Fruit
Research

REVIEW

Open Access

https://doi.org/10.48130/frures-0024-0025
Fruit Research **2024**, 4: e031

Accumulation and regulation of malate in fruit cells

Li-Hua Zhang[1,2], Ao-Ning Zhang[1], Yu Xu[1], Ling-Cheng Zhu[2], Bai-Quan Ma[2] and Ming-Jun Li[2*]

[1] *The Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (Northeast Region), Ministry of Agriculture and Rural Affairs/National-Local Joint Engineering Research Center for Development and Utilization of Small Fruits in Cold Regions, College of Horticulture & Landscape Architecture, Northeast Agricultural University, Harbin 150030, PR China*

[2] *State Key Laboratory of Crop Stress Biology for Arid Areas/Shaanxi Key Laboratory of Apple, College of Horticulture, Northwest A&F University, Yangling 712100, PR China*

* Corresponding author, E-mail: limingjun@nwsuaf.edu.cn

Abstract

Fruit acidity is an important component of flavor quality in fleshy fruit. The accumulation of malate, the dominant organic acid in the acidity formation of most mature fruit, is highly regulated by metabolism and transportation during fruit development. The knowledge on the mechanism of fruit acidification, as well as the major genes and substances is however still limited. In the present paper, the research advances on the relevance between malate accumulation and the genes associated with malate metabolism and transportation, as well as the transcriptional regulation of malate in fruit was reviewed. Furthermore, positive future research could provide a theoretical reference for optimizing fruit quality and genetic improvement.

Citation: Zhang LH, Zhang AN, Xu Y, Zhu LC, Ma BQ, et al. 2024. Accumulation and regulation of malate in fruit cells. *Fruit Research* 4: e031 https://doi.org/10.48130/frures-0024-0025

Introduction

Organic acid, serving as the primary contributor of fruit acidity measured by titrable acidity and pH, influences synergistically taste and flavor quality with soluble sugars in many fleshy fruits[1,2]. Flavor quality is believed to be a vital driver of consumer preference and its formation and regulation mechanism is one of the strategic requirements for the quality optimization and variety genetic improvement of fruit[3]. Knowing the accumulation mechanism of organic acid in fruit cells is necessary to improve fruit quality.

Fruit acidity is due to the accumulation of organic acids such as malic acid, citric acid, and tartaric acid in vacuoles. The composition and content of organic acids in different fruits are significantly different, while malate is the main organic acid presented in most mature fruits[1,4]. Malate content in fleshy fruits is affected gravely by acid metabolism and transport[2,5]. In the cytoplasm, the intermediate product phosphoenolpyruvate (PEP) from glycolysis is catalyzed sequentially to malic acid by PEPC (PEP carboxylase) and NAD-cyt-MDH (NAD–dependent malate dehydrogenase)[1,6–8]. The function of NAD-cyt-MDH on increasing fruit acidity has been defined[8,9], which is believed to be the major pathway for malic acid synthesis[6,10]. Part of the organic acids accumulated in the cytoplasm are consumed as substances and energy to maintain the normal physiological activities of the cells, while the rest are transported and stored in the vacuole[2,11,12]. However, whether it can accumulate in large quantities in vacuoles is highly dependent on specific transmembrane transporters[4,12,13]. Currently, major fruit malate-regulated transporters include aluminum-activated malate transporter ALMT4 and ALMT9/Ma1[14−17] and tonoplast dicarboxylate transporter tDT[4,17,18]. Additionally, H^+ pumped into the cell *via* proton pump protein on the vacuole membrane combine with malate^{2-} to form protonated malate for storage in the vacuole[1,12,13,19], providing continuous driving force for malate^{2-} to enter the cell.

The genetic mechanism of fruit acidity, a quantitative trait regulated by multiple genes, is relatively complex[2,20]. The function and regulatory mechanism analysis of malate metabolism and transport genes during fruit acidity accumulation is of great significance for scientific regulation of fruit quality. With the development of transcriptome, proteomics, and the application of gene fine-mapping technology in forward genetics, researchers have identified a series of metabolic and transport genes related to malate accumulation in apple[20,21], citrus[22,23], peach[24], watermelon[25], jujube[15], and loquat[26], and verified their regulatory functions of fruit acidity. This review focuses on the recent advances in metabolism, transport genes and upstream regulators related to malate accumulation in horticultural fruits and the future research direction of improving fruit quality by molecular biological methods are prospected.

Malate-related metabolic activities in fruit cells

Malate metabolism in the cytoplasm

The accumulation of malate in fruit cells is a complex phenomenon involving many metabolic pathways. The first step of malate synthesis in the cytoplasm is to fix CO_2 to the carbon skeleton from hexose catabolism. The photocontracted products are delivered to the sink fruit over long distance transport of phloem[27], and the glucose produced by its decomposition is converted into the key substrate phosphoenolpyruvate (PEP) of malate metabolism in glycolysis. Then, PEP is converted to malate catalyzed by phosphoenolpyruvate carboxylase (PEPC) and subsequent cytoplasmic NAD-dependent malate dehydrogenase (NAD-cyMDH), and the reverse reaction is

© The Author(s)

mediated by phosphoenolpyruvate carboxykinase (PEPCK)[1]. In addition, malate accumulated in the cytoplasm can be degraded to pyruvate *via* the NADP-dependent malic enzyme (NADP-cyME), and the remaining malate is transported and stored in the vacuole[28]. Therefore, the major enzymes involved in malate metabolism include PEPC, NAD-cyMDH, PEPCK, and NADP-cyME (Fig. 1).

PEPC and NAD-cyMDH are involved in the synthesis and accumulation of malate in fruit. There was a positive correlation between the malate content and the expression levels of *PEPC* in apple and grape fruit during the early developmental stage[29−31]. Similarly, *NAD-cyMDH* has also been shown to promote malate synthesis in young grape fruits[10,32]. In apple, *NAD-cyMDH1* could improve malate accumulation in apple calli and fruit, as well as increase the salt tolerance of apple plants[9,28,33,34]. Recent studies have also demonstrated the important function of another apple gene *NAD-cyMDH5* in enhancing malate accumulation in fruit[35].

Physiological studies have revealed that the malate content of fleshy fruits such as apple, peach, and grape decreases significantly in the later stages of development[10,36], which is closely related to the malate decomposition genes *NADP-cyME* and *PEPCK*. Sweetman et al. pointed out that NADP-ME had low activity in the early stage of grape fruit development and was involved in the malate generating by fixing CO_2[10]. As fruits gradually matured, the activity of NADP-ME increased significantly and began to catalyze the degradation of malate. Additionally, *NADP-cyME* is thought to be involved in the reduction of malate content during the ripening of loquat, apple, and grape fruit[6,37,38].

In the later stage of fruit development, soluble sugars accumulated in large quantities, sugar catabolic metabolism slowed down, and vacuolar malate was released into the cytoplasm as a carbon source to participate in energy metabolism and compound biosynthesis through gluconeogenesis[10]. The first step of gluconeogenesis is the production of PEP catalyzed by PEPCK or phosphopyruvate dikinase (PPDK)[39,40]. Due to the bare expression of *PPDK* in fruits, *PEPCK* is believed to be more essential in the gluconeogenesis of fruits, which has been validated in grape skins and ripe peach, blueberry, redcurrant, and raspberry fruits[41−43]. Compared with high-acid varieties, PEPCK activity in low-acid apples increased threefold, and ^{14}C-labeled malate consumption was higher, suggesting that gluconeogenesis might increase malate consumption[36]. Recent studies have suggested that the large accumulation of malate in the cytoplasm altered soluble sugar content in apple fruit, which might be caused by the up-regulating expression of *MdPEPCK* in gluconeogenesis[8]. In conclusion, PEPCK plays a key role in the regulation of malate accumulation in fruit.

Malate metabolism in mitochondria and glyoxysome

Malate in the TCA cycle can be oxidized *via* two competing metabolic pathways. It is converted reversibly to OAA by NAD-mtMDH[10], or converted to pyruvate by NAD-mtME[44], which ultimately results in citrate synthesis and an altered ratio of malate and citrate. Omics analysis showed that NAD-mtMDH in mitochondria mainly catalyzed malate degradation during fruit ripening[10,32]. While NAD-mtMDH can also catalyze the malate synthesis when the conditions exist for the reversible reaction of the TCA[7,45]. In addition, the regulation of NAD-mtMDH varies among species. NAD-mtMDH appears to be regulated by the gene expression in loquat[46], while regulated at the post-transcriptional level in strawberry[47].

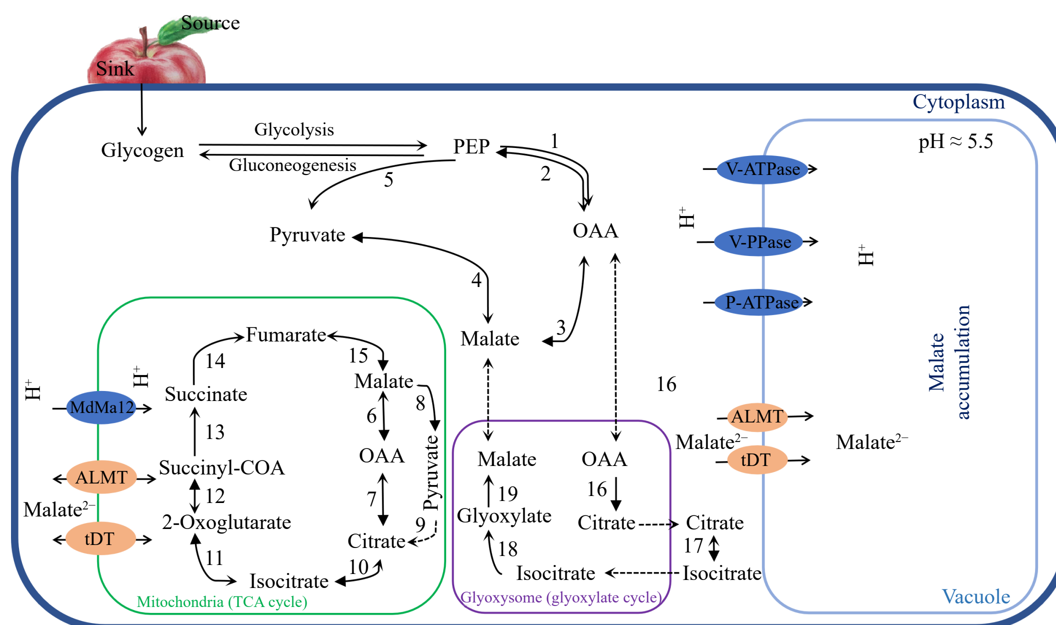

Fig. 1 A simplified model for malate metabolism, transport and accumulation in fruit. Major enzymes involved in malate metabolism in fruit: 1, phosphoenolpyruvate carboxylase (PEPC); 2, phosphoenolpyruvate carboxykinase (PEPCK); 3, NAD-cyMDH (NAD-cytoplasmic malate dehydrogenase); 4, NADP-cytoplasmic malate enzyme (NADP-cyME); 5, pyruvate kinase (PK); 6, NAD-mitochondrial malate dehydrogenase (NAD-mtMDH); 7, mitochondrial citrate synthase (mtCS); 8, NAD-mitochondrial malate enzyme (NAD-mtME); 9, pyruvate dehydrogenase (PDH); 10, mitochondrial aconitase (mtACO); 11, isocitrate dehydrogenase (ICDH); 12, α-oxoglutarate dehydrogenase (α-OGDH); 13, succinyl-coa synthase (SCS); 14, succinate dehydrogenase (SuDH); 15, fumarase; 16, cytoplasmic citrate synthase (cyCS); 17, glyoxylate aconitase (glyACO); 18, isocitrate lyase (ICL); 19, malate synthase (MS).

Malate synthase glyMS located in the glyoxysome is involved in malate synthesis in the glyoxylic acid cycle[48] (Fig. 1). Studies have shown that the glyoxylic acid cycle is activated during the ripening of banana fruit after harvest, providing substrates for gluconeogenesis[49]. Pua et al.[50] detected the expression of *glyMS* only in banana fruit tissues, its expression was up-regulated during the whole ripening stage, whose change was similar to the trend of malate accumulation. However, ICL proteins required for glyoxylate (MS catalytic substrates) synthesis in the glyoxylate cycle was not detected at any stage of development in raspberry, blueberry, strawberry or redcurrant fruits[41], suggesting that the malate accumulation involved in the glyoxylate cycle during fruit ripening may be species-specific.

Malate transport

Malate synthesized in the cytoplasm and mitochondria will be transported and stored in vacuoles[7,35,51]. The passage of malate into or out of the vacuole requires specific anion channels or transporters located on the vacuole membrane as carriers, which can specifically recognize and transport malate[52,53]. In the cytoplasm with near-neutral pH, most malic acid exists in anion forms, while they will combine with cations (such as K^+, Na^+, Ca^{2+}), forming salt in the acidic vacuole, which maintains an electrochemical gradient between the vacuole, allowing subsequent organic acids to enter the vacuole[1,53]. The main transporters associated with malate transmembrane delivery identified in horticultural crops include malate channel protein ALMT (aluminum-activated malate transporter), malate transporter tDT (tonoplast dicarboxylate transporter), and proton pump.

Aluminum-activated malate transporter

ALMT encodes aluminum-activated channel protein with typical transmembrane domains that transport $malate^{2-}$ in different organelles[54,55]. *ALMT1* was initially identified in wheat root tip tissue and its malate transport properties were confirmed by the heterologous expression of xenopus cells[56]. Subsequently, researchers identified successively a series of ALMT proteins in the model plant *Arabidopsis*[57,58], major crops[59,60], vegetables[61,62] and fruit trees[63,64].

The ALMT family is mainly divided into four subfamilies (ALMT I–IV) and the protein functions of each subfamily are various, mainly including aluminum tolerance, symbiotic nitrogen fixation, fertilization, ion transport, stomatal regulation, and fruit flavor[65−67]. Therein, most of the ALMTs of the subfamily I and II are mainly located on the vacuole membrane and participate in malate transportation in plant cells. For example, the ALMT subfamily I genes *AtALMT1*, *BnALMT1/2, HvALMT1* and *ZmALMT2* from respectively *Arabidopsis* (*Arabidopsis thaliana*), rape (*Brassica napus*), barley (*Hordeum vulgare* L.), and maize (*Zea mays* L.) are primarily expressed in root and function on the regulation of malate secretion[68−71]. The interference of *LaALMT1* (*Lupinus albus*) led to a decrease in malate concentration in xylem sap[72], and the ALMT subfamily II genes *SlALMT5* significantly increased malate and citrate contents in tomato seeds[73,74]. SlALMT11 located in leave guard cells transports malate to mediate stomatal closure in tomato[75]. *AtALMT6* and *AtALMT9* mediated the transcellular transport of malate or fumaric acid[57,75,71]. Moreover, it was found that AtALMT6 and AtALMT9 have strong inward rectification *via* membrane electrophysiology techniques, and malate transport only occurred under the condition of positive potential inside the vacuolar membrane[75,76]. As the vacuole pH decreases, the pathway by which *AtALMT6* and *AtALMT9* transport malate might be closed, which may be an active protection against excessive acidification of the vacuole.

ALMT family genes are closely related to the regulation of fruit acid quality. Therefore, the function of *ALMT9* on fruit vacuole acidification in various horticultural crops is the most widely studied. The *Ma1* located on chromosome 16 in apple encodes an *ALMT9* homologous gene, which is considered to be the main gene controlling the acidity of apple fruit[16,63,77]. The single nucleotide polymorphism (SNP) of *Ma1* at 1,455 bases causes the premature termination of its protein translation, thereby losing the capability to transport malate into the vacuole, which is closely related to the decrease of fruit acidity[77]. In general, the acidity of mature apple fruit of genotype *ma1/ma1* was significantly lower than that of genotype *Ma1/Ma1* or *Ma1/ma1*[55,78]. While vacuolar membrane-localized *VvALMT9* and *SlALMT9* also are the homologs of *AtALMT9* in grapes and tomatoes, respectively. *VvALMT9* mediates the transport of malic acid and tartaric acid in grape fruit[79]. *SlALMT9*, located on chromosome 6 of tomato, is a major gene leading to the variation of malate content in tomato fruit, and the deletion of 3-bp in the promoter region of *SlALMT9* destroys the W-box binding site and prevents the binding of its upstream transcriptional suppressor *SlWRKY42*, resulting in a high accumulation of malate in fruit[62]. Recent studies have also indicated that *PpALMT9* mediated the accumulation of malate in pear fruit under salt stress[14]. Additionally, overexpression of *ZjALMT4 and AcALMT1* significantly promoted the increase of organic acid content in sour jujube (*Ziziphus jujuba* Mill.) and in kiwifruit (*Actinidia* spp.) fruit[15,80]. Allogeneic expression of the ALMT family gene *Pbr020270.1* of pear (*Pyrus bretschneideri*) could increase malate accumulation in tomato fruit[64]. While, a *CitALMT* gene of citrus (*Citrus reticulata* B.) negatively affects citrate accumulation in citrus fruit[81]. These results indicate that the aluminum-activated channel protein ALMT regulates malate accumulation in fruit vacuoles.

Tonoplast dicarboxylate transporter

Tonoplast dicarboxylate transporters (tDT) are the first class of transmembrane transporters with malate transport properties discovered in plants[82]. In contrast to malate ion channel proteins, tDT has little rectification and plays an essential role in maintaining intracellular pH homeostasis[83]. Researchers first identified and demonstrated the acid transport function of *AttDT* in *Arabidopsis thaliana*. Overexpression of *AttDT* significantly increased malate content and decreased citrate accumulation in *Arabidopsis* leaves[84,85]. Further studies confirm that *AttDT* can also transport fumaric acid and succinic acid, and participate in the regulation of cytoplasmic pH homeostasis[83,85,86].

So far, *AttDT* homologous genes have been isolated from various fruit such as apple (*Malus domestica*), tomato (*Solanum lycopersicum*), grape (*Vitis vinifera*), and citrus (*Citrus sinensis*). During citrus maturation, the *AttDT* homologous gene, *CsCit1*, encodes a vacuolar $citrate^{3-}/H^+$ symporter that mediates the effluence of H^+ and $CitH^{2-}$ in vacuole to maintain vacuolar acidic pH and citrate balance[87]. Lin et al.[88] pointed out that CitDIC, a dicarboxylate transporter, and CitCHX, a cation/H^+ exchange protein, were involved in the degradation of citrate during fruit development and the reduction of citrate in fruit after harvest triggered by hot air. The content of malate in *SltDT* overexpressed tomato fruit was significantly increased, while

the citrate accumulation was inhibited[89]. Similarly, *MdtDT* negatively regulates the citrate content[90] and positively participates in the accumulation of malate in cultivated apple fruit[11,91,92]. The *AttDT* homologous gene in grape fruit is actively transporting tartaric acid into the vacuole[93]. In addition, mitochondrial dicarboxylate transporters *VvDTC2* and *VvDTC3* identified in grape are likely responsible for malate transport to mitochondria in grape fruit[94]. These studies indicate that *tDT* positively regulates malate and negatively regulates citrate accumulation in most fruit.

Proton pump gene family

The transmembrane transport of malate is also affected by the vacuole pH and the electrochemical gradient ($\Delta\psi$) inside and outside the vacuole[1], while the activity and function of the proton pump greatly affects the vacuole pH and $\Delta\psi$. Proton pump is a kind of membrane-integrated glycoprotein that can transport H^+ across membranes against the concentration gradient, which mostly exists in the vacuolar membrane and plasma membrane, mainly including V-ATPase/H^+-ATPase, V-PPase/H^+-PPiase and P-ATPase[1,12,13,19], they pump H^+ into the vacuole by hydrolyzing ATP or pyrophosphate, reducing the vacuole pH while increasing the $\Delta\psi$ on both sides of the membrane, thereby providing power for the transport of organic acids.

V-ATPase and V-PPase are widely present in a variety of horticultural crops and are involved in secondary metabolite transport, vacuole acidification, ion homeostasis, and stress tolerance[95−98]. Although V-ATPase and V-PPase are both effective in acidifying vacuoles, their activity varies in different plants and at different developmental stages of the same plant. V-ATPase is the main proton pump in the vacuoles of most horticultural plants, but V-PPase is more active than V-ATPase in some C4 plants. A large amount of highly active V-PPase is enriched in the early development stage of young tissue, hydrolyzing and removing pyrophosphate to inhibit the polymerization reactions such as RNA and starch synthesis. While, the synthesis of pyrophosphate in mature tissues is reduced and cell respiration continues to provide ATP, so V-ATPase activity dominates[4,99]. The activity analysis of the proton pump during the development of pear (*Pyrus pyrifolia*) fruit supported the above conclusion. V-PPase activity was highest in young fruit and decreased with the maturation of pear fruit, whereas V-ATPase activity was highest in mature fruit[100]. However, V-PPase is also the main vacuolar proton pump in grape berries whose vacuoles are strongly acidic (pH < 3)[93].

The expression patterns of *V-ATPase* and *V-PPase* were similar in high-acid and low-acid loquat varieties, but their expression levels were higher in low-acid varieties[26]. Etienne et al.[101] found that the expression of *V-ATPase* and *V-PPase* in the fruit of different peach varieties were positively correlated with organic acid accumulation, indicating that *V-ATPase* and *V-PPase* were involved in the regulation of organic acid accumulation in fruit. Overexpression of V-ATPase proton pump *MdVHP1* in apple calli increased the accumulation of malate and soluble sugar in vacuoles[91]. Further studies confirmed the active function of *MdVHA-A3*, *MdVHA-D2*, *MdVHA-B1*, *MdVHA-B2*, *MdVHA-E2*, *CitAHA10*, and *CitVHA-c4* in fruit vacuole acidification[92,102−104]. Moreover, a mitochondria-targeted PPase gene, *Ma12*, was identified in apple and its overexpression increased malate accumulation in apple calli and tomato fruit by up-regulating the expression of mitochondrial malate dehydrogenase *mMDH12*[7]. These results fully demonstrated the indispensable functions of V-ATPase and V-PPase in fruit vacuole acidification.

P-ATPase is a new class of proton pump genes with proton transport and vacuolar acidification properties, which are divided into five subfamilies (P1−P5), among which the P_3 subfamily ATPase is involved in the transport of organic acids. P_{3A}-ATPase proton pump *PhPH5*, which is localized in the vacuolar membrane in petunias interacts with the P_{3B}-ATPase proton pump *PhPH1* to form a complex, which affects petal color by acidifying the vacuole[105,106]. Interestingly, the vast majority of P_{3A} subfamily members are located in the plasma membrane, while only *PhPH5* belongs to the vacuole membrane localization gene, exhibiting a strong ability to transport protons across membranes. It is unclear how *PhPH5* acquired this unique cellular localization during evolution. The pH1−pH5 complex can reduce the stoichiometric value of H^+/ATP from 1.0 to 0.5 for super acidifying vacuoles[107].

The function of PH1−PH5 complex highly acidifying vacuoles exists only in a few angiosperms, and PH1 homologs are lacking in most plants[106]. How the independent loss of PH1 homologs occurs in multiple plants are unclear. Studies have shown that *PH1* and *PH5* can be expressed ectopically in plants where certain tissues do not express them, resulting in a decrease of vacuole pH in the corresponding tissues[108]. At present, the researchers have begun to explore whether the pH1−pH5 complex has the function of acidifying fruit vacuoles. The hyper-acidification of citrus fruit is regulated by the P-ATPase complex CitPH1-CitPH5[109]. Similarly, the *PhPH5* homolog *Ma10* and the *PhPH1* homolog *Ma13* in apple were reported to regulate the malate accumulation in apple calli and tomato fruit[12,110], however, it is not clear whether there is an interactive relationship between the *Ma10* and *Ma13*. The interference of *VvWRKY26* and *VvMYB5* in grape leaves significantly decreased the transcriptional expression of *VvPH5* and *VvPH1*, causing increased vacuole pH[111]. In the same year, researchers identified and proved that the expression level of *CsPH8*, a homologous gene of *PhpH5*, was highly consistent with the changing trend of citrate content in various citrus fruits at different developmental stages, and overexpression of *CsPH8* significantly increased the citrate accumulation in strawberry fruit[22]. Similarly, overexpression of the P_{3A}-ATPase proton pump gene *PbPH5* also significantly increased the malate accumulation in pear fruit[112]. It can be seen that P-ATPase alone or in the complex form, both play an important roles in vacuole acidifying.

Transcriptional regulation of malate accumulation

Transcriptional regulation of malate metabolism

Many transcription factors play essential roles in the regulation of malate content by influencing the expression of genes related to malate metabolism and transport (Table 1). *MdcyMDH1* is identified as a major gene associated with malate accumulation *via* MapQTL in 'Honeycrisp' × 'Qinguan' F1 hybrids, and after *MdcyMDH1* is overexpressed, the malate concentration of fruit is enhanced[8,9,28]. MdbHLH3 and MdWRKY126 directly activated the transcriptional expression of *MdcyMDH1*, and also increased the expression of malate transport-related genes such as *MdtDT*, increasing malate content in fruit[34,35]. The indel of a repeat sequence in *MdcyMDH1* (*MA7*) promoter region in 'Gala' (*MA7/MA7*) and 'Fuji' (*ma7/ma7*) apple

Table 1. The crucial genes and their upstream regulatory factors of fruit acidity regulation.

Gene family	Gene name	Protein name	Activation (+)/ inhibition (−)	Module control	Species
MYB transcription factor	*MdMa1*	MdMYB73	+	MdBT2-MdClbHLH1 -MdMYB73	*Malus domestica*
	MdMa1/MdMa11	MdMYB123	+	—	*Malus domestica*
	MdMa1	MdMYB44	−	MdbHLH49-MdMYB44	*Malus domestica*
	MdMa1	MdMYB21	−	—	*Malus domestica*
	MdVHA-A3/D2 Ma10	MdMYB44	−	WD40-MdbHLH49 -MdMYB44	*Malus domestica*
	MdVHA-B1/E MdVHP1 MdtDT	MYB1/10	+	MdTTG1-MdbHLH3 -MdMYB1/10	*Malus domestica*
	MdVHA-A MdVHP1	MdMYB73	+	MdBT2- WD40-MdbHLH1 -MdMYB73	*Malus domestica*
	CitPH5	CitPH4	+	CitTRL-CitPH4	*Citrus reticulata*
WRKY transcription factor	*SlALMT9*	SlWRKY42	−	—	*Solanum lycopersicum*
	PpALMT9	PpWRKY44	+	PpABF3-PpWRKY44	*Pyrus spp.*
	ZjALMT4	ZjWRKY7	+	—	*Ziziphus jujuba*
	MdMa1	MdWRKY31	+	—	*Malus domestica*
	MdMDH1	MdWRKY126	+	—	*Malus domestica*
bHLH transcription factor	*MdMDH1*	MdbHLH3	+	—	*Malus domestica*
NAC transcription factor	*AcALMT1*	AcNAC1	+	—	*Actinidia spp.*
ERF transcription factor	*MdMa1*	MdERF72	−	MdWRKY31-MdERF72	*Malus domestica*
	CitVHA-C4	CiERF13	+	—	*Citrus reticulata*
PP2C family	*MdVHA-A3/B2/D2 Ma10*	MdPP2CH	−	SAUR37-MdPP2CH	*Malus domestica*

varieties were named respectively '*MA7*' and '*ma7*', and the upstream regulator, MdbHLH74, could enhance the expression of *MdcyMDH1* in apple with *MA7/MA7* genotype, but not with *ma7/ma7* genotype, affecting the malate content in different varieties[9]. Additionally, *TRXL1* up-regulates NADP-cyMDH activity, increases malate accumulation, and inhibits superoxide radical formation in response to high-temperature stress, and the expression of *TRXL1* is positively regulated by *CPN60A* and negatively regulated by *CLPC1*[113]. Based on current research, the main object of transcriptional regulation of malate metabolism is cytoplasmic malate dehydrogenase, and the regulatory network of other malate metabolism-related genes need to be further studied.

Transcriptional regulation of malate transportation

Jia et al.[20] identified three major genes associated with malate transport (*MdPP2CH*, *MdMYB44*, and *MdSAUR37*) *via* MapQTL and BSA-seq and verified their functions. MdPP2CH reduced the malate accumulation by phosphorylating the proton pump gene in apple calli, while MdSAUR37 could inhibit the phosphorylation activity of MdPP2CH and positively regulate malate content. Another acid accumulation major gene, MdMYB44, negatively regulated proton pump gene *Ma10*, *MdVHA-A3*, and *MdVHA-D2* and malate transporter *Ma1* to inhibit fruit vacuole acidification. A further study indicated that the presence of SNP (A/T) in the *MdMYB44* promoter affected the ability of its upstream transcription factor MdbHLH49 to regulate the activity of the *MdMYB44* promoter and malate accumulation of fruit[104]. Additionally, other MYB transcription factors also play an essential role in proton pump regulation. Apple MdMYB1/10 directly binds and activates the expression of proton pump genes *MdVHA-B1*, *MdVHA-B2*, *MdVHA-E2*, and *MdVHP1*, accelerating the malate accumulation of vacuoles[92]. The MdClbHLH1-MdMYB73 module regulates downstream proton pump genes *MdVHA-A*, and *MdVHP1* for the acidification of fruit vacuole[11], while the MdBT2 response to nitrate treatment could ubiquititatively degrade MdClbHLH1, and malate content in *MDBT2*-silenced apple calli is significantly upregulated[114].

Studies on the transcriptional regulation of malate transporters focus on tDT and ALMT proteins. *tDT* positively regulates the malate content and negatively participates in the accumulation of citrate in tomato and apple fruit[89,90], which was regulated by transcription factor MdMYB1[92], MdMYB73[11] and MdbHLH3[34]. The latest study revealed that AP2 domain-containing transcription factor MdESE3 activate their expression of *MdtDT*, *MdMa11*, and *MdMDH12* to increase malate accumulation in apple[18]. While, ALMT9, the major contributor of fruit malate accumulation, has constant attention from reseachers, and an increasing number of transcriptional regulatory mechanisms regarding the influence of *ALMT9* on fruit acidity have emerged. The *ALMT9* homologous genes have been identified as a crucial gene functioning in enhancing malate transport, and vacuole acidification in various horticultural crops such as apple[77], tomato[62], grape[79], and pear[14], where expression is regulated respectively by the transcription factors MdWRKY31-MdERF72[115], MdMYB73[11,114], MdMYB21[116], MdMYB123[117], MdMYB44[104], SlWRKY42[62], and PpABF3-PpWRKY44[14] (Fig. 2). Interestingly, the malate content in stable *Ma1*-overexpressed apple fruits was significantly reduced. Further studies showed that alternative splicing generates two *Ma1* isoforms (higher expression of MA1α and lower expression of Ma1β). Ma1β is only able to form polymers with MA1α protein for strong malate transport function and the absence or reduced transport activity of MA1α/Ma1β polymers in *Ma1* transgenic fruits decreased malate accumulation, which was regulated by MdMYB73[16].

Protein post-translational modification

Protein post-translational modification is a kind of chemical modification existing in the late stage of protein biosynthesis that affects the protein stability and activity by changing different biochemical functional groups on amino acid residues of proteins, including protein phosphorylation, ubiquitination,

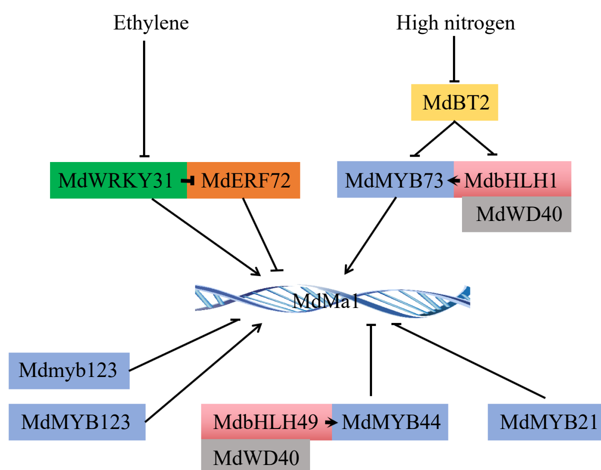

Fig. 2 Upstream regulators of major gene *Ma1* regulating apple fruit acidity. The arrows represent positive regulation and the rest represent negative regulation.

acetylation and methylation. MdPP2CH decreased the malate content *via* phosphorylating the proton pump MdVHA-A3, MdVHA-B2, MdVHA-D2 and ALMT transporter MdALMTII, and its dephosphatase activity was inhibited by MdSAUR37 in apple[20]. Some enzymes involved in malate metabolism, such as malate dehydrogenase (MDH2) and phosphoenolpyruvate carboxykinase (PEPCK1), are also regulated by either the glucose-induced degradation-deficient pathway or the vacuole import and degradation pathway[118], but the specific protein modification mechanisms remain to be investigated.

Posttranslational modification except for the above directly modified functional proteins associated with malate accumulation, can also modify upstream regulatory proteins of functional genes to affect fruit acidity. The ubiquitin E3 ligase MdCOP1 degrades MdMYB1 in the dark through a ubiquitin-dependent pathway to regulate anthocyanins and malate accumulation[92,119]. Similar, high glucose-inhibited U-box-type E3 ubiquitin ligase MdPUB29 and glucose sensor MdHXK1 ubiquitinates and phosphorylates MdbHLH3, respectively, affecting malate concentration[120,121]. It was found that the apple transcription factor MdClbHLH1 acidified fruit vacuole by enhancing the activity of MdMYB73, which promoted the up-regulated expression of *MdVHA-A*, *MdVHP1* and *MdALMT9*. While BTB-BACK-TAZ domain protein MdBT2 degrades ubiquitiatively MdClbHLH1 and MdMYB73 *via* ubiquitin/26S proteasome pathway to regulate the malate content of vacuole in apple plants under nitrate stress[114,122]. These studies provide groundbreaking insights into the direct posttranslational modification of organic acid-related functional proteins and their upstream regulatory proteins, which is helpful to cultivate high-quality horticultural crop varieties from the perspective of post-translational modification.

Future research

To date, researchers have performed some basic research on the accumulation of malate in horticultural crops. Members of the acid metabolism and transport families have been screened and identified at the genome-wide level in most horticultural crops, and their function on vacuole acidification has been demonstrated in apple, tomato, pear, and *Arabidopsis*. However, the systematic regulatory network of malate accumulation and the cross-regulation between sugar and acid metabolism and transport remain to be further explored. Therefore, the following suggestions are put forward for the future research direction of malate accumulation regulation in horticultural crops.

Epigenetic regulation

Epigenetics is a kind of 'post-genetics' that can achieve genetic heritability under the premise that the nuclear DNA sequence remains unchanged through the methylation modification or histones acetylation, phosphorylation, and ubiquitination of gene promoter DNA. This regulatory mechanism of epigenetic modification has been elucidated to some extent in fruit soluble sugar and anthocyanin accumulation[123−125], indicating epigenetic modification plays a crucial role in fruit quality formation. In pummelo (*Citrus maxima* LCA) and lemon (*Citrus limon* (L.) Burm f.), DNA methylation changes in promoters of key genes involved in citrate synthesis and accumulation directly affect the citrate content in the flesh[23,126], revealing a previously unexplored link between epigenetic regulation and organic acid accumulation of horticultural fruits. A recent study identified a *CgAN1*, BHLH-type regulator coupling citrate and anthocyanin, from citrus varieties with high citrate, anthocyanin, and low citrate, anthocyanin, and confirmed that the reduction of the methylation level of the gene promoter can enhance the citrate accumulation of fruit[23]. However, the epigenetic mechanism related to fruit acidity, especially malate, is relatively limited, the urgent task is to uncover the complex DNA methylation mechanisms controlling key genes in malate synthesis, and accumulation pathways in horticultural crops.

Cross-regulation on the accumulation of soluble sugars and organic acids

The differences in the composition and content of soluble sugars and organic acids play a decisive role in fruit quality, and flavor[127]. Physiological studies have revealed that during the ripening process of fleshy fruit such as apple, peach, and grape, organic acid content decreases, and soluble sugar accumulation increases[10,28,36,128]. The variousness in malate content caused by the overexpression of *MDH* or *ME* genes could lead to a change in the redox state of the plastid thus affecting the accumulation of starch and sugar in tomato fruit cells[129,130]. Yao et al.[28,91] pointed out that the overexpression of *MdVHP1* and *MdcyMDH1* in apple calli both increased malate and soluble sugar contents, and the effect of *MdcyMDH1* on malate and soluble sugar accumulation was regulated by transcription factor MdbHLH3[34]. While high glucose-inhibited U-box-type E3 ubiquitin ligase MdPUB29 and glucose sensor MdHXK1 ubiquitinates and phosphorylates MdbHLH3, respectively, affecting the expression of its downstream genes[120,121]. FaMYB44.2 could inhibit the expression of *FaSPS*, reducing both the sucrose and malate content in banana fruit[131]. Similarly, the increased accumulation of malate, citrate, glucose, and fructose was observed in *SlAREB1* overexpressed red ripe peel compared to antisense-inhibited lines[132]. A recent study has also indicated that the accumulation of malate in the cytoplasm mediated by *MdcyMDH1* increased the sucrose content in apple fruit by up-regulating the expression of *MdSPS*, which is likely to be achieved *via* starch cleavage or gluconeogenesis[8]. These above studies indicate that there is an interactive relationship between carbohydrate and organic acid accumulation in fruit. However, the spatiotemporal crosstalk between sugars and acids during fruit development remains unclear. Therefore, elucidation of the potential cross-regulatory mechanisms of

sugars and acids in fruit is important to optimize the ratio of sugars to acids in fruit and improve fruit quality.

The exploration of a clear and thorough regulatory network of malate accumulation and regulation in horticultural crops relies on the functional identification of major genes in malate metabolism and transport in fruit and the in-depth study of the above directions, which lays an important foundation for the improvement of fruit quality *via* molecular-assisted breeding.

Author contributions

The authors confirm contribution to the paper as follows: study conception and design: Zhang LH; data collection: Li MJ, Zhang LH, Zhang AN, Xu Y; analysis and interpretation of results: Zhang LH, Zhang AN, Xu Y, Zhu LC, Ma BQ; draft manuscript preparation: Zhang LH, Zhang AN, Xv Y, Zhu LC, Ma BQ. All authors reviewed the results and approved the final version of the manuscript.

Data availability

All data generated or analyzed during this study are included in this published article.

Acknowledgments

This work was supported by the Outstanding Youth Science Foundation of Heilongjiang Province (YQ2023C006), the China Postdoctoral Science Foundation (2023MD744175), the Talent Introduction Program of Northeast Agricultural University of China, and Modern Agricultural Industrial Technology Collaborative Innovation and Promotion System of Heilongjiang Province. The authors would like to thank Mr. Li Dalong from Northeast Agricultural University for providing the instruments.

Conflict of interest

The authors declare that they have no conflict of interest.

Dates

Received 1 May 2024; Revised 2 July 2024; Accepted 13 July 2024; Published online 2 September 2024

References

1. Etienne A, Génard M, Lobit P, Mbeguié-A-Mbéguié D, Bugaud C. 2013. What controls fleshy fruit acidity? A review of malate and citrate accumulation in fruit cells. *Journal of Experimental Botany* 64:1451−69
2. Wu W, Chen F. 2016. Malate transportation and accumulation in fruit cell. *Endocytobiosis and Cell Research* 27:107−12
3. Jaeger SR, Andani Z, Wakeling IN, MacFie HJH. 1998. Consumer preferences for fresh and aged apples: a cross-cultural comparison. *Food Quality and Preference* 9:355−66
4. Huang XY, Wang CK, Zhao YW, Sun CH, Hu DG. 2021. Mechanisms and regulation of organic acid accumulation in plant vacuoles. *Horticulture Research* 8:227
5. Yang M, Hou G, Peng Y, Wang L, Liu X, et al. 2023. *FaGAPC2/FaPKc2.2* and *FaPEPCK* reveal differential citric acid metabolism regulation in late development of strawberry fruit. *Frontiers in Plant Science* 14:1138865
6. Yao YX, Li M, Liu Z, You CX, Wang DM, et al. 2009. Molecular cloning of three malic acid related genes *MdPEPC*, *MdVHA-A*, *MdcyME* and their expression analysis in apple fruits. *Scientia Horticulturae* 122:404−8
7. Gao M, Zhao H, Zheng L, Zhang L, Peng Y, et al. 2022. Overexpression of apple *Ma12*, a mitochondrial pyrophosphatase pump gene, leads to malic acid accumulation and the upregulationof malate dehydrogenase in tomato and apple calli. *Horticulture Research* 9:uhab053
8. Zhang L, Wang C, Jia R, Yang N, Jin L, et al. 2022. Malate metabolism mediated by the cytoplasmic malate dehydrogenase gene *MdcyMDH* affects sucrose synthesis in apple fruit. *Horticulture Research* 9:uhac194
9. Gao M, Yang N, Shao Y, Shen T, Li W, et al. 2024. An insertion in the promoter of a malate dehydrogenase gene regulates malic acid content in apple fruit. *Plant Physiology* 00:kiae303
10. Sweetman C, Deluc LG, Cramer GR, Ford CM, Soole KL. 2009. Regulation of malate metabolism in grape berry and other developing fruits. *Phytochemistry* 70:1329−44
11. Hu DG, Li YY, Zhang QY, Li M, Sun CH, et al. 2017. The R2R3-MYB transcription factor MdMYB73 is involved in malate accumulation and vacuolar acidification in apple. *The Plant Journal* 91:443−54
12. Ma B, Liao L, Fang T, Peng Q, Ogutu C, et al. 2019. A *Ma10* gene encoding P-type ATPase is involved in fruit organic acid accumulation in apple. *Plant Biotechnology Journal* 17:674−86
13. Martinoia E. 2018. Vacuolar transporters – companions on a longtime journey. *Plant Physiology* 176:1384−407
14. Alabd A, Cheng H, Ahmad M, Wu X, Peng L, et al. 2023. ABRE-BINDING FACTOR3-WRKY DNA-BINDING PROTEIN44 module promotes salinity-induced malate accumulation in pear. *Plant Physiology* 192:1982−96
15. Zhang C, Geng Y, Liu H, Wu M, Bi J, et al. 2023. Low-acidity *ALUMINUM-DEPENDENT MALATE TRANSPORTER4* genotype determines malate content in cultivated jujube. *Plant Physiology* 191:414−27
16. Li C, Krishnan S, Zhang M, Hu D, Meng D, et al. 2024. Alternative splicing underpins the ALMT9 transporter function for vacuolar malic acid accumulation in apple. *Advanced Science* 11:2310159
17. Li W, Lu X, Li J. 2022. The effect of organic nutrient solution on flavor in ripe cherry tomato fruit—Transcriptome and metabolomic analyses. *Environmental and Experimental Botany* 194:104721
18. Zheng L, Ma W, Liu P, Song S, Wang L, et al. 2024. Transcriptional factor MdESE3 controls fruit acidity by activating genes regulating malic acid content in apple. *Plant Physiology* 00:kiae282
19. Cohen S, Itkin M, Yeselson Y, Tzuri G, Portnoy V, et al. 2014. The *PH* gene determines fruit acidity and contributes to the evolution of sweet melons. *Nature Communications* 5:4026
20. Jia D, Shen F, Wang Y, Wu T, Xu X, et al. 2018. Apple fruit acidity is genetically diversified by natural variations in three hierarchical epistatic genes: *MdSAUR37*, *MdPP2CH* and *MdALMTII*. *The Plant Journal* 95:427−43
21. Ban S, Xu K. 2020. Identification of two QTLs associated with high fruit acidity in apple using pooled genome sequencing analysis. *Horticulture Research* 7:171
22. Shi CY, Hussain SB, Yang H, Bai YX, Khan MA, et al. 2019. CsPH8, a P-type proton pump gene, plays a key role in the diversity of citric acid accumulation in citrus fruits. *Plant Science* 289:110288
23. Lu Z, Huang Y, Mao S, Wu F, Liu Y, et al. 2022. The high-quality genome of pummelo provides insights into the tissue-specific regulation of citric acid and anthocyanin during domestication. *Horticulture Research* 9:uhac175
24. Zheng B, Zhao L, Jiang X, Cherono S, Liu J, et al. 2021. Assessment of organic acid accumulation and its related genes in peach. *Food Chemistry* 334:127567
25. Umer MJ, Bin Safdar L, Gebremeskel H, Zhao S, Yuan P, et al. 2020. Identification of key gene networks controlling organic acid and sugar metabolism during watermelon fruit development by integrating metabolic phenotypes and gene expression profiles. *Horticulture Research* 7:193

26. Yang J, Zhang J, Niu XQ, Zheng XL, Chen X, et al. 2021. Comparative transcriptome analysis reveals key genes potentially related to organic acid and sugar accumulation in loquat. *PLoS One* 16:e0238873
27. Li M, Li P, Ma F, Dandekar AM, Cheng L. 2018. Sugar metabolism and accumulation in the fruit of transgenic apple trees with decreased sorbitol synthesis. *Horticulture Research* 5:60
28. Yao YX, Li M, Zhai H, You CX, Hao YJ. 2011. Isolation and characterization of an apple *cytosolic malate dehydrogenase* gene reveal its function in malate synthesis. *Journal of Plant Physiology* 168:474−80
29. Diakou P, Svanella L, Raymond P, Gaudillère JP, Moing A. 2000. Phosphoenolpyruvate carboxylase during grape berry development: protein level, enzyme activity and regulation. *Australian Journal of Plant Physiology* 27:221−29
30. Terrier N, Glissant D, Grimplet J, Barrieu F, Abbal P, et al. 2005. Isogene specific oligo arrays reveal multifaceted changes in gene expression during grape berry (*Vitis vinifera* L.) development. *Planta* 222:832−47
31. Shi J, Li FF, Ma H, Li ZY, Xu JZ. 2015. Effects of different dwarfing interstocks on key enzyme activities and the expression of genes related to malic acid metabolism in Red Fuji apples. *Genetics and Molecular Research* 14:17673−83
32. Martínez-Esteso MJ, Sellés-Marchart S, Lijavetzky D, Pedreño MA, Bru-Martínez R. 2011. A DIGE-based quantitative proteomic analysis of grape berry flesh development and ripening reveals key events in sugar and organic acid metabolism. *Journal of Experimental Botany* 62:2521−69
33. Wang QJ, Sun H, Dong QL, Sun TY, Jin ZX, et al. 2016. The enhancement of tolerance to salt and cold stresses by modifying the redox state and salicylic acid content via the *cytosolic malate dehydrogenase* gene in transgenic apple plants. *Plant Biotechnology Journal* 14:1986−97
34. Yu JQ, Gu KD, Sun CH, Zhang QY, Wang JH, et al. 2021. The apple bHLH transcription factor MdbHLH3 functions in determining the fruit carbohydrates and malate. *Plant Biotechnology Journal* 19:285−99
35. Zhang L, Ma B, Wang C, Chen X, Ruan YL, et al. 2022. MdWRKY126 modulates malate accumulation in apple fruit by regulating cytosolic malate dehydrogenase (*MdMDH5*). *Plant Physiology* 188:2059−72
36. Mignard P, Beguería S, Reig G, Font i Forcada C, Moreno MA. 2021. Genetic origin and climate determine fruit quality and antioxidant traits on apple (*Malus* × *domestica* Borkh). *Scientia Horticulturae* 285:110142
37. Chen F, Liu X, Chen L. 2009. Developmental changes in pulp organic acid concentration and activities of acid-metabolising enzymes during the fruit development of two loquat (*Eriobotrya japonica* Lindl.) cultivars differing in fruit acidity. *Food Chemistry* 114:657−64
38. Plotto A, Bai J, Baldwin E. 2020. Effect of CA/MA on sensory quality. In *Controlled and Modified Atmospheres for Fresh and Fresh-Cut Produce*, eds Gil MI, Beaudry R. Amsterdam: Elsevier. pp. 109−30. https://doi.org/10.1016/B978-0-12-804599-2.00007-7
39. Walker RP, Chen ZH. 2002. Phosphoenolpyruvate carboxykinase: structure, function and regulation. *Advances in Botanical Research* 38:93−189
40. Famiani F, Battistelli A, Moscatello S, Cruz-Castillo JG, Walker RP. 2015. The organic acids that are accumulated in the flesh of fruits: occurrence, metabolism and factors affecting their contents–a review. *Revista Chapingo Serie Horticultura* 21:97−128
41. Famiani F, Cultrera NGM, Battistelli A, Casulli V, Proietti P, et al. 2005. Phosphoenolpyruvate carboxykinase and its potential role in the catabolism of organic acids in the flesh of soft fruit during ripening. *Journal of Experimental Botany* 56:2959−69
42. Famiani F, Farinelli D, Frioni T, Palliotti A, Battistelli A, et al. 2016. Malate as substrate for catabolism and gluconeogenesis during ripening in the pericarp of different grape cultivars. *Biologia Plantarum* 60:155−62
43. Famiani F, Farinelli D, Moscatello S, Battistelli A, Leegood RC, et al. 2016. The contribution of stored malate and citrate to the substrate requirements of metabolism of ripening peach (*Prunus persica* L. Batsch) flesh is negligible. Implications for the occurrence of phosphoenolpyruvate carboxykinase and gluconeogenesis. *Plant Physiology and Biochemistry* 101:33−42
44. MacRae AR, Moorhouse R. 1970. The oxidation of malate by mitochondria isolated from cauliflower buds. *European Journal of Biochemistry* 16:96−102
45. Ollat N, Gaudillère JP. 2000. Carbon balance in developing grapevine berries. *Acta Horticulturae* 526:345−50
46. Yang LT, Xie CY, Jiang HX, Chen LS. 2011. Expression of six malate-related genes in pulp during the fruit development of two loquat (*Eriobotrya japonica*) cultivars differing in fruit acidity. *African Journal of Biotechnology* 10:2414−22
47. Iannetta PPM, Escobar NM, Ross HA, Souleyre EJF, Hancock RD, et al. 2004. Identification, cloning and expression analysis of strawberry (*Fragaria* × *ananassa*) mitochondrial citrate synthase and mitochondrial malate dehydrogenase. *Physiologia Plantarum* 121:15−26
48. Pracharoenwattana I, Smith SM. 2008. When is a peroxisome not a peroxisome? *Trends in Plant Science* 13:522−25
49. Liu S, Yang Y, Murayama H, Taira S, Fukushima T. 2004. Effects of CO_2 on respiratory metabolism in ripening banana fruit. *Postharvest Biology and Technology* 33:27−34
50. Pua EC, Chandramouli S, Han P, Liu P. 2003. Malate synthase gene expression during fruit ripening of Cavendish banana (*Musa acuminata* cv. Williams). *Journal of Experimental Botany* 54:309−16
51. Fontes N, Gerós H, Delrot S. 2011. Grape berry vacuole: a complex and heterogeneous membrane system specialized in the accumulation of solutes. *American Journal of Enology and Viticulture* 62:270−78
52. Shiratake K, Martinoia E. 2007. Transporters in fruit vacuoles. *Plant Biotechnology* 24:127−33
53. Martinoia E, Maeshima M, Neuhaus HE. 2007. Vacuolar transporters and their essential role in plant metabolism. *Journal of Experimental Botany* 58:83−102
54. Ligaba A, Dreyer I, Margaryan A, Schneider DJ, Kochian L, et al. 2013. Functional, structural and phylogenetic analysis of domains underlying the Al sensitivity of the aluminum-activated malate/anion transporter, TaALMT1. *The Plant Journal* 76:766−80
55. Ma B, Liao L, Zheng H, Chen J, Wu B, et al. 2015. Genes encoding aluminum-activated malate transporter II and their association with fruit acidity in apple. *The Plant Genome* 8:eplantgenome 2015.03.0016
56. Sasaki T, Yamamoto Y, Ezaki B, Katsuhara M, Ahn SJ, et al. 2004. A wheat gene encoding an aluminum-activated malate transporter. *The Plant Journal* 37:645−53
57. Kovermann P, Meyer S, Hörtensteiner S, Picco C, Scholz-Starke J, et al. 2007. The *Arabidopsis* vacuolar malate channel is a member of the ALMT family. *The Plant Journal* 52:1169−80
58. Liu J, Magalhaes JV, Shaff J, Kochian LV. 2009. Aluminum-activated citrate and malate transporters from the MATE and ALMT families function independently to confer *Arabidopsis* aluminum tolerance. *The Plant Journal* 57:389−99
59. Ligaba A, Maron L, Shaff J, Kochian L, Piñeros M. 2012. Maize ZmALMT2 is a root anion transporter that mediates constitutive root malate efflux. *Plant, Cell & Environment* 35:1185−200
60. Peng W, Wu W, Peng J, Li J, Lin Y, et al. 2018. Characterization of the soybean GmALMT family genes and the function of GmALMT5 in response to phosphate starvation. *Journal of Integrative Plant Biology* 60:216−31
61. Ligaba A, Katsuhara M, Ryan PR, Shibasaka M, Matsumoto H. 2006. The *BnALMT1* and *BnALMT2* genes from rape encode aluminum-activated malate transporters that enhance the aluminum resistance of plant cells. *Plant Physiology* 142:1294−303

62. Ye J, Wang X, Hu T, Zhang F, Wang B, et al. 2017. An InDel in the promoter of *Al-ACTIVATED MALATE TRANSPORTER9* selected during tomato domestication determines fruit malate contents and aluminum tolerance. *The Plant Cell* 29:2249−68
63. Bai Y, Dougherty L, Li M, Fazio G, Cheng L, et al. 2012. A natural mutation-led truncation in one of the two aluminum-activated malate transporter-like genes at the Ma locus is associated with low fruit acidity in apple. *Molecular Genetics and Genomics* 287:663−78
64. Xu L, Qiao X, Zhang M, Zhang S. 2018. Genome-wide analysis of aluminum-activated malate transporter family genes in six rosaceae species, and expression analysis and functional characterization on malate accumulation in Chinese white pear. *Plant Science* 274:451−65
65. Barbier-Brygoo H, De Angeli A, Filleur S, Frachisse JM, Gambale F, et al. 2011. Anion channels/transporters in plants: from molecular bases to regulatory networks. *Annual Review of Plant Biology* 62:25−51
66. Eisenach C, Baetz U, Huck NV, Zhang J, De Angeli A, et al. 2017. ABA-induced stomatal closure involves ALMT4, a phosphorylation-dependent vacuolar anion channel of *Arabidopsis*. *The Plant Cell* 29:2552−69
67. Luu K, Rajagopalan N, Ching JCH, Loewen MC, Loewen ME. 2019. The malate-activated ALMT12 anion channel in the grass *Brachypodium distachyon* is co-activated by Ca^{2+}/calmodulin. *Journal of Biological Chemistry* 294:6142−56
68. Hoekenga OA, Maron LG, Piñeros MA, Cançado GMA, Shaff J, et al. 2006. *AtALMT1*, which encodes a malate transporter, is identified as one of several genes critical for aluminum tolerance in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America* 103:9738−43
69. Gruber BD, Delhaize E, Richardson AE, Roessner U, James RA, et al. 2011. Characterisation of *HvALMT1* function in transgenic barley plants. *Functional Plant Biology* 38:163−75
70. Ding ZJ, Yan JY, Xu XY, Li GX, Zheng SJ. 2013. WRKY46 functions as a transcriptional repressor of *ALMT1*, regulating aluminum-induced malate secretion in *Arabidopsis*. *The Plant Journal* 76:825−35
71. Piñeros MA, Cançado GMA, Maron LG, Lyi SM, Menossi M, et al. 2008. Not all ALMT1-type transporters mediate aluminum-activated organic acid responses: the case of *ZmALMT1* – an anion-selective transporter. *The Plant Journal* 53:352−67
72. Zhou Y, Neuhäuser B, Neumann G, Ludewig U. 2020. LaALMT1 mediates malate release from phosphorus-deficient white lupin root tips and metal root to shoot translocation. *Plant, Cell & Environment* 43:1691−706
73. Sasaki T, Tsuchiya Y, Ariyoshi M, Nakano R, Ushijima K, et al. 2016. Two members of the aluminum-activated malate transporter family, *SlALMT4* and *SlALMT5*, are expressed during fruit development, and the overexpression of *SlALMT5* alters organic acid contents in seeds in tomato (*Solanum lycopersicum*). *Plant and Cell Physiology* 57:2367−79
74. Sasaki T, Ariyoshi M, Yamamoto Y, Mori IC. 2022. Functional roles of ALMT-type anion channels in malate-induced stomatal closure in tomato and *Arabidopsis*. *Plant, Cell & Environment* 45:2337−50
75. Meyer S, Scholz-Starke J, De Angeli A, Kovermann P, Burla B, et al. 2011. Malate transport by the vacuolar AtALMT6 channel in guard cells is subject to multiple regulation. *The Plant Journal* 67:247−57
76. Hafke JB, Hafke Y, Smith JAC, Lüttge U, Thiel G. 2003. Vacuolar malate uptake is mediated by an anion-selective inward rectifier. *The Plant Journal* 35:116−28
77. Li C, Dougherty L, Coluccio AE, Meng D, El-Sharkawy I, et al. 2020. Apple ALMT9 requires a conserved C-terminal domain for malate transport underlying fruit acidity. *Plant Physiology* 182:992−1006
78. Xu K, Wang A, Brown S. 2012. Genetic characterization of the *Ma* locus with pH and titratable acidity in apple. *Molecular Breeding* 30:899−912
79. De Angeli A, Baetz U, Francisco R, Zhang J, Chaves MM, et al. 2013. The vacuolar channel VvALMT9 mediates malate and tartrate accumulation in berries of *Vitis vinifera*. *Planta* 238:283−91
80. Fu BL, Wang WQ, Li X, Qi TH, Shen QF, et al. 2023. A dramatic decline in fruit citrate induced by mutagenesis of a NAC transcription factor, *AcNAC1*. *Plant Biotechnology Journal* 21:1695−706
81. Liu S, Liu X, Gou B, Wang D, Liu C, et al. 2022. The interaction between CitMYB52 and CitbHLH2 negatively regulates citrate accumulation by activating *CitALMT* in *Citrus* fruit. *Frontiers in Plant Science* 13:848869
82. Yamaki S. 1984. Isolation of vacuoles from immature apple fruit flesh and compartmentation of sugars, organic acids, phenolic compounds and amino acids. *Plant and Cell Physiology* 25:151−66
83. Hurth MA, Suh SJ, Kretzschmar T, Geis T, Bregante M, et al. 2005. Impaired pH homeostasis in *Arabidopsis* lacking the vacuolar dicarboxylate transporter and analysis of carboxylic acid transport across the tonoplast. *Plant Physiology* 137:901−10
84. Emmerlich V, Linka N, Reinhold T, Hurth MA, Traub M, et al. 2003. The plant homolog to the human sodium/dicarboxylic cotransporter is the vacuolar malate carrier. *Proceedings of the National Academy of Sciences of the United States of America* 100:11122−26
85. Frei B, Eisenach C, Martinoia E, Hussein S, Chen XZ, et al. 2018. Purification and functional characterization of the vacuolar malate transporter tDT from *Arabidopsis*. *Journal of Biological Chemistry* 293:4180−90
86. Medeiros DB, Barros KA, Barros JAS, Omena-Garcia RP, Arrivault S, et al. 2017. Impaired malate and fumarate accumulation due to the mutation of the tonoplast dicarboxylate transporter has little effects on stomatal behavior. *Plant Physiology* 175:1068−81
87. Shimada T, Nakano R, Shulaev V, Sadka A, Blumwald E. 2006. Vacuolar citrate/H^+ symporter of citrus juice cells. *Planta* 224:472−80
88. Lin Q, Li S, Dong W, Feng C, Yin X, et al. 2015. Involvement of *CitCHX* and *CitDIC* in developmental-related and postharvest-hot-air driven citrate degradation in citrus fruits. *PLoS One* 10:e0119410
89. Liu R, Li B, Qin G, Zhang Z, Tian S. 2017. Identification and functional characterization of a tonoplast dicarboxylate transporter in tomato (*Solanum lycopersicum*). *Frontiers in Plant Science* 8:186
90. Liao L, Zhang W, Zhang B, Fang T, Wang XF, et al. 2021. Unraveling a genetic roadmap for improved taste in the domesticated apple. *Molecular Plant* 14:1454−71
91. Yao YX, Dong QL, You CX, Zhai H, Hao YJ. 2011. Expression analysis and functional characterization of apple *MdVHP1* gene reveals its involvement in Na^+, malate and soluble sugar accumulation. *Plant Physiology and Biochemistry* 49:1201−8
92. Hu DG, Sun CH, Ma QJ, You CX, Cheng L, et al. 2016. MdMYB1 regulates anthocyanin and malate accumulation by directly facilitating their transport into vacuoles in apples. *Plant Physiology* 170:1315−30
93. Terrier N, Sauvage FX, Ageorges A, Romieu C. 2001. Changes in acidity and in proton transport at the tonoplast of grape berries during development. *Planta* 213:20−28
94. Regalado A, Pierri CL, Bitetto M, Laera VL, Pimentel C, et al. 2013. Characterization of mitochondrial dicarboxylate/tricarboxylate transporters from grape berries. *Planta* 237:693−703
95. Mohammed SA, Nishio S, Takahashi H, Shiratake K, Ikeda H, et al. 2012. Role of vacuolar H^+-inorganic pyrophosphatase in tomato fruit development. *Journal of Experimental Botany* 63:5613−21
96. Hussain SB, Shi CY, Guo LX, Du W, Bai YX, et al. 2020. Type I H^+-pyrophosphatase regulates the vacuolar storage of sucrose in citrus fruit. *Journal of Experimental Botany* 71:5935−47
97. Jiang YT, Tang RJ, Zhang YJ, Xue HW, Ferjani A, et al. 2020. Two tonoplast proton pumps function in *Arabidopsis* embryo development. *New Phytologist* 225:1606−17
98. Gao MY, Liang J, li H, Zhong R, Di-an N. 2021. Loss-of-function of vacuolar-type H+ pyrophosphatase gene lead to reduce in

stomatal aperture and density. *IOP Conference Series: Earth and Environmental Science* 657:012024
99. Maeshima M. 2000. Vacuolar H$^+$-pyrophosphatase. *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1465:37−51
100. Lu XP, Liu YZ, Zhou GF, Wei QJ, Hu HJ, et al. 2011. 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. *Scientia Horticulturae* 129:680−87
101. Etienne C, Moing A, Dirlewanger E, Raymond P, Monet R, et al. 2002. Isolation and characterization of six peach cDNAs encoding key proteins in organic acid metabolism and solute accumulation: involvement in regulating peach fruit acidity. *Physiologia Plantarum* 114:259−70
102. Aprile A, Federici C, Close TJ, De Bellis L, Cattivelli L, et al. 2011. Expression of the H+-ATPase AHA10 proton pump is associated with citric acid accumulation in lemon juice sac cells. *Functional & Integrative Genomics* 11:551−63
103. Li SJ, Yin XR, Xie XL, Allan AC, Ge H, et al. 2016. The *Citrus* transcription factor, CitERF13, regulates citric acid accumulation via a protein-protein interaction with the vacuolar proton pump, CitVHA-c4. *Scientific Reports* 6:20151
104. Jia D, Wu P, Shen F, Li W, Zheng X, et al. 2021. Genetic variation in the promoter of an R2R3−MYB transcription factor determines fruit malate content in apple (*Malus domestica* Borkh.). *Plant Physiology* 186:549−68
105. Faraco M, Spelt C, Bliek M, Verweij W, Hoshino A, et al. 2014. Hyperacidification of vacuoles by the combined action of two different P-ATPases in the tonoplast determines flower color. *Cell Reports* 6:32−43
106. Li Y, Provenzano S, Bliek M, Spelt C, Appelhagen I, et al. 2016. Evolution of tonoplast P-ATPase transporters involved in vacuolar acidification. *New Phytologist* 211:1092−107
107. Eisenach C, Baetz U, Martinoia E. 2014. Vacuolar proton pumping: more than the sum of its parts? *Trends in Plant Science* 19:344−46
108. Faraco M, Di Sansebastiano GP, Spelt K, Koes RE, Quattrocchio FM. 2011. One protoplast is not the other! *Plant Physiology* 156:474−78
109. Strazzer P, Spelt CE, Li S, Bliek M, Federici CT, et al. 2019. Hyperacidification of *Citrus* fruits by a vacuolar proton-pumping P-ATPase complex. *Nature Communications* 10:744
110. Zheng L, Ma W, Deng J, Peng Y, Tian R, et al. 2022. A *MdMa13* gene encoding tonoplast P_{3B}-type ATPase regulates organic acid accumulation in apple. *Scientia Horticulturae* 296:110916
111. Amato A, Cavallini E, Walker AR, Pezzotti M, Bliek M, et al. 2019. The MYB5-driven MBW complex recruits a WRKY factor to enhance the expression of targets involved in vacuolar hyperacidification and trafficking in grapevine. *The Plant Journal* 99:1220−41
112. Song JX, Chen YC, Lu ZH, Zhao GP, Wang XL, et al. 2022. PbPH5, an H$^+$ P-ATPase on the tonoplast, is related to malic acid accumulation in pear fruit. *Journal of Integrative Agriculture* 21:1645−57
113. Pant BD, Oh S, Lee HK, Nandety RS, Mysore KS. 2020. Antagonistic regulation by CPN60A and CLPC1 of TRXL1 that regulates MDH activity leading to plant disease resistance and thermotolerance. *Cell Reports* 33:108512
114. Zhang QY, Gu KD, Wang JH, Yu JQ, Wang XF, et al. 2020. BTB-BACK-TAZ domain protein MdBT2-mediated MdMYB73 ubiquitination negatively regulates malate accumulation and vacuolar acidification in apple. *Horticulture Research* 7:151
115. Wang JH, Gu KD, Zhang QY, Yu JQ, Wang CK, et al. 2023. Ethylene inhibits malate accumulation in apple by transcriptional repression of *aluminum-activated malate transporter 9* via the WRKY31-ERF72 network. *New Phytologist* 239:1014−34
116. Peng Y, Yuan Y, Chang W, Zheng L, Ma W, et al. 2023. Transcriptional repression of *MdMa1* by MdMYB21 in *Ma* locus decreases malic acid content in apple fruit. *The Plant Journal* 115:1231−42
117. Zheng L, Liao L, Duan C, Ma W, Peng Y, et al. 2023. Allelic variation of *MdMYB123* controls malic acid content by regulating *MdMa1* and *MdMa11* expression in apple. *Plant Physiology* 192:1877−91
118. Nakatsukasa K, Okumura F, Kamura T. 2015. Proteolytic regulation of metabolic enzymes by E3 ubiquitin ligase complexes: lessons from yeast. *Critical Reviews in Biochemistry and Molecular Biology* 50:489−502
119. Li YY, Mao K, Zhao C, Zhao XY, Zhang HL, et al. 2012. MdCOP1 ubiquitin E3 ligases interact with MdMYB1 to regulate light-induced anthocyanin biosynthesis and red fruit coloration in apple. *Plant Physiology* 160:1011−22
120. Hu DG, Sun CH, Zhang QY, An JP, You CX, et al. 2016. Glucose sensor MdHXK1 phosphorylates and stabilizes MdbHLH3 to promote anthocyanin biosynthesis in apple. *PLoS Genetics* 12:e1006273
121. Hu DG, Yu JQ, Han PL, Xie XB, Sun CH, et al. 2019. The regulatory module MdPUB29-MdbHLH3 connects ethylene biosynthesis with fruit quality in apple. *New Phytologist* 221:1966−82
122. Zhang QY, Gu KD, Cheng L, Wang JH, Yu JQ, et al. 2020. BTB-TAZ domain protein MdBT2 modulates malate accumulation and vacuolar acidification in response to nitrate. *Plant Physiology* 183:750−64
123. Chen Q, Xu X, Xu D, Zhang H, Zhang C, et al. 2019. WRKY18 and WRKY53 coordinate with HISTONE ACETYLTRANSFERASE1 to regulate rapid responses to sugar. *Plant Physiology* 180:2212−26
124. Li X, Guo W, Li J, Yue P, Bu H, et al. 2020. Histone acetylation at the promoter for the transcription factor PuWRKY31 affects sucrose accumulation in pear fruit. *Plant Physiology* 182:2035−46
125. Sicilia A, Scialò E, Puglisi I, Lo Piero AR. 2020. Anthocyanin biosynthesis and DNA methylation dynamics in sweet orange fruit [*Citrus sinensis* L. (osbeck)] under cold stress. *Journal of Agricultural and Food Chemistry* 68:7024−31
126. Yu H, Zhang C, Lu C, Wang Y, Ge C, et al. 2024. The lemon genome and DNA methylome unveil epigenetic regulation of citric acid biosynthesis during fruit development. *Horticulture Research* 11:uhae005
127. Giné Bordonaba J, Terry LA. 2010. Manipulating the taste-related composition of strawberry fruits (*Fragaria* × *ananassa*) from different cultivars using deficit irrigation. *Food Chemistry* 122:1020−26
128. Wu BH, Quilot B, Génard M, Kervella J, Li SH. 2005. Changes in sugar and organic acid concentrations during fruit maturation in peaches, *P. davidiana* and hybrids as analyzed by principal component analysis. *Scientia Horticulturae* 103:429−39
129. Centeno DC, Osorio S, Nunes-Nesi A, Bertolo ALF, Carneiro RT, et al. 2011. Malate plays a crucial role in starch metabolism, ripening, and soluble solid content of tomato fruit and affects postharvest softening. *The Plant Cell* 23:162−84
130. Osorio S, Vallarino JG, Szecowka M, Ufaz S, Tzin V, et al. 2013. Alteration of the interconversion of pyruvate and malate in the plastid or cytosol of ripening tomato fruit invokes diverse consequences on sugar but similar effects on cellular organic acid, metabolism, and transitory starch accumulation. *Plant Physiology* 161:628−43
131. Wei L, Mao W, Jia M, Xing S, Ali U, et al. 2018. FaMYB44.2, a transcriptional repressor, negatively regulates sucrose accumulation in strawberry receptacles through interplay with FaMYB10. *Journal of Experimental Botany* 69:4805−20
132. Bastías A, López-Climent M, Valcárcel M, Rosello S, Gómez-Cadenas A, et al. 2011. Modulation of organic acids and sugar content in tomato fruits by an abscisic acid-regulated transcription factor. *Physiologia Plantarum* 141:215−26

Copyright: © 2024 by the author(s). Published by Maximum Academic Press, Fayetteville, GA. This article is an open access article distributed under Creative Commons Attribution License (CC BY 4.0), visit https://creativecommons.org/licenses/by/4.0/.