柠檬酸含量差异较大,积累模式也不尽相同^[1-2]。由

于柠檬酸在柑橘果实品质形成和调控中的重要意义,国内外学者在不同种类柑橘果实上开展了柠檬酸积累机制研究。针对柑橘果实柠檬酸积累的研究在转录、蛋白和代谢水平均已开展,发现和报道了一批柠檬酸积累相关的重要途径和重要基因^[3]。笔者从报道的柑橘果实柠檬酸积累相关基因的角度,综述了目前国内外柑橘果实柠檬酸研究的相关结果和报道的相关基因,以期对柑橘果实品质形成及其调控提供参考。

1 柑橘果实柠檬酸的来源及积累模式

柑橘果实中的柠檬酸是三羧酸循环(TCA,又称柠檬酸循环)的中间产物(图1),在果实中有有机酸有2个来源,一是果实自身合成或转化^[4],二是从根和叶中运输到果实。研究表明,柑橘果实在转色前可以独立进行光合作用^[5],可为果实三羧酸循环提供底物;此外,通过柠檬、伏令夏橙^[6]和甜橙^[7]果实上的¹⁴CO₂饲喂试验表明,柑橘果实的确可以自身合成



Cytosol. 细胞质; Mitochondria. 线粒体; Vacuole. 液泡; Sucrose. 蔗糖; PEP. 磷酸烯醇式丙酮酸; Pyruvate. 丙酮酸; Acetyl-CoA. 乙酰辅酶 A; Citrate. 柠檬酸; Isocitrate. 异柠檬酸; 2-Oxoglutarate. 2-酮戊二酸; Succinyl-CoA. 琥珀酰辅酶 A; Succinate. 琥珀酸; Fumarate. 延胡索酸; Malate. 苹果酸; Oxaloacetate. 草酰乙酸; GABA. γ -氨基丁酸; V-ATPase. 液泡膜 H⁺-ATP 酶; V-PPase. H⁺-焦磷酸化酶; ATP. 三磷酸腺苷; ADP. 二磷酸腺苷; Cys. 半胱氨酸; Met. 蛋氨酸; Asp. 天冬氨酸; Arg. 精氨酸; Pro. 脯氨酸; Glu. 谷氨酸; Phe. 苯丙氨酸; CS. 柠檬酸合酶; IDH. 异柠檬酸脱氢酶; ACO. 乌头酸酶; α -KGDH. α 酮戊二酸脱氢酶; Succinate thiokinase. 琥珀酸硫激酶; SDH. 琥珀酸脱氢酶; Fumarase. 延胡索酸酶; MDH. 苹果酸脱氢酶。

图 1 柑橘果实柠檬酸来源和代谢途径^[8-10]

Fig. 1 Source of citrate in citrus fruit and its metabolism pathway^[8-10]

柠檬酸。另一方面,研究表明柑橘叶片影响果实柠檬酸积累^[8],但针对柠檬酸由根或叶转运入果实的直接证据较少。

与其他果实类似,柑橘果实中柠檬酸含量在果实发育前期迅速积累升高,后期持续降低的积累模式被广泛认可。不同类型柑橘中,甜橙类果实柠檬酸在盛花后 100 d 前达到最高,温州蜜柑约在盛花后 100 d 达到最高,椪柑和杂柑约在盛花后 120 d 达到最高^[11],柚类约在盛花后 120 d 达到最高^[11]。不同类

型柑橘果实柠檬酸积累模式及成熟期果实柠檬酸含量不同,同一类型不同品种柑橘果实柠檬酸积累模式也有较大差异。如表 1 所示,过去 20 a 国内外学者对柑橘果实柠檬酸代谢开展了广泛研究,研究材料涉及柠檬、甜橙、脐橙、温州蜜柑、克里曼丁橘、椪柑和杂柑等柑橘类型。不同类型或同一类型不同品种柑橘果实柠檬酸积累模式差异显著,尤其是许多芽变材料在果实柠檬酸含量方面差异显著(表 1),为开展柑橘果实柠檬酸积累和代谢研究奠定了基础。

表 1 国内外柑橘果实柠檬酸相关研究涉及的材料

Table 1 Citrus materials in fruit citrate studies

材料/表型 Materials/Phenotype	柑橘种类/遗传关系 <i>Citrus</i> species/Genetic relationship	柠檬酸含量比(高酸/低酸) Ratio of citrate level (high level /low level)	参考文献 Reference
1 大红甜橙/高酸 <i>Citrus sinensis</i> Osbeck Dahongtiancheng/high acidity 冰糖橙/低酸 <i>C. sinensis</i> Osbeck Bingtangcheng/low acidity	甜橙/均为黔阳甜橙芽变 Sweet orange/mutants of <i>C. sinensis</i> Osbeck Qianyangtiancheng	3.5	[8]
2 纽荷尔破色期/常酸 Fruit peel color break stage of <i>C. sinensis</i> Osbeck Newhall/normal acidity 新会橙/常酸 <i>C. sinensis</i> Osbeck Xinhuicheng/normal acidity 冰糖橙/低酸 <i>C. sinensis</i> Osbeck Bingtangcheng/low acidity 糖橙/低酸 <i>C. sinensis</i> Osbeck Succari/low acidity	脐橙 Navel orange 甜橙 Sweet orange 甜橙 Sweet orange 甜橙 Sweet orange	~ 2.5-4.7(总酸比) (Ratio of total acidity)	[12]
3 椪柑-烟草、拟南芥验证 Ponkan-tobacco, <i>Arabidopsis</i> confirmation			[13]
4 椪柑-烟草验证 Ponkan-tobacco confirmation			[14]
5 高橙/高酸 <i>Citrus</i> sp. Gaocheng/high acidity 温州蜜柑/低酸 <i>C. unshiu</i> Marc./low acidity	甜橙 Sweet orange 温州蜜柑 Satsuma mandarin	3	[15]
6 纽荷尔破色期/高酸 Fruit peel color break stage of <i>C. sinensis</i> Osbeck Newhall/high acidity 纽荷尔成熟期/低酸 Harvesting stage of <i>C. sinensis</i> Osbeck Newhall /low acidity 尾张破色期/高酸 Fruit peel color break stage of <i>C. unshiu</i> Owari/high acidity 尾张成熟期/低酸 Harvesting stage of <i>C. unshiu</i> Owari/low acidity 鄂柑1号破色期/高酸 Fruit peel color break stage of <i>C. reticulata</i> Egan No. 1/high acidity 鄂柑1号成熟期/低酸 Middle ripening stage of <i>C. reticulata</i> Egan No. 1/low acidity 伊予柑破色期/高酸 Fruit peel color break stage of <i>C. iyo</i> Hort. ex Tanaka/high acidity 伊予柑成熟期/低酸 Middle ripening stage of <i>C. iyo</i> Hort. ex Tanaka/low acidity	脐橙 Navel orange 温州蜜柑 Satsuma mandarin 椪柑 Ponkan 杂柑 Hybrid citrus	~ 1.8 ~ 1.6 ~ 1.6 ~ 1.5	[16]
7 普通椪柑/高酸 Normal Ponkan/high acidity 早熟椪柑/低酸 Earlier-ripening mutant Ponkan/low acidity	椪柑/芽变 Ponkan/bud mutant	~ 1.6	[17]
8 暗柳橙/高酸 <i>C. sinensis</i> Osbeck Anliu/high acidity 红暗柳橙/低酸 <i>C. sinensis</i> Osbeck Honganliu/low acidity	甜橙/芽变 Sweet orange/bud mutant		[18]
9 暗柳橙花后 178 d/高酸 <i>C. sinensis</i> Osbeck Anliu at 178 days after florescence/high acidity 暗柳橙花后 212 d/低酸 <i>C. sinensis</i> Osbeck Anliu at 212 days after florescence/low acidity 国庆1号花后 154 d/高酸 <i>C. unshiu</i> Guoqing No.1 at 154 days after florescence/high acidity 国庆1号花后 176 d/低酸 <i>C. unshiu</i> Guoqing No.1 at 176 days after florescence/low acidity 纽荷尔花后 150 d/高酸 <i>C. sinensis</i> Osbeck Newhall at 150 days after florescence/high acidity 纽荷尔花后 184 d/低酸 <i>C. sinensis</i> Osbeck Newhall at 184 days after florescence/low acidity	甜橙/芽变 Sweet orange/bud mutant 温州蜜柑 Satsuma mandarin 脐橙 Navel orange	~ 1.1 ~ 1.2 ~ 1.2	[19]
10 松阳纽荷尔/高酸 <i>C. sinensis</i> Osbeck Newhall planting in Sonyang city/high acidity 赣州纽荷尔/低酸 <i>C. sinensis</i> Osbeck Newhall planting in Ganzhou city/low acidity 松阳朋娜/高酸 <i>C. sinensis</i> Osbeck Skaggs Bonanza planting in Sonyang city/high acidity 赣州朋娜/低酸 <i>C. sinensis</i> Osbeck Skaggs Bonanza planting in Ganzhou city/low acidity	脐橙 Navel orange 脐橙 Navel orange	~ 1.9 ~ 1.2	[20]
11 椪柑 10 °C贮藏/高酸 Ponkan in storage at 10 °C/high acidity 椪柑 42 °C加热 2 d后 10 °C贮藏/低酸 Ponkan treated with 42 °C for two days and then stored at 10 °C/low acidity	椪柑 Ponkan	~ 1.2	[21]

表 1(续) Table 1(continued)

材料/表型 Materials/Phenotype	柑橘种类/遗传关系 <i>Citrus</i> species/Genetic relationship	柠檬酸含量比(高酸/低酸) Ratio of citrate level (high level /low level)	参考文献 Reference
12 Ori 喷施二甲基苹果酸/高酸 <i>C. reticulata</i> Ori treated with citramalate/high acidity Ori/低酸 <i>C. reticulata</i> Ori/low acidity	椪柑 Ponkan	~ 1.4	[22]
13 Faris 酸柠檬/高酸 <i>C. limon</i> Faris/high acidity Faris 甜柠檬/低酸 <i>C. limon</i> Faris/low acidity	柠檬/嵌合体 Lemon/chimera	pH 比值>1 Ratio of pH>1	[23]
14 Clemenules/高酸 Clemenules/high acidity Fortune/低酸 Fortune/low acidity	克里曼丁橘 Clementine	~ 3.5(总酸比) (Ratio of total acidity)	[24]
Comuna/高酸 Comuna/high acidity Valencia late/高酸 Valencia late/high acidity Sucrena/低酸 Sucrena/low acidity	甜橙 Sweet orange	~ 17-18(总酸比) (Ratio of total acidity)	
Fino/高酸 Fino/high acidity Dulce/低酸 Dulce/low acidity	柠檬 Lemon	~ 18.7(总酸比) (Ratio of total acidity)	
15 暗柳/高酸 <i>C. sinensis</i> Osbeck Anliu/high acidity 红暗柳橙/低酸 <i>C. sinensis</i> Osbeck Honganliu/low acidity	甜橙 Sweet orange	4.2	[25]
16 奉节 72-1/高酸 <i>C. sinensis</i> Osbeck Fengjie 72-1/high acidity 奉节脐橙/低酸 <i>C. sinensis</i> Osbeck Fengjie/low acidity	脐橙/芽变 Navel orange/bud mutant	~ 1.2	[26]
17 尤力克柠檬酸果实成熟期/高酸 <i>C. limon</i> Eureka fruit at ripening stage/high acidity 尤力克柠檬酸果实发育前期/低酸 <i>C. limon</i> Eureka fruit at early development satge/low acidity	柠檬 Lemon	~ 6	[27]
18 酸柠檬/高酸 Sour lemon (<i>Citrus limon</i> Eureka)/high acidity 巴勒斯坦甜来檬/低酸 Palestine sweet lime (<i>C. limettioides</i> Tan.)/low acidity	柠檬 Lemon	~ 6	[10]

注: ~ 数据表示根据参考文献图表推测。

Note: ~ means the data was estimated from the chart or figure in reference.

2 参与调控柑橘果实柠檬酸积累的功能基因

不同果实积累的优势有机酸类型不同,如苹果、梨以苹果酸为主,葡萄以酒石酸为主,柑橘以柠檬酸为主要有机酸类型,柠檬酸含量高低直接影响柑橘果实总有机酸含量^[28]。对多种柑橘果实的分析表明,不同类型柑橘果实柠檬酸和总有机酸积累模式及终含量有较大差异^[1]。因此,围绕柑橘类果实柠檬酸差异积累的原因及参与的关键因子这一问题,国内外一直在开展相关研究,但研究结论尚不统一。

2.1 柠檬酸合成相关基因在柑橘果实柠檬酸积累中的作用

柠檬酸合酶(CS)是三羧酸循环的重要酶之一,在细胞线粒体中催化草酰乙酸(OAA)与乙酰辅酶A(乙酰-CoA)结合生成柠檬酸^[29]。早期研究通过在柑橘上喷施砷酸盐抑制了CS活性进而降低了果实柠檬酸含量,表明CS在果实柠檬酸积累中有重要作用^[30-31];同时报道也表明,砷酸盐抑制CS活性但诱导了该基因的表达^[31]。文涛等^[32]在脐橙上的研究表明,PEPC和CS活性在果实发育过程中与有机酸含

量呈显著正相关,说明CS是柑橘果实柠檬酸合成的关键酶之一。但也有许多报道认为CS与柠檬酸的积累没有明显相关性。Canel等^[33]研究CS基因表达与柚果实酸含量的关系后认为,其与果实高柠檬酸和低柠檬酸表型没有显著相关性;对甜柠檬、酸柠檬和‘沙莫蒂甜橙’3个酸度有差异的果实在基因表达和酶活性水平的分析也表明,柠檬酸含量的差异与CS的变化没有直接关系^[34];Luo等^[35]在柠檬、锦橙、冰糖橙和奉节脐橙中的研究也认为,CS活性变化与各类型柑橘果实中柠檬酸含量差异没有明显联系。因此,CS虽是已明确的直接参与柠檬酸合成的重要酶,但其在果实柠檬酸积累中的地位仍不明确。

2.2 柠檬酸分解相关基因在柑橘果实柠檬酸积累中的作用

顺乌头酸酶(ACO)在三羧酸循环中催化柠檬酸分解为异柠檬酸,从而影响果实中柠檬酸的积累。酸柠檬和甜来檬果实发育过程中ACO活性和基因表达与柠檬酸积累关系的研究表明,果实发育早期线粒体ACO活性的降低对柠檬酸积累有重要作用,成熟期细胞质ACO活性的增加有利于降低果实柠檬酸水平^[10]。Degu等^[22]通过抑制ACO活性使柑橘果

实柠檬酸含量升高,也从另一个侧面表明ACO在柠檬酸积累中的重要作用。在甜橙和温州蜜柑上的研究表明,温州蜜柑低柠檬酸积累与酸降解途径中的*CitAco3* - *CitGS2* - *CitGDU1* 基因及转运途径中的*CitCHX*和*CitDIC*基因高量表达相关^[15]。异柠檬酸脱氢酶(IDH)在三羧酸循环中催化异柠檬酸转化为 α -酮戊二酸,影响柠檬酸的分解进而影响果实柠檬酸积累。在柠檬中关于IDH的研究指出,线粒体NADP-IDH活性和基因表达在果实发育早期较高,随果实成熟降低,而细胞质NADP-IDH则在早期较低,随果实成熟升高,两者的协同作用调节果实柠檬酸含量^[27]。龚荣高等^[36]对不同生态栽培区脐橙有机酸积累差异的研究表明,NAD-IDH活性差异是引起果实柠檬酸及总机酸含量差异的主要原因。Chen等^[21]指出‘纽荷尔’和‘朋娜’脐橙在江西赣州栽培酸含量显著低于浙江松阳,究其原因为赣州脐橙果实发育期*CitAco3*、*CitIDH1*和*CitGS2*基因表达量均较高,柠檬酸分解途径活跃从而使果实酸含量较低。也有其他研究认为柑橘果实PEPC与NAD-IDH活性比值较低则果实有机酸含量高^[37]。‘HB柚’和‘Fairchild’杂交后代果实出现酸度性状分离,进而针对高酸和低酸个体的深入研究表明,*m-ACO*和*NAD-IDH*基因表达量较高是促进低酸性状的原因之一^[38]。

2.3 柠檬酸消耗利用途径相关基因在果实柠檬酸积累过程中作用的研究

果实中的柠檬酸参与其他代谢途径时被消耗掉也会影响柑橘果实柠檬酸积累。Cercós等^[9]提出了柑橘果实成熟期柠檬酸代谢新途径:柠檬酸经2步代谢形成 α -酮戊二酸和谷氨酸,谷氨酸可在谷氨酸脱羧酶(GAD)的作用下生成 γ -氨基丁酸(GABA),进入GABA途径(GABA \rightarrow 琥珀酸半醛 \rightarrow 琥珀酸)最终消耗了柠檬酸,由此认为GABA途径在柠檬酸的降解过程中起着重要作用。Katz等^[39-40]在蛋白组学水平对脐橙果实柠檬酸代谢进行研究,发现果实柠檬酸含量下降阶段氨基酸合成相关酶及GABA途径相关酶蛋白表达^[21]增加,下游相应物质积累,表明GABA途径在柠檬酸的降解过程有作用。Chen等^[21]通过加热椪柑使其降酸的研究表明,柠檬酸含量降低与GABA代谢途径显著上调有密切关系。在脐橙、温州蜜柑、椪柑和柚等多种柑橘果实中的研究显示,*CsGAD1*基因表达参与调控果实柠檬酸积累^[19]。针对‘HB柚’和‘Fairchild’杂交后代果实酸性性状分

离的研究指出,包括GAD1、GABP和GABA-T在内的一系列GABA途径基因上调是低酸性状形成的主要原因^[38];类似地,通过外源喷施GABA抑制GAD基因表达、降低柠檬酸消耗从而导致果实柠檬酸剧增的研究也表明,GABA途径在影响柑橘果实柠檬酸含量方面有重要作用^[41]。GABA途径正成为研究柑橘果实柠檬酸积累的热点之一。ATP-柠檬酸裂解酶(ACL)存在于细胞质,催化柠檬酸分解为乙酰-CoA和草酰乙酸,也影响柠檬酸的积累^[42]。Cercós等^[9]首次报道克里曼丁橘果实发育过程中ACL基因下调表达与柠檬酸积累有明显的关系;Katz等^[40]报道了‘华盛顿脐橙’果实成熟过程中ACL参与柠檬酸的裂解;Hu等^[16]的研究表明ACL基因的下调表达可导致柠檬酸积累,其表达对不同柑橘果实柠檬酸含量的影响略有不同。综上,柑橘果实柠檬酸分解和消耗途径研究较为集中,多数报道支持柠檬酸分解利用直接影响果实柠檬酸含量;在分解利用途径研究中,除经典的TCA途径分解柠檬酸影响果实柠檬酸含量外,GABA途径和柠檬酸裂解途径也被大量报道参与调控柑橘果实柠檬酸含量,而这些途径中的主效调控途径或调控基因尚待进一步明确。

2.4 柠檬酸转运积累相关基因在柑橘果实柠檬酸积累中的作用

果实细胞中有液泡膜H⁺-ATP酶(V-ATPase)^[43]和液泡膜H⁺-焦磷酸化酶(V-PPase)^[44]两种类型的液泡膜质子泵,由于2者在有机酸向液泡转运贮藏过程中提供动力,故对果实有机酸的积累有重要的作用。柑橘果实汁胞中柠檬酸的积累伴随着大量H⁺输入,H⁺输入提高了液胞膜内外的电学梯度,是驱动柠檬酸跨液胞膜运输、贮藏于液胞中的主要动力^[45]。Aprile等^[23]通过甜柠檬和酸柠檬的比较研究,揭示了甜柠檬的无酸性状是由于H⁺-ATPase基因*AHA10*不表达造成的果实柠檬酸积累缺陷,表明质子泵在柑橘果实柠檬酸积累中有重要作用。柑橘*CsPH*基因家族编码H⁺-ATPase,在‘红暗柳橙’和无酸柚中对柑橘*CsPH*基因家族的研究表明,*CsPH8*基因表达可能调控柑橘果实柠檬酸的积累^[18]。此外,阳离子氢离子交换蛋白(CHX)也可促使形成液泡膜内外电学梯度,也有相应报道显示*CitCHX*基因调控柑橘果实柠檬酸积累^[15]。高等植物细胞的柠檬酸由细胞质经液泡膜转运到液泡贮藏,是由转运体介导的被动运输过程^[46-47]。目前,包括柑橘在内的

果实柠檬酸转运体报道较少,多数柠檬酸转运体研究集中在模式植物胁迫条件下及根系柠檬酸转运。Shimada等^[48]报道了一个柑橘液泡膜柠檬酸转运体 *CsCit1*,不仅调控柑橘果实柠檬酸积累,且具有 H⁺和柠檬酸共转运的特性。相反地,介导苹果酸转运进入液泡内的转运体在多种果实上研究较为深入,结论较为明确,如二羧酸转运蛋白(DT)、铝激活苹果酸转运蛋白(ALMT)和 Multidrug and Toxin Efflux (MATE)等转运体已被证实在葡萄^[49]、苹果^[50-51]或番茄^[52-54]果实中转运调控苹果酸。在苹果果实苹果酸含量研究中,先通过遗传群体构建找到了大量与苹果酸含量连锁的分子标记^[55-56],后证实 ALMT 基因是控制果实苹果酸含量的主效基因^[50-51],成为木本果树果实有机酸含量调控基因研究中的典范。也有报道认为,上述苹果酸转运体有转运柠檬酸的功能,如 DT 基因参与柑橘果实柠檬酸转运^[15],MATE 基因参与大麦^[57]、拟南芥^[58]和水稻^[59]根系的柠檬酸转运等。总体上,参与果实柠檬酸转运积累的基因报道较少,果实柠檬酸转运积累机制研究尚不清晰。

3 调控柑橘果实柠檬酸积累的相关转录因子

2015 和 2016 年,同一实验室的 2 篇报道显示 ERF 和 MYB 转录因子参与调控果实柠檬酸代谢。Li 等^[13]从椪柑中分离到参与柠檬酸积累的重要基因 *CitVHA-c4*,再通过酵母双杂交的方式获得 *CitERF13* 转录因子,并在烟草和拟南芥上通过瞬时过量表达的方式验证其正调控柠檬酸积累。Li 等^[14]从椪柑果

实中分离出 *CrMYB73* 转录因子,并在烟草上通过瞬时过量表达的方式验证其正调控柠檬酸积累。Lu 等^[8]以柠檬酸积累差异显著的甜橙果实为材料,通过转录组研究检测到差异表达的转录因子,再以 *CsAco1* 启动子为基础,通过酵母单杂交验证筛选到 5 个可能调控柠檬酸积累的转录因子。Wu 等^[60]以‘奉节 72-1’脐橙及其芽变‘奉节晚橙’为材料,在全基因组水平筛选差异表达转录因子,通过生物信息学分析其中 49 个转录因子可能参与调控柠檬酸积累,这些转录因子涉及 bZIP、ERF、bHLH、MYB 等多个转录因子家族。类似地,在苹果酸积累研究较为成熟的苹果上,Hu 等^[61-62]在 2016 和 2017 年先后报道了转录因子 *MdMYB1* 和 *MdMYB73* 调控果实苹果酸的转运和积累。上述研究表明,果实有机酸积累研究正在深入推进,有机酸代谢和调控网络正在逐步完善。

4 结语和展望

柑橘果实柠檬酸积累是一个复杂的过程,其含量受到柠檬酸合成、降解、贮藏、利用等多个方面的影响。通过前人的研究和报道,大家对于柑橘柠檬酸的代谢途径、积累模式和规律等几方面形成了共识,但在影响果实柠檬酸含量的关键途径和关键代谢步骤等方面有待进一步研究和验证。笔者认为开展柑橘果实柠檬酸积累研究还应注意以下几点:(1)栽培柑橘包含了不同类型的果实,不同类型果实柠檬酸积累模式和总体水平有较大差异,柠檬酸积累机制可能不同(表 2),故对不同类型柑橘应区分对待和开展研究。(2)参与调控柑橘果实柠檬酸积累的

表 2 国内外报道的不同柑橘果实中参与调控柠檬酸积累的基因

Table 2 The genes involved in regulating citrate accumulation in various citrus fruits

果实 Fruit	参与调控柠檬酸积累的基因 Genes involved in regulating citrate accumulation	参考文献 Reference
1 甜橙 Sweet orange	<i>CsAco</i> , <i>CsIDH</i> , <i>CsbHLH</i>	[8]
2 脐橙、甜橙 Navel orange and sweet orange	<i>AHA</i> , <i>APD</i> , <i>AIL</i>	[12]
3 温州蜜柑、甜橙 Satsuma mandarin and sweet orange	<i>CitCHX</i> , <i>CitDIC</i>	[15]
4 温州蜜柑、脐橙、椪柑、杂柑 Satsuma mandarin, navel orange, Ponkan and hybrid citrus	<i>ACL</i>	[16]
5 椪柑 Ponkan	<i>CitAco3</i> , <i>CitIDH1/3</i> , <i>CitGAD4/5</i> , <i>CitGS2</i>	[17]
6 甜橙 Sweet orange	<i>CsPH8</i>	[18]
7 温州蜜柑、甜橙、脐橙 Satsuma mandarin, sweet orange and Navel orange	<i>CsGAD1</i>	[19]
8 脐橙 Navel orange	<i>CitAco3</i> , <i>CitIDH1</i> , <i>CitGS2</i>	[20]
9 椪柑 Ponkan	<i>CitAco3</i> , <i>CitIDH2/3</i> , <i>CitGAD4</i>	[21]
10 Ori	<i>Aco</i>	[22]
11 柠檬 Lemon	<i>AHA10</i>	[23]
12 克里曼丁橘、甜橙、柠檬 Clementine, sweet orange and lemon	<i>CcAco1/2</i>	[24]
13 脐橙 Navel orange	<i>CsCS</i> , <i>CsACO</i>	[26]
14 柠檬 Lemon	<i>NADP-IDH</i>	[27]
15 柠檬 Lemon	<i>cyt-Aco</i>	[10]

代谢步骤研究中,在基因表达、蛋白表达、酶活性等同一步骤的多个水平表现不一致的情况常有报道,许多报道推测转录后修饰或调控可能参与该过程,而证实转录后修饰或调控的直接证据较少。(3)果实柠檬酸转运机制研究尚未明确,柑橘果实柠檬酸转运体较其他果实苹果酸转运体研究明显滞后。苹果酸转运体DT、ALMT、MATE等是否兼有柠檬酸转运体的功能,模式植物报道较多的根系柠檬酸转运体是否在果实液泡膜上工作进而调控柠檬酸含量等系列研究亟需加强。(4)果实柠檬酸代谢调控机制研究正在深入,相关转录因子和互作基因的发现对完善柠檬酸代谢基础理论、揭示柠檬酸调控网络有重要意义。(5)柑橘果实柠檬酸积累是受多因素影响的过程,影响柑橘果实柠檬酸含量的报道从转录因子调控、相关代谢途径基因表达达到外界生物或非生物胁迫均有涉及,果实柠檬酸积累机制的研究需要对多个生物学过程总体考虑。

参考文献 References:

- [1] 赵淼. 柑橘果实有机酸代谢及调控研究[D]. 合肥: 安徽农业大学, 2008.
ZHAO Miao. Study on the metabolism of organic acid and regulation in *Citrus* fruits[D]. Hefei: Anhui Agricultural University, 2008.
- [2] 曾祥国. 不同种类和产区柑橘糖酸含量及组成研究[D]. 武汉: 华中农业大学, 2005.
ZENG Xiangguo. Studies on contents and composition of sugar and acid in citrus from different species and production areas[D]. Wuhan: Huazhong Agricultural University, 2005.
- [3] ETIENNE A, GENARD M, LOBIT P, MBEGUIE A, MBEGUIE D, BUGAUD C. What controls fleshy fruit acidity? A review of malate and citrate accumulation in fruit cells[J]. *Journal of Experimental Botany*, 2013, 64(6): 1451-1469.
- [4] 张上隆, 陈昆松. 果实品质形成与调控的分子生理[M]. 北京: 中国农业出版社, 2007.
ZHANG Shanglong, CHEN Kunsong. Molecular physiology of fruit quality formation and regulation[M]. Beijing: China Agriculture Press, 2007.
- [5] HIRATSUKA S, SUZUKI M, NISHIMURA H, NADA K. Fruit photosynthesis in *Satsuma mandarin*[J]. *Plant Science*, 2015, 241: 65-69.
- [6] BEAN R C, TODD G W. Photosynthesis and respiration in developing fruits. I. $^{14}\text{CO}_2$ uptake by young oranges in light and in dark [J]. *Plant Physiology*, 1960, 35(4): 425.
- [7] HAFFAKER R C, WALLACE A. Dark fixation of CO_2 in homogenates from citrus leaves, fruits, and roots[J]. *Proceedings of the Society for Horticultural Science*, 1959, 74: 348-357.
- [8] LU X P, CAO X J, LI F F, LI J, XIONG J, LONG G Y, CAO S Y, XIE S X. Comparative transcriptome analysis reveals a global insight into molecular processes regulating citrate accumulation in sweet orange (*Citrus sinensis*) [J]. *Physiologia Plantarum*, 2016, 158(4): 463-482.
- [9] CERCÓS M, SOLER G, IGLESIAS D J, GADEA J, FORMENT J, TALÓN M. Global analysis of gene expression during development and ripening of citrus fruit flesh. A proposed mechanism for citric acid utilization[J]. *Plant Molecular Biology*, 2006, 62(4/5): 513-527.
- [10] SADKA A, DAHAN E, COHEN L, MARSH K B. Aconitase activity and expression during the development of lemon fruit[J]. *Physiologia Plantarum*, 2000, 108(3): 255-262.
- [11] 陈青英, 陈俊伟, 徐红霞, 李晓颖, 卢方良, 杨希宏. 大棚栽培‘玉环柚’果实发育与糖酸积累特性[J]. *福建农业学报*, 2015, 30(5): 492-497.
CHEN Qingying, CHEN Junwei, XU Hongxia, LI Xiaoying, LU Fangliang, YANG Xihong. Fruit development and sugar/acids accumulation of ‘Yuhuangyou’ pomelos cultivated in greenhouse [J]. *Fujian Journal of Agricultural Sciences*, 2015, 30(5): 492-497.
- [12] HUANG D, ZHAO Y, CAO M, QIAO L, ZHENG Z L. Integrated systems biology analysis of transcriptomes reveals candidate genes for acidity control in developing fruits of sweet orange (*Citrus sinensis* L. Osbeck) [J]. *Frontiers in Plant Science*, 2016, 7(59): 486.
- [13] LI S J, YIN X R, XIE X L, ALLAN A C, GE H, SHEN S L, CHEN K S. The *Citrus* transcription factor, CitERF13, regulates citric acid accumulation via a protein-protein interaction with the vacuolar proton pump, CitVHA-c4[J]. *Scientific Reports*, 2016, 6: 20151.
- [14] LI S J, LIU X J, XIE X L, SUN C D, GRIERSON D, YIN X R, CHEN K S. CrMYB73, a PH-like gene, contributes to citric acid accumulation in citrus fruit[J]. *Scientia Horticulturae*, 2015, 197: 212-217.
- [15] LIN Q, LI S, DONG W, FENG C, YIN X, XU C, SUN C, CHEN K. Involvement of *CitCHX* and *CitDIC* in developmental-related and postharvest-hot-air driven citrate degradation in citrus fruits [J]. *PLoS One*, 2015, 10(3): e119410.
- [16] HU X M, SHI C Y, LIU X, JIN L F, LIU Y Z, PENG S A. Genome-wide identification of citrus ATP-citrate lyase genes and their transcript analysis in fruits reveals their possible role in citrate utilization[J]. *Molecular Genetics and Genomics*, 2015, 290(1): 29-38.
- [17] 陈明. 椪柑和脐橙果实柠檬酸合成与降解相关基因表达及其调控研究[D]. 杭州: 浙江大学, 2013.
CHEN Ming. Expression and its regulation of citric acid synthesis and degradation related genes in the fruits of Ponkan and Na-

- vel orange[D]. Hangzhou: Zhejiang University, 2013.
- [18] SHI C Y, SONG R Q, HU X M, LIU X, JIN L F, LIU Y Z. *Citrus PH5*-like H⁺-ATPase genes: identification and transcript analysis to investigate their possible relationship with citrate accumulation in fruits[J]. *Frontiers in Plant Science*, 2015, 6: 135.
- [19] LIU X, HU X M, JIN L F, SHI C Y, LIU Y Z, PENG S A. Identification and transcript analysis of two glutamate decarboxylase genes, *CsGAD1* and *CsGAD2*, reveal the strong relationship between *CsGAD1* and citrate utilization in citrus fruit[J]. *Molecular Biology Reports*, 2014, 41(9): 6253-6262.
- [20] CHEN M, XIE X, LIN Q, CHEN J Y, GRIERSON D, CHEN K S. Differential expression of organic acid degradation-related genes during fruit development of Navel Oranges (*Citrus sinensis*) in two habitats[J]. *Plant Molecular Biology Reporter*, 2013, 31(5): 1131-1140.
- [21] CHEN M, JIANG Q, YIN X R, LIN Q, CHEN J Y, ALLAN A C, XU C J, CHEN K S. Effect of hot air treatment on organic acid- and sugar-metabolism in Ponkan (*Citrus reticulata*) fruit[J]. *Scientia Horticulturae*, 2012, 147(4): 118-125.
- [22] DEGU A, HATEW B, NUNES-NESE A, SHLIZERMAN L, ZURN, KATZ E, FERNIE A R, BLUMWALD E, SADKA A. Inhibition of aconitase in citrus fruit callus results in a metabolic shift towards amino acid biosynthesis[J]. *Planta*, 2011, 234(3): 501-513.
- [23] APRILE A, FEDERICI C, CLOSE T J, DE BELLIS L, CATTIVELLI L, ROOSE M L. Expression of the H⁺-ATPase AHA10 proton pump is associated with citric acid accumulation in lemon juice sac cells[J]. *Functional and Integrative Genomics*, 2011, 11(4): 551-563.
- [24] TEROL J, SOLER G, TALON M, CERCOS M. The aconitate hydratase family from *Citrus*[J]. *BMC Plant Biology*, 2010, 10(1): 222.
- [25] 顾建芹. 暗柳橙及其突变体红暗柳橙果实发育过程中糖酸组分的变化[D]. 武汉:华中农业大学, 2007.
GU Jianqin. Changing of sugar and acid contents of Anliu sweet orange and its mutant Red Anliu sweet orange during fruit development[D]. Wuhan: Huazhong Agricultural University, 2007.
- [26] 刘永忠. 脐橙(*Citrus sinensis* Osbeck)晚熟芽变性状形成机理研究[D]. 武汉:华中农业大学, 2006.
LIU Yongzhong. Formation mechanism of a late-ripening bud mutant of Navel orange (*Citrus sinensis* Osbeck) [D]. Wuhan: Huazhong Agricultural University, 2006.
- [27] SADKA A, DAHAN E, OR E, COHEN L. NADP(+)-isocitrate dehydrogenase gene expression and isozyme activity during citrus fruit development[J]. *Plant Science*, 2000, 158(1/2): 173-181.
- [28] LU X P, LIU Y Z, ZHOU G F, WEI Q J, HU H J, PENG S A. Identification of organic acid-related genes and their expression profiles in two pear (*Pyrus pyrifolia*) cultivars with difference in predominant acid type at fruit ripening stage[J]. *Scientia Horticulturae*, 2011, 129(4): 680-687.
- [29] POPOVA T N, DE CARVALHO M. Citrate and isocitrate in plant metabolism[J]. *Biochimica et Biophysica Acta*, 1998, 1364(3): 307-325.
- [30] YAMAKI Y T. Effect of lead arsenate on citrate synthase activity in fruit pulp of Satsuma mandarin[J]. *Journal of the Japanese Society for Horticultural Science*, 1990, 58(4): 899-905.
- [31] SADKA A, ARTZI B, COHEN L, DAHAN E, HASDAI D, TAGARI E, ERNER Y. Arsenite reduces acid content in citrus fruit, inhibits activity of citrate synthase but induces its gene expression[J]. *Journal of the American Society for Horticultural Science*, 2000, 125(3): 288-293.
- [32] 文涛, 熊庆娥, 曾伟光, 刘远鹏. 脐橙果实发育过程中有机酸合成代谢酶活性的变化[J]. *园艺学报*, 2001, 28(2): 161-163.
WEN Tao, XIONG Qing'e, ZENG Weiguang, LIU Yuanpeng. Changes of organic acid synthetase activity during fruit development of navel orange[J]. *Acta Horticulturae Sinica*, 2001, 28(2): 161-163.
- [33] CANEL C, BAILEYSERRES J N, ROOSE M L. Molecular characterization of the mitochondrial citrate synthase gene of an acidless pummelo (*Citrus maxima*) [J]. *Plant Molecular Biology*, 1996, 31(1): 143-147.
- [34] SADKA A, DAHAN E, OR E, ROOSE M L, MARSH K B, COHEN L. Comparative analysis of mitochondrial citrate synthase gene structure, transcript level and enzymatic activity in acidless and acid-containing *Citrus* varieties[J]. *Australian Journal of Plant Physiology*, 2001, 28(5): 383-390.
- [35] LUO A C, YANG X H, DENG Y Y, LI C F, XIANG K S, LI D G. Organic acid concentrations and the relative enzymatic changes during the development of the citrus fruits[J]. *Agricultural Sciences in China*, 2003, 2(6): 653-657.
- [36] 龚荣高, 吕秀兰, 张光伦, 曾秀丽, 罗楠, 胡强. 罗伯逊脐橙在不同生境下果实有机酸代谢相关酶的研究[J]. *果树学报*, 2006, 23(6): 805-808.
GONG Ronggao, LÜ Xiulan, ZHANG Guanglun, ZENG Xiuli, LUO Nan, HU Qiang. Study on the organic acid-metabolizing enzymes in Robertson Navel orange fruit collected from different habitats[J]. *Journal of Fruit Science*, 2006, 23(6): 805-808.
- [37] 罗安才, 杨晓红, 邓英毅, 李纯凡, 向可术, 李道高. 柑橘果实发育过程中有机酸含量及相关代谢酶活性的变化[J]. *中国农业科学*, 2003, 36(8): 941-944.
LUO Ancai, YANG Xiaohong, DENG Yingyi, LI Chunfan, XIANG Keshu, LI Daogao. Organic acid concentrations and the relative enzymatic changes during the development of citrus fruits [J]. *Scientia Agricultura Sinica*, 2003, 36(8): 941-944.
- [38] SHENG L, SHEN D D, YANG W, ZHANG M F, ZENG Y L, XU J, DENG X X, CHENG Y J. GABA pathway rate-limit citrate degradation in postharvest citrus fruit evidence from HB pummelo (*Citrus grandis*) × Fairchild (*Citrus reticulata*) hybrid population[J].

- Journal of Agricultural and Food Chemistry, 2017, 65(8): 1669–1676.
- [39] KATZ E, BOO K H, KIM H Y, EIGENHEER R A, PHINNEY B S, …, BLUMWALD E. Label-free shotgun proteomics and metabolite analysis reveal a significant metabolic shift during citrus fruit development[J]. Journal of Experimental Botany, 2011, 62(15): 5367–5384.
- [40] KATZ E, FON M, LEE Y, PHINNEY B, SADKA A, BLUMWALD E. The citrus fruit proteome: insights into citrus fruit metabolism[J]. Planta, 2007, 226(4): 989–1005.
- [41] SHENG L, SHEN D D, LUO Y, SUN X H, WANG J Q, LUO T, ZENG Y L, XU J, DENG X X, CHENG Y J. Exogenous γ -aminobutyric acid treatment affects citrate and amino acid accumulation to improve fruit quality and storage performance of postharvest citrus fruit[J]. Food Chemistry, 2017, 216: 138–145.
- [42] CHYPRE M, ZAIDI N, K S. ATP-citrate lyase: a mini-review[J]. Biochemical and Biophysical Research Communications, 2012, 422(1): 1–4.
- [43] RATAJCZAK R. Structure, function and regulation of the plant vacuolar H^+ -translocating ATPase[J]. Biochimica et Biophysica Acta-Biomembranes, 2000, 1465(1/2): 17–36.
- [44] MAESHIMA M. Vacuolar H^+ -pyrophosphatase[J]. Biochimica et Biophysica Acta, 2000, 1465(1/2): 37–51.
- [45] MULLER M L, TAI Z L. Regulation of the lemon-fruit V-ATPase by variable stoichiometry and organic acids[J]. Journal of Membrane Biology, 2002, 185(3): 209–220.
- [46] GOUT E, BLIGNY R, PASCAL N, DOUCE R. ^{13}C nuclear magnetic resonance studies of malate and citrate synthesis and compartmentation in higher plant cells[J]. Journal of Biological Chemistry, 1993, 268(6): 3986–3992.
- [47] OLESKI N, MAHDAVI P, BENNETT A B. Transport properties of the tomato fruit tonoplast II. Citrate transport[J]. Plant Physiology, 1987, 84(4): 997–1000.
- [48] SHIMADA T, NAKANO R, SHULAEV V, SADKA A, BLUMWALD E. Vacuolar citrate/ H^+ symporter of citrus juice cells[J]. Planta, 2006, 224(2): 472–480.
- [49] DE ANGELI A, BAETZ U, FRANCISCO R, ZHANG J B, CHAVES M M, REGALADO A. The vacuolar channel VvALMT9 mediates malate and tartrate accumulation in berries of *Vitis vinifera*[J]. Planta, 2013, 238(2): 283–291.
- [50] BAI Y, DOUGHERTY L, CHENG L L, ZHONG G Y, XU K N. Uncovering co-expression gene network modules regulating fruit acidity in diverse apples[J]. BMC Genomics, 2015, 16(1): 612.
- [51] XU K N, WANG A D, BROWN S. Genetic characterization of the *Ma* locus with pH and titratable acidity in apple[J]. Molecular Breeding, 2012, 30(2): 899–912.
- [52] SASAKI T, TSUCHIYA Y, ARIYOSHI M, NAKANO R, USHIJIMA K, KUBO Y, MORI I C, HIGASHIZUMI E, GALIS I, YAMAMOTO Y. Two members of the aluminum-activated malate transporter family, *SIALMT4* and *SIALMT5*, are expressed during fruit development, and the overexpression of *SIALMT5* alters organic acid contents in seeds in tomato (*Solanum lycopersicum*)[J]. Plant and Cell Physiology, 2016, 57(11): 2367–2379.
- [53] LIU R L, LI B Q, QIN G Z, ZHANG Z Q, TIAN S P. Identification and functional characterization of a tonoplast dicarboxylate transporter in tomato (*Solanum lycopersicum*) [J]. Frontiers in Plant Science, 2017, 8(472480): 186.
- [54] YE J, WANG X, HU T X, ZHANG F X, WANG B, LI C X, YANG T X, LI H X, LU Y G, GIOVANNONI J J, ZHANG Y Y, YE Z B. An InDel in the promoter of *Al-activated malate transporter 9* selected during tomato domestication determines fruit malate contents and aluminum tolerance[J]. The Plant Cell, 2017, 29(9): 2249–2268.
- [55] KENIS K, KEULEMANS J, DAVEY M W. Identification and stability of QTLs for fruit quality traits in apple[J]. Tree Genetics and Genomes, 2008, 4(4): 647–661.
- [56] LIEBHARD R, KELLERHALS M, PFAMMATTER W, JERTMINI M, GESSLER C. Mapping quantitative physiological traits in apple (*Malus × domestica* Borkh.) [J]. Plant Molecular Biology, 2003, 52(3): 511–526.
- [57] FURUKAWA J, YAMAJI N, WANG H, MITANI N, MURATA Y, SATO K, KATSUHARA M, TAKEDA K, MA J F. An aluminum-activated citrate transporter in barley[J]. Plant and Cell Physiology, 2007, 48(8): 1081–1091.
- [58] DURRETT T P, GASSMANN W, ROGERS E E. The FRD3-mediated efflux of citrate into the root vasculature is necessary for efficient iron translocation[J]. Plant Physiology, 2007, 144(1): 197–205.
- [59] YOKOSHO K, YAMAJI N, UENO D, MITANI N, MA J F. OsFRDL1 is a citrate transporter required for efficient translocation of iron in rice[J]. Plant Physiology, 2009, 149(1): 297–305.
- [60] WU J X, FU L L, YI H L. Genome-wide identification of the transcription factors involved in *Citrus* fruit ripening from the transcriptomes of a late-ripening sweet orange mutant and its wild type[J]. PLoS One, 2016, 11(4): e0154330.
- [61] HU D G, SUN C H, MA Q J, YOU C X, CHENG L, HAO Y J. MdMYB1 regulates anthocyanin and malate accumulation by directly facilitating their transport into vacuoles in apples[J]. Plant Physiology, 2016, 170(3): 1315–1330.
- [62] HU D G, LI Y Y, ZHANG Q Y, LI M, SUN C H, YU J Q, HAO Y J. The R2R3-MYB transcription factor MdMYB73 is involved in malate accumulation and vacuolar acidification in apple[J]. The Plant Journal, 2017, 91(3): 443–454.