

# 园艺作物果实苹果酸代谢与转运及其调控研究进展

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**摘要:** 番茄、苹果、梨、枣等园艺作物是典型的苹果酸型果实, 其果实酸度主要取决于液泡中苹果酸的积累量。苹果酸不仅决定果实的风味品质, 还可作为呼吸底物为植物体提供必需的物质和能量, 在调节植物细胞渗透势、酸碱平衡、抗逆性等方面起着重要作用。苹果酸代谢途径比较复杂, 涉及众多结构催化酶的参与, 而苹果酸主要贮存于液泡中, 从细胞质向液泡的跨膜运输和储存是复杂的生物学过程, 需要多种转运蛋白、质子泵的参与。总结了苹果酸型果实酸度性状遗传研究、转运蛋白及质子泵在苹果酸跨膜转运中的作用, 并将转录因子对苹果酸的代谢调控进行了概述, 以深入理解苹果酸代谢调控网络, 为园艺作物品质育种提供理论基础。

**关键词:** 园艺作物; 果实; 苹果酸; 代谢; 质子泵; 转运蛋白; 调控

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## Advances in research of malate metabolism and regulation in fruit of horticultural crops

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**Abstract:** Acidity is an important part of the sensory quality of fruit. Malic acid is the main organic acid in ripe fruits of tomatoes, apples, pears, and jujubes. Malic acid not only determines fruit acidity and quality but also has multiple important functions in the plant. Malate is well known as a key intermediate in the tricarboxylic acid (TCA) cycle and is imported into mitochondria as a respiratory substrate. Malate also participates the glyoxalate cycle pathway and is closely related to plant primary metabolism, carbon cycling, and carbohydrate accumulation. Malate plays an important role in regulating the osmotic potential, pH balance, and stress resistance in horticultural crops. Thus, it is of important theoretical significance and practical value for high-quality breeding programs as well as the study of the mechanism underlying malic acid biosynthesis and transport in fruits. Malic acid is synthesized in the cytoplasm, accumulated in the vacuole during the early stages of fruit development, and used as a respiratory substrate during fruit ripening. Malate accumulation is affected by synthesis, transport, and metabolism, and involves the participation of numerous catalytic enzymes. Malate metabolism is a complex biological system influenced not only by genetic factors but also by environmental factors, agronomic practices, and post-harvest treatments. In the cytoplasm of fruit, glycogen is converted to phosphoenolpyruvate (PEP) through the glycolytic pathway. PEP is carboxylated by phosphoenolpyruvate carboxylase (PEPC) to produce oxaloacetate (OAA), which is the first step of malic acid synthesis. Then, malate synthesis is catalyzed by cytosolic NAD-dependent malate dehydrogenase (cyMDH) and cytosolic NADP-dependent malic enzyme (cyME). The cyMDH is a key enzyme involved in malate synthesis and catalyzes the conversion reaction from OAA to malate, while cyME is an important malate-degrading enzyme that catalyzes the conversion of malate to pyruvate in the cytoplasm. In addition,

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malate accumulation is regulated by transmembrane transport between the vacuole and cytoplasm. The transmembrane transport of malic acid requires not only a proton pump to provide energy but also the assistance of channel proteins or transmembrane transporters. The main vacuolar transporters, such as the tonoplast-localized malate transporter (tDT) and aluminum-activated malate transporter (ALMT), participate in the transmembrane transport and accumulation of malate in the fruit. Among the ALMT family members, *ALMT9* is the most widely studied gene. Apple *Mal* gene is a key malate transporter responsible for differences in malic acid content between wild and cultivated fruits. *SLALMT9* is considered to be responsible for variation in malate content in the fruit among tomato genotypes. *VvALMT9*, a homolog of *AtALMT9* in grapes, is a vacuolar malate channel that mediates the accumulation of malate and tartrate in the vacuoles of grape berries. Tonoplast proton pumps such as vacuolar-type H<sup>+</sup>-ATPase (V-ATPase, VHA), vacuolar-type H<sup>+</sup>-pumping pyrophosphatase (V-PPase, VHP), and P-ATPase (PHA) generate the driving force for vacuolar acidification by transporting protons across the membrane into the vacuole. In petunia flowers, the P-type proton pump genes *PhPH1* and *PhPH5* interact with each other and form a complex to promote vacuolar acidification. *MdPH1* and *MdPH5*, homologs of *PhPH1* and *PhPH5* in apples, have been identified and shown to be involved in vacuolar acidification and malate accumulation. Another P-type proton pump gene *Ma10* in apples was found to be significantly correlated with malic acid accumulation, explaining about 8% of the variation in fruit acidity phenotypes in natural apple populations. Increasing evidences showed that transcription factors, such as MYB, bHLH, WRKY, and ERF family members, participate in the regulation of malate transporters and proton pumps. In apples, *MdMYB1*, *MdMYB44*, and *MdMYB73* regulate malate accumulation and vacuolar acidification in fruits by activating or repressing the promoter activities of the malate transporter and proton pump genes. Apart from MYB transcription factors, other transcription factors, such as bHLH and WRKY, are also involved in the regulation of malic acid accumulation and vacuolar acidification. In petunia, AN1 (bHLH transcription factor) can form a complex with *AN1-Ph4* to positively regulate vacuolar acidification and thus affects pH. In apples, *MdbHLH3*, a homolog of *AN1* regulates malate accumulation in fruit by directly activating the expression of the malate dehydrogenase gene *MdcyMDH*. *MdbHLH3* forms a complex with *MdMYB1* to promote pulp anthocyanin and malate accumulation. In tomatoes, *SlWRKY42* directly binds to the promoter of *SLALMT9*, repressing its transcription, and thereby inhibiting malate accumulation in tomato fruit. *ZjWRKY7* transcription factor activates the transcription of *ZjALMT4* by the W-box region of the high-acidity genotype in sour jujube, thereby promoting malate accumulation, whereas the binding ability was weakened in jujube. This paper summarizes the mechanism of malate accumulation in horticultural crops, such as tomato, apple, pear, and jujube, and provides an overview of the role of transporters, proton pumps, and upstream transcription factors responsible for malate accumulation and vacuolar acidification, which will provide a theoretical basis for quality breeding in horticultural crops.

**Key words:** Horticultural crops; Fruit; Malate; Metabolism; Proton pump; Transporter protein; Regulation

有机酸是影响园艺作物果实风味品质的重要因素,番茄、苹果、梨、枣等园艺作物属于苹果酸型果实,苹果酸是成熟果实有机酸的主要成分,其果实酸度主要取决于液泡中苹果酸的积累量。苹果酸不但决定着果实的风味品质,同时作为呼吸代谢底物参与到细

胞质的糖酵解、线粒体中三羧酸循环(TCA)、乙醛酸循环等过程,为植物体提供能量物质<sup>[1-2]</sup>。此外,苹果酸对果实花青苷具有共色作用,可以通过增强花青苷的稳定性影响果实色泽形成<sup>[3-4]</sup>。目前研究表明,液泡膜苹果酸转运蛋白与质子泵对苹果酸跨液泡膜转运

起重要作用<sup>[5-7]</sup>。苹果酸转运蛋白主要负责苹果酸的跨液泡膜转运<sup>[8]</sup>；而质子泵将H<sup>+</sup>转运到液泡内，促使液泡内外形成较大的pH梯度和电化学梯度，为苹果酸跨液泡膜运输提供动力<sup>[9]</sup>。笔者结合前人研究，从果实酸度遗传学研究、苹果酸合成降解途径、苹果酸转运蛋白和质子泵类型及功能、转录调控因子等方面进行总结，阐述苹果酸代谢转运机制，对园艺作物优质品种的选育具有重要理论意义与应用价值。

## 1 植物苹果酸功能

苹果酸主要以苹果酸酯的形式广泛存在植物体中，分布于根、茎、叶、果实等多种组织器官，不但决定果实风味和品质，同时作为呼吸代谢的底物参与细胞质的糖酵解、线粒体中三羧酸循环(TCA)、乙醛酸循环等过程，为植物体提供能量物质。此外，苹果酸还参与植物体内pH平衡、植物细胞渗透势调节等代谢过程<sup>[1-2]</sup>。植物根系分泌的苹果酸，可以解除铝离子的毒害作用，促进对营养成分的吸收，为根际土壤微生物提供良好的环境<sup>[10]</sup>。在苹果和枣中研究发现，野生型果实苹果酸含量显著高于栽培型品种，这种现象可能是自然选择的结果，果实中较高的酸度水平能够帮助植物抵御外界不良环境而生存下来<sup>[7,10-12]</sup>。

## 2 园艺作物果实苹果酸遗传学水平研究进展

在园艺作物中，果实酸度属于数量性状遗传，受自身和外界等多种因素的影响，其中遗传因素是影响果实酸度的重要因素。以桃、苹果、番茄和杏为代表的苹果酸型果实有关果实酸度的遗传研究中表明，果实酸度由多个基因协同控制，遗传机制较为复杂。控制桃果实酸度的主效基因位于第5号染色体顶端，又命名为D位点，且低酸为显性性状<sup>[13]</sup>；Wang等<sup>[14]</sup>结合全基因组关联分析和BSA-seq技术发现第5号染色体上存在调控桃果实有机酸积累的主效基因*PpTST1*。在苹果中，多数研究者一致认为苹果果实酸含量由一对主效基因(*Ma/ma*)和其他多基因控制，位于16号染色体顶端的Ma位点是控制苹果成熟果实酸度的主效QTL，其中编码铝诱导的苹果酸转运蛋白的*Mal*基因是主效基因，且相对于*mal*具有不完全显性特征，显性纯合体*Mal/Mal*为高酸，杂合体*Mal/mal*表现为中酸，在同一基因型内株系间表现出连续性酸度变异，则是多基因控制的结

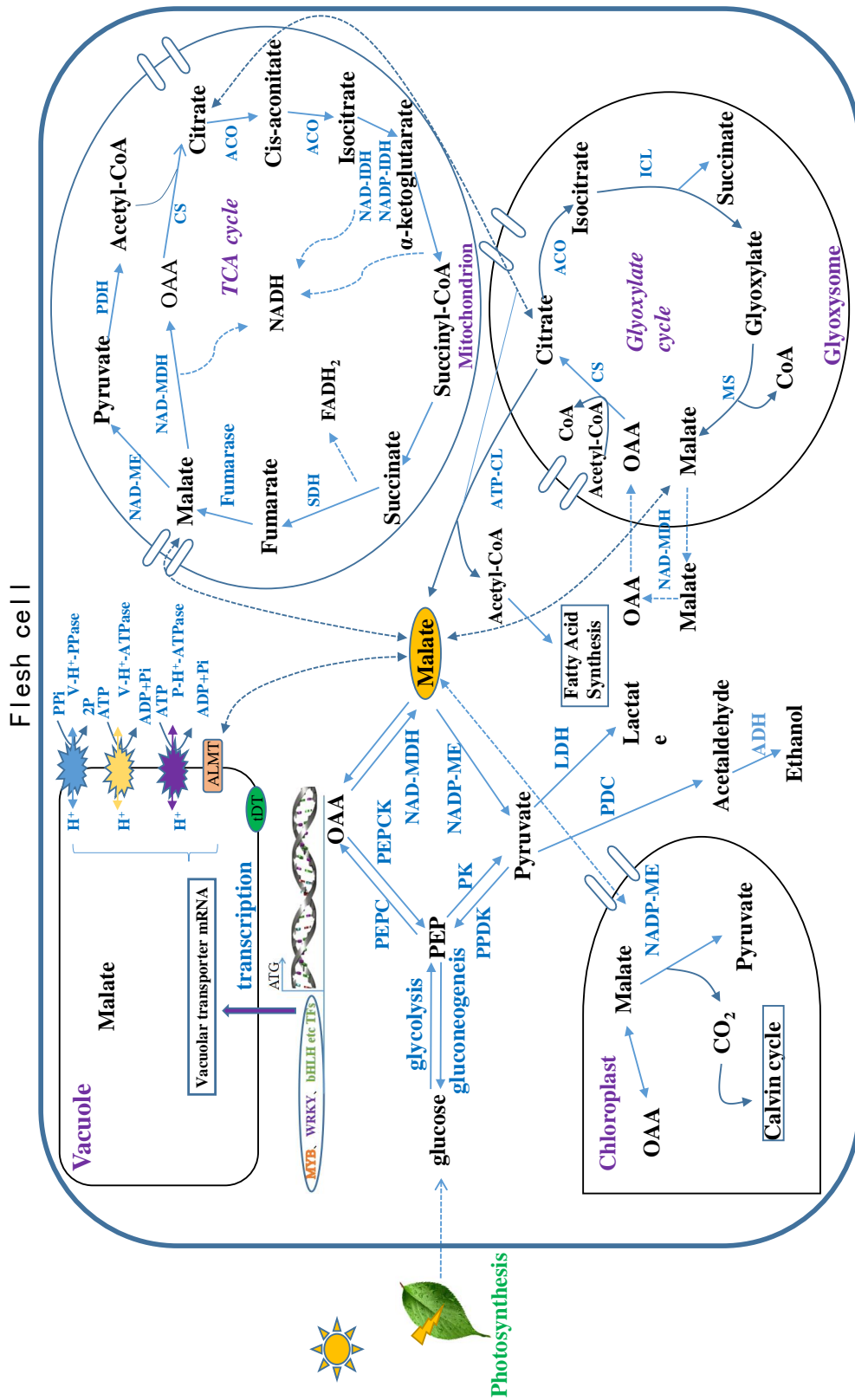
果<sup>[5,15-16]</sup>；除*Mal*基因外，在多个遗传群体中均检测到位于8号染色体的另一个主效QTL位点，其中编码P型质子泵的*Ma10*基因对果实苹果酸的积累起重要作用<sup>[17]</sup>。Sauvage等<sup>[18]</sup>利用163份番茄种质中的19种主要代谢物和5995个SNPs进行mGWAS研究，发现第6号染色体的SNP位点与果实苹果酸含量相关。Ye等<sup>[6]</sup>结合全基因组关联分析和BSA技术进一步证实第6号染色体上存在调控果实苹果酸的主效基因*SLALMT9*，该基因与苹果*Mal*基因具有较高的同源性。Dondini等<sup>[19]</sup>基于F<sub>1</sub>群体进行QTL定位，发现杏果实酸度是多基因控制的数量性状，在4、5、6、7、8号染色体上均检测到QTL位点。

综上所述，苹果酸型果实酸度有两种遗传方式：一种是主效基因控制的数量性状，高酸/中酸/低酸由一对主效基因控制，低酸性状的显隐性因树种而异；另一种是多基因控制的数量性状，多数品种杂交后代果实酸度性状表现出连续变异。

## 3 园艺作物果实苹果酸合成与降解

在果实发育前期，苹果酸在细胞质中合成，在液泡中积累；在果实发育后期，苹果酸从液泡中释放出来，在细胞质中降解。苹果酸代谢途径比较复杂，涉及众多酶参与(图1)：叶片经光合作用制造的光合产物，通过韧皮部运输到果实。在果实细胞质中，糖通过糖酵解途径生成磷酸烯醇式丙酮酸(PEP)，PEP经磷酸烯醇式丙酮酸羧化酶(PEPC)羧化后生成草酰乙酸(OAA)，这是苹果酸合成第一步，OAA在NAD-苹果酸脱氢酶(NAD-cyMDH)的催化下形成苹果酸<sup>[20]</sup>。部分苹果酸被转运到液泡中储存，形成果实风味品质的重要组成因素。PEP和NAD-cyMDH是苹果酸合成的关键酶<sup>[21-22]</sup>。对不同酸度类型的桃<sup>[23]</sup>、苹果<sup>[24]</sup>、枇杷<sup>[25]</sup>、杏<sup>[26]</sup>果实分析发现，PEPC虽然催化苹果酸的合成，但其表达量和酶活性与果实中苹果酸含量没有显著相关性，在苹果愈伤和番茄中超表达*MdcyMDH*会导致苹果酸含量显著增加，同时诱导苹果酸代谢相关基因上调表达，表明*MdcyMDH*直接参与苹果酸合成<sup>[27]</sup>。

在果实成熟后期，部分苹果酸通过跨膜转运从液泡释放出来，在细胞质内降解后重新合成PEP。降解过程关键酶包括磷酸烯醇式丙酮酸羧激酶(PEPCK)和苹果酸酶(NADP-cytME)。一方面，苹果酸可以通过NADP-cytME催化脱羧形成丙酮酸，



Photosynthesis. 光合作用; Malate. 苹果酸; Glucose. 葡萄糖; Glycolysis. 糖酵解; Gluconeogenesis. 糖异生; PEP. 磷酸烯醇式丙酮酸; OAA. 草酰乙酸; Pyruvate. 丙酮酸; Citrate. 柠檬酸; Isocitrate. 异柠檬酸; α-ketoglutarate. α-酮戊二酸; Succinyl-CoA. 琥珀酰辅酶 A; Succinate. 琥珀酸; Glyoxylate. 乙醛酸; Mitochondrion. 线粒体; Glyoxysome. 乙醛酸体; Vacuole. 液泡; Chloroplast. 叶绿体; PEPCK. 磷酸烯醇式丙酮酸羧化酶; PEPCK. 磷酸烯醇式丙酮酸羧化酶; NADP-MDH. NADP-苹果酸脱氢酶; NADP-ME. NADP-苹果酸酶; Acetyl-CoA. 乙酰辅酶 A.

图 1 园艺作物果实苹果酸代谢途径(改编自文献[2, 20])

Fig. 1 Metabolic pathway of malate in the mesocarp of fruits (Adapted from references [2, 20])



再由丙酮酸正磷酸盐二激酶(PPDK)反向催化生成 PEP;另一方面,苹果酸还可以通过 NAD-cyMDH 反向转化为 OAA,然后在 PEPCK 的作用下生成 PEP。PEP 是糖酵解和糖异生作用的中间产物,当果肉细胞内没有足够的葡萄糖进行糖酵解时,PEP 可在果糖 1,6-二磷酸酶和葡萄糖激酶等的作用下反向合成葡萄糖,实现果实苹果酸向可溶性糖的转变,此转变过程通过糖异生途径来实现<sup>[28-30]</sup>。

## 4 园艺作物果实苹果酸转运

苹果酸主要贮存于液泡中,液泡中的跨膜转运与果实酸度密切相关,迄今为止,控制果实苹果酸含量的关键基因多为苹果酸跨膜转运相关基因。苹果酸的跨膜转运不仅需要质子泵来提供能量,还需要苹果酸转运蛋白和离子通道蛋白的协助,目前研究较多的是液泡膜二羧酸转运蛋白(tonoplast dicarboxylate transporter, tDT/TDT)和铝诱导的苹果酸转运蛋白(aluminum-activated malate transporter, ALMT)(表 1)。

### 4.1 苹果酸转运蛋白

拟南芥液泡膜二羧酸转运蛋白(AttDT)是最早发现的一类具有苹果酸转运特性的蛋白,定位于液泡膜上,主要参与苹果酸在液泡和细胞质之间的跨膜转运,还参与调节植物细胞 pH 动态平衡<sup>[37]</sup>。现已在番茄(*SITDT*)<sup>[34]</sup>、柑橘(*CsCit1*)<sup>[38]</sup>、梨(*PbrTDT1*)<sup>[39]</sup>等园艺作物中克隆到 *AttDT* 的同源基因。在拟南芥中过表达 *AttDT* 显著提高了叶片苹果酸含量,但降低了柠檬酸含量<sup>[40]</sup>。在番茄中同源过表达番茄 *SITDT* 和异源过表达梨 *PbrTDT1* 后显著提高了番茄果实苹果酸含量,但降低了柠檬酸含量,说明其与拟南芥 *AttDT* 具有相似功能<sup>[34, 39]</sup>。柑橘 *CsCit1* 则属于柠檬酸/H<sup>+</sup> 同向转运载体,主要介导柠檬酸从液泡流出<sup>[38]</sup>。

### 4.2 苹果酸离子通道蛋白

铝诱导的苹果酸转运蛋白(ALMT)是普遍存在于植物体内的一类阴离子通道蛋白,其部分成员能够参与到苹果酸跨膜转运<sup>[6, 15, 41]</sup>。拟南芥 ALMT 家族被分为 3 个亚家族,其中 ALMTII 家族成员是一类位于液泡膜上、具有苹果酸盐转运功能的通道蛋白,已经发现参与苹果酸转运的成员有 *AtALMT6* 和 *AtALMT9*<sup>[42-43]</sup>,其中 ALMT9 是发现最早且被广泛研究的液泡膜苹果酸通道蛋白<sup>[44]</sup>。在葡萄中,

*AtALMT9* 同源基因 *VvALMT9* 被证明可以调控果实苹果酸和酒石酸积累<sup>[35]</sup>。在苹果中,控制果实酸度的主效候选基因 *Mal* 编码 ALMT,其编码框尾端单碱基 G 突变为 A 时,翻译提前终止,少了 84 个氨基酸,造成编码的蛋白质不完整,丧失苹果酸转运功能,不利于有机酸积累,导致低酸性状形成<sup>[41]</sup>。番茄中控制果实酸度的主要候选基因 *SLALMT9* 同样编码 ALMT 蛋白。*SLALMT9* 基因启动子区 GTC 插入/缺失与自然群体中番茄果实苹果酸含量完全连锁<sup>[6]</sup>。枣中导致果实苹果酸含量自然变异的主要候选基因 *ZjALMT4* 编码 ALMT 蛋白。*ZjALMT4* 基因启动子区 W-box 元件中存在 SNP 位点,在高酸型酸枣中,*ZjWRKY7* 转录因子与 *ZjALMT4* 启动子 W-box 元件相结合,正向调控其转录,促进苹果酸积累;而栽培枣中 W-box 位点突变后降低 *ZjWRKY7* 与之结合的能力,导致苹果酸积累减少。在栽培枣长期驯化过程中低酸突变基因型被选择固定下来<sup>[7]</sup>。番茄 *SLALMT9* 基因、葡萄 *VvALMT9* 基因、苹果 *Mal* 基因与枣 *ZjALMT4* 基因序列同源性较高,表明园艺作物果实苹果酸代谢调控具有一定的保守性。

### 4.3 其他参与苹果酸转运的蛋白

除 tDT 和 ALMT 两种苹果酸转运蛋白之外,最近一个编码液泡膜糖转运蛋白的 *PpTST1* 基因在桃中被证明与果实酸度有关。前人研究表明,液泡膜糖转运蛋白 TST 是负责细胞质葡萄糖向液泡的跨膜运输,部分成员还具备蔗糖转运功能<sup>[45-46]</sup>。我国科学家通过全基因组关联分析确定了控制桃果实非酸/酸含量的关键基因 *PpTST1*,该基因第三个外显子区的单碱基突变被证实与桃果实有机酸含量连锁。在桃和番茄中超量表达 *PpTST1*<sup>46s</sup> 导致果实总糖含量增加、有机酸含量减少,同时导致苹果酸转运相关基因下调表达,表明 *PpTST1* 具备参与桃果实有机酸和糖积累的双重功能<sup>[14]</sup>。

### 4.4 质子泵对苹果酸跨膜转运的作用

质子泵在液泡积累有机酸的过程中起着重要作用。目前植物中与酸度有关的是位于液泡膜上的 V 型[V-H<sup>+</sup>-ATPase(VHA)和 V-H<sup>+</sup>-PPase(VHP)]和 P 型质子泵[P-H<sup>+</sup>-ATPase(PHA)]<sup>[47-51]</sup>。

4.4.1 V 型质子泵 V 型质子泵 VHA 和 VHP 分别通过水解 ATP 或 PPi 产生能量,可将 H<sup>+</sup> 从细胞质转运到液泡致使液泡酸化,也能够为次级转运蛋白的跨膜运输提供能量<sup>[9, 52]</sup>。VHA 结构较为复杂,是由

表1 园艺作物苹果酸转运载体  
Table 1 Malate transport carriers for horticultural crops

| 物种<br>Species                     | 基因<br>Genes    | 登录号<br>Accession numbers          | 基因功能<br>Gene function                                    | 亚细胞定位<br>Subcellular localization         | 转运方向<br>Transport direction | 主要生理功能<br>Main physiological function                               | 调控因子<br>Regulatory Factors  | 参考文献<br>Reference |
|-----------------------------------|----------------|-----------------------------------|--|---|-----------------------------|---|---|-------------------|
| 苹果<br><i>Malus domestica</i>      | <i>Mal</i>     | MDP0000252114                     | 苹果酸转运蛋白<br>Malate transporter protein                    | 液泡膜<br>Tonoplast                          | 流入<br>Influx                | 苹果酸离子通道<br>Malate channel   | <i>MdMYB44</i> 负调控因子<br><i>MdMYB44</i> can inhibit the promoter activity    | [5]               |
|                                   | <i>Mal10</i>   | MDP0000810883                     | 质子泵<br>Proton pumps                                      | 液泡膜<br>Tonoplast                          | 流入<br>Influx                | 质子泵基因,促进苹果酸转运<br>Proton pump gene that facilitates malate transport | -   | [31]              |
|                                   | <i>MdtDT1</i>  | HM641022                          | 跨膜转运体<br>Transmembrane transporter                       | 可能定位于液泡膜<br>May be localized on tonoplast | 可能流入<br>Probably for influx | 促进苹果酸积累<br>Promote malate accumulation                              | -   | [32]              |
|                                   | <i>MdPH1</i>   | MD15G1317200                      | 质子泵<br>Proton pumps                                      | 液泡膜<br>Tonoplast                          | 可能流入<br>Probably for influx | 质子泵基因,促进苹果酸转运<br>Proton pump gene that facilitates malate transport | <i>MdMYB73</i> 正调控因子<br><i>MdMYB73</i> can activate the promoter activity   | [33]              |
|                                   | <i>MdPH5</i>   | MD17G1155800                      | 质子泵<br>Proton pumps                                      | 液泡膜<br>Tonoplast                          | 可能流入<br>Probably for influx | 质子泵基因,促进苹果酸转运<br>Proton pump gene that facilitates malate transport | -   | [33]              |
| 番茄<br><i>Solanum lycopersicum</i> | <i>SLALMT9</i> | Solyc06g072910/<br>Solyc06g072920 | 苹果酸转运蛋白<br>Malate transporter protein                    | 液泡膜<br>Tonoplast                          | 可能流入<br>Probably for influx | 苹果酸离子通道<br>Malate channel   | <i>SlWRKY42</i> 负调控因子<br><i>SlWRKY42</i> can inhibit its promoter activity  | [6]               |
|                                   | <i>SITDT</i>   | KC733165                          | 跨膜转运体<br>Transmembrane transporter                       | 液泡膜<br>Tonoplast                          | 可能流入<br>Probably for influx | 促进苹果酸积累<br>Promote malate accumulation                              | -   | [34]              |
| 桃<br><i>Prunus persica</i> L.     | <i>PpTST1</i>  | Prupe.5G006300                    | 液泡膜糖转运蛋白<br>Vesicular membrane sugar transporter protein | 液泡膜<br>Tonoplast                          | 可能流出<br>Probably for efflux | 调控有机酸的积累<br>Regulation of organic acid accumulation                 | -   | [14]              |
|                                   | <i>VvALMT9</i> | GSVIVG01008270001                 | 苹果酸转运蛋白<br>Malate transporter protein                    | 液泡膜<br>Tonoplast                          | 流入<br>Influx                | 苹果酸(酒石酸)离子通道<br>Malate (tartrate) ion channel                       | -   | [35]              |
| 枣<br><i>Zizyphus jujuba</i>       | <i>ZjALMT4</i> | Zijuj10G0147600                   | 苹果酸转运蛋白<br>Malate transporter protein                    | 液泡膜<br>Tonoplast                          | 流入<br>Influx                | 苹果酸离子通道<br>Malate channel   | <i>ZjWRKY7</i> 正调控因子<br><i>ZjWRKY7</i> can activate its promoter activity   | [7]               |
|                                   | <i>PpALMT9</i> | Pbr020270.1                       | 苹果酸转运蛋白<br>Malate transporter protein                    | 液泡膜<br>Tonoplast                          | 流入<br>Influx                | 苹果酸离子通道<br>Malate channel   | <i>PpWRKY44</i> 正调控因子<br><i>PpWRKY44</i> can activate its promoter activity | [36]              |

注:- 表示目前没有相关报道与相应基因相互关联的调控因子。

Note: - Indicates that there are currently no relevant reported regulatory factors that are interrelated with the corresponding genes.

10多个不同亚基组成的复合物,而VHP仅由单一多肽组成<sup>[48,53]</sup>。然而,关于VHA和VHP在苹果酸积累方面的研究较少。Hu等<sup>[54]</sup>在苹果中过表达VHA亚基*MdVHA-B1*发现果实苹果酸含量升高,盐胁迫处理诱导*MdVHA-B1*蛋白磷酸化从而驱动苹果酸向液泡运输致使果肉细胞苹果酸含量升高<sup>[55]</sup>。Yao等<sup>[52]</sup>发现*MdVHP1*过表达显著促进转基因苹果愈伤组织与番茄果实中Na和苹果酸盐积累。Krebs等<sup>[56]</sup>研究表明拟南芥VHA突变株的叶片pH显著高于野生型,同时突变VHA和VHP后突变体叶片pH显著升高,说明在拟南芥中VHA和VHP共同调控着有机酸的积累,值得注意的是,在同时缺乏V-ATPase和V-PPase活性的突变体中,液泡仍保留着酸化能力,这说明可能还有其他因素参与液泡有机酸的积累<sup>[57]</sup>。

**4.4.2 P型质子泵** P型质子泵是另外一类参与质子转运和液泡酸化的质子泵家族,其中P3亚家族主要参与维持液泡内外的pH平衡和提供跨膜运输驱动力<sup>[58-59]</sup>。位于拟南芥细胞膜上的P型质子泵基因*AHA10*最先被证实参与液泡形成和酸化过程<sup>[60]</sup>。在矮牵牛花中,Faraco等<sup>[61]</sup>证实质子泵基因*PhPH1*和*PhPH5*可以相互作用形成复合体,对液泡中有机酸的积累有调控作用,使花瓣呈现不同的颜色;苹果中*PhPH1*和*PhPH5*同源基因*MdPH1*和*MdPH5*可能参与了液泡酸化和苹果酸积累<sup>[33]</sup>,研究还发现苹果中另外一个P型质子泵基因*Ma10*表达量与苹果酸的积累显著相关,可解释苹果自然群体果实酸度8%左右的表型变异<sup>[31]</sup>;此外,在柠檬酸型果实柑橘中也发现了类似矮牵牛的酸度调控机制,定位于液泡膜上的P型质子泵基因*CitPH1*和*CitPH5/CsPH8*对液泡中酸的积累有调控作用<sup>[62-64]</sup>。拟南芥*AHA10*基因、矮牵牛*PhPH5*基因、苹果*Ma10*基因与柑橘*CsPH8*基因序列同源性较高,表明液泡膜上P型质子泵基因在参与有机酸积累调控方面具有一定的保守性。

## 5 园艺作物果实苹果酸代谢和转运的调控

### 5.1 转录因子对园艺作物果实苹果酸代谢和转运的调控

越来越多研究表明苹果酸转运蛋白和质子泵相关基因的表达受到MYB、bHLH、WRKY多种转录因子的调控<sup>[6,21,33,48,65-68]</sup>。

**5.1.1 R2R3-MYB 转录因子** 在苹果中,R2R3-MYB转录因子*MdMYB1/10*、*MdMYB44*和*MdMYB73*通过直接调控液泡膜苹果酸转运蛋白和质子泵基因表达来调控果实苹果酸积累和液泡酸化<sup>[33,66-67]</sup>。其中*MdMYB1/10*和*MdMYB73*是正调控因子,而*MdMYB44*是负调控因子,它们分别作用于不同的下游基因。*MdMYB1*直接结合并激活质子泵基因*MdVHA-B1*、*MdVHA-B2*、*MdVHA-E*和*MdVHP1*表达,促进苹果酸在液泡中积累<sup>[66]</sup>。*MdMYB73*直接激活下游质子泵基因*MdVHA-A*、*MdVHP1*和苹果酸转运蛋白*MdALMT9*,从而促进果实液泡的酸化<sup>[33]</sup>。*MdMYB44*通过抑制*Ma1*、*MdVHA-A3*、*MdVHA-D2*、*Ma10*和*MaALMT9*启动子活性,负调控苹果果实苹果酸积累,*MdMYB44*启动子区2个遗传变异位点被证实与苹果果实苹果酸含量显著相关<sup>[66]</sup>。同时,MYB转录因子还可以与WD40蛋白和bHLH转录因子形成MBW蛋白复合体,通过直接结合苹果酸转运蛋白相关基因和液泡型质子泵基因启动子,转录激活或抑制其表达,最终影响苹果酸含量<sup>[33,66-67]</sup>。

**5.1.2 bHLH和WRKY 转录因子** 近年,除了MYB转录因子外,bHLH和WRKY转录因子在有机酸代谢中的功能也被发掘<sup>[6-7,36,62,69-70]</sup>。在矮牵牛中,*ANI*(bHLH转录因子)可以与*ANII-PH4*形成复合体正向调节液泡酸化,从而影响pH<sup>[47,71]</sup>。在苹果中,*ANI*的同源基因*MdbHLH3*可以直接激活苹果酸脱氢酶基因*MdcyMDH*表达,促进果实苹果酸积累,也可以与*MdMYB1*形成复合体,促进果肉花青素和苹果酸积累<sup>[21,67]</sup>。在柑橘中,*ANI*的同源基因*CitANI*可以与*CitPH4*形成复合体直接激活P型质子泵基因*CitPH1*和*CitPH5*表达,*CitANI*基因突变会导致柑橘果实酸度降低<sup>[62]</sup>。在拟南芥中,*AtWRKY46*转录因子通过负调控*AtALMT1*基因表达,调控苹果酸跨膜转运<sup>[72]</sup>。在矮牵牛中,编码WRKY的*PH3*基因能够被*ANII-ANI-PH4*复合物诱导转录,通过形成*PH3-ANII-ANI-PH4*复合物,诱导P型质子泵基因*PhPH5*转录从而控制液泡酸度<sup>[60]</sup>。在番茄中,*SIWRKY42*转录因子通过结合W-box元件来负调控*SIALMT9*表达,抑制番茄果实苹果酸积累<sup>[6]</sup>。在枣中,*ZjWRKY7*转录因子通过结合W-box元件正调控*ZjALMT4*表达,促进酸枣果实苹果酸积累,而栽培枣中*ZjWRKY7*与*ZjALMT4*的结合能力较弱<sup>[7]</sup>。



## 5.2 外界环境和栽培条件对园艺作物果实苹果酸的调控

园艺作物果实苹果酸的积累受很多因素的影响,包括温度、水分、光照、矿物营养及土壤盐分胁迫等<sup>[20,73]</sup>。温度是影响果实苹果酸积累和代谢的关键因素,其对园艺作物果实苹果酸含量的影响因树种而异,在桃、苹果等果实发育或者贮藏期间环境温度升高导致果实苹果酸含量降低,而草莓果实发育过程暴露在较高温度下果实苹果酸含量升高<sup>[74-77]</sup>;在葡萄和猕猴桃中研究发现,温度对果实苹果酸积累的影响因发育时期而异,果实发育前期暴露在较高温度下果实苹果酸含量升高,但在果实发育后期暴露在较高温度下果实苹果酸含量减少<sup>[78-80]</sup>。水分是影响果实苹果酸代谢的另一关键因子。研究表明,在大多数情况下,果实发育过程中水分供应量与成熟果实苹果酸含量呈负相关<sup>[81]</sup>。在苹果、葡萄等果实发育过程中,适度干旱胁迫会提高果实中可溶性糖和苹果酸含量<sup>[2,82-83]</sup>。光照度与苹果酸积累关系密切,在蓝莓、葡萄、苹果等研究中发现,光周期延长或光照增强会降低果实苹果酸含量,如苹果成熟时树冠上部和外围的果实酸度较低<sup>[84-86]</sup>,葡萄套袋后造成的弱光胁迫抑制苹果酸降解,导致果实苹果酸含量增加<sup>[87]</sup>。适当增施Ca、P、K肥可降低果实苹果酸含量,而微量元素如铁、铜的缺乏同样也能够使果实酸度升高<sup>[88-89]</sup>。

综上所述,园艺作物果实苹果酸代谢是一个复杂的过程,外部环境条件对果实苹果酸含量的影响错综复杂,因此,园艺作物果实苹果酸的含量受自身遗传因素、环境条件和栽培条件的共同影响。

## 6 总结与展望

通过数十年遗传学研究和多组学技术的应用,目前在以番茄、苹果、梨、枣等为代表的苹果酸型果实园艺作物中研究发现,果实酸含量是一种数量性状,受多种因素(自身和外界)的影响,而遗传因素是影响果实酸度的重要因素,其中位于液泡膜上的苹果酸转运蛋白与质子泵对苹果酸跨液泡膜转运起到重要作用,苹果酸转运蛋白和质子泵相关基因的转录水平受到多种转录因子的调控。综合已有的研究内容,未来研究领域重点可以集中在以下几个方面:(1)利用正向遗传学与反向遗传学研究相结合的技术手段开展果实苹果酸代谢基因挖掘及功能分析;

(2)开发果实酸度性状相关分子标记,用于分子标记辅助育种;(3)挖掘调控苹果酸代谢的转录因子,解析苹果酸代谢机制;(4)研究环境因子(如温度、水分、光照等)对苹果酸代谢和转运的调控机制。

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