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‘蜂糖李’果实发育过程中有机酸含量变化及其与苹果酸代谢相关酶的关系

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摘 要：【目的】明确‘蜂糖李’果实有机酸组成与含量特点，揭示其发育过程中有机酸含量的变化规律及其与苹果酸代谢相关酶的关系，阐明有机酸积累的关键时期和关键酶。【方法】以‘蜂糖李’及对照‘四月李’为材料，采用高效液相色谱法（high performance liquid chromatograph，HPLC）分析测试李果实发育过程中有机酸组分及含量，并测定苹果酸代谢相关酶的活性。【结果】利用高效液相色谱分析李果实中有机酸组分及含量，发现‘蜂糖李’果实成熟时总酸含量（ω，后同）为5.94 mg·g^{-1}，包括苹果酸、酒石酸、柠檬酸、草酸、莽草酸和琥珀酸6种，其中以苹果酸含量最高（占总酸含量的88%），‘四月李’果实中有机酸组分与‘蜂糖李’一致，均属于苹果酸型。2个李品种果实中总酸含量的差异主要是由苹果酸含量的差异所致。通过分析李果实发育过程中有机酸含量的变化，发现‘蜂糖李’果实中苹果酸含量大量积累的关键时期在果实发育前期，整体呈先增加后降低的趋势，草酸、酒石酸含量逐渐降低，而柠檬酸含量逐渐升高。与‘四月李’相比，‘蜂糖李’果实中苹果酸、草酸、酒石酸及总酸含量的变化趋势与之一致，且苹果酸及总酸含量在整个过程中均显著低于‘四月李’，而柠檬酸含量的变化趋势与之相反。最后通过分析苹果酸含量与相关代谢酶活性之间的相关性，表明‘蜂糖李’果实中苹果酸在果实发育前期大量积累主要是该时期磷酸烯醇式丙酮酸羧化酶（PEPC）活性增强促进了苹果酸的大量合成以及NADP-苹果酸酶（NADP-ME）活性降低减少苹果酸的分解，与NAD-苹果酸脱氢酶（NAD-MDH）关系不大，而‘四月李’苹果酸积累的关键酶是NAD-MDH。在果实发育后期，‘蜂糖李’及‘四月李’NADP-ME活性迅速升高，前者PEPC活性减弱，后者NAD-MDH活性下降，使得2者果实中苹果酸的降解大于合成而呈降低趋势。【结论】‘蜂糖李’是以苹果酸为主要有机酸的低酸型李品种，其果实中苹果酸积累的关键时期为果实发育前期，苹果酸含量的变化由PEPC和NADP-ME协同调控，而对照‘四月李’由NAD-MDH和NADP-ME起主要的调控作用。

关键词：李果实；有机酸；苹果酸；磷酸烯醇式丙酮酸羧化酶；NADP-苹果酸酶；NAD-苹果酸脱氢酶；高效液相色谱

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Changes in organic acids content during ‘Fengtang’ plum (*Prunus salicina*) fruit development in relation to malic acid metabolism related enzymes

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Abstract:【Objective】In order to understand the mechanisms regulating acidity in plum fruit, the composition and contents of organic acids in ‘Fengtang’ plum (*Prunus salicina* ‘Fengtang’) fruit were determined, and the changes in organic acids content during the fruit development and their relationships with the activities of enzymes associated with malic acid metabolism were analyzed. The critical period and key enzymes of organic acid accumulation were elucidated.【Methods】‘Fengtang’ and ‘Siyue’ fruits were used as experimental material. The composition and contents of organic acids in plum fruits

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during the development were analyzed by high performance liquid chromatography (HPLC) and the activities of enzymes related with malic acid metabolism were determined by spectrophotometer. The correlations between the changes in malic acid content and the activities of malic acid metabolism enzymes were analyzed in this study. 【Results】The total acid content was 5.94 mg· g^{-1} at maturity in ‘Fengtang’, including 6 organic acids, malic acid, tartaric acid, citric acid, oxalic acid, shikimic acid and succinic acid. Among them, malic acid was the dominant acid accounting for 88% of the total acid content. ‘Siyue’ fruit had the same composition of organic acids. Both cultivars belong to the malic acid type. During the growth and development of plum, the content of malic acid increased first and decreased at the late phase. The malic acid content in ‘Fengtang’ reduced to a minimum when the fruit was ripe, and was always significantly lower than ‘Siyue’. The content of oxalic acid and tartaric acid gradually decreased with the growth and development of fruit, and the amplitude was small at the ripening period, but the content of citric acid gradually increased. The changes in total acid content and malic acid content in the fruit of ‘Siyue’ were similar to those of ‘Fengtang’. The difference in total acid content in the two cultivars was mainly due to the difference in malic acid content. The changes in malic acid content were closely related to the changes in NAD-malate dehydrogenase (NAD-MDH), NADP-malosterase (NADP-ME) and phosphoenolpyruvate carboxylase (PEPC) activities. The activity of NAD-MDH in ‘Fengtang’ and ‘Siyue’ had a significant difference. The activity of NAD-MDH in the ‘Fengtang’ fruit fluctuated within the range of 82.24–169.12 U· mg^{-1}. NAD-MDH activity in ‘Siyue’ showed an M pattern and was higher than that in ‘Fengtang’. There was a significant positive correlation between malic acid content and NAD-MDH activity in the two cultivars with a correlation coefficient of 0.97** and 0.95**, respectively. The change pattern of the activity of NADP-ME in ‘Fengtang’ was similar to that ‘Siyue’, increasing during young fruit enlargement period and decreasing during maturation. However, the activity of NADP-ME in the fruit of ‘Fengtang’ was always significantly lower than that of ‘Siyue’. The activity of NADP-ME was significantly negatively correlated with malic acid content with a correlation coefficient of -0.86– -0.87, respectively. The change in PEPC activity in the fruit ‘Fengtang’ exhibited an M pattern. Except for the fruit ripening period, the enzyme activity was always significantly higher in ‘Fengtang’. The activity of PEPC in ‘Siyue’ fruit changed smoothly during the whole fruit development period. There was a significant positive correlation between the activity of PEPC and the content of malic acid in ‘Fengtang’ and ‘Siyue’ with a coefficient of 0.75* and 0.73*, respectively. The early stage of fruit development was the critical period of malic acid accumulation in ‘Fengtang’, when enhanced activity of PEPC promoted the synthesis of malic acid and the decreased activity of NADP-ME reduced the decomposition of malic acid, while NAD-MDH had little effect. However the key enzyme for accumulation of malic acid in ‘Siyue’ was NAD-MDH. At the later stage of fruit development, the activity of NADP-ME increased rapidly in both cultivars, while the activity of PEPC in ‘Fengtang’ was weakened and the NAD-MDH in ‘Siyue’ activity decreased. As a result, the degradation of malic acid was greater than synthesis and the content of malic acid became lower in the both cultivars. 【Conclusion】‘Fengtang’ is a low-acid plum variety, in which malic acid is the main organic acid. The critical period of malic acid accumulation is the early stages of fruit development. The change in malic acid content is coordinated by PEPC and NADP-ME. However, in ‘Siyue’ malic acid content is mainly controlled by NAD-MDH and NADP-ME.

Key words: Plum (*Prunus salicina*) fruit; Organic acids; Malic acid; PEPC; NADP-ME; NAD-MDH; HPLC

有机酸含量及组分比例是决定果实酸度及风味的重要组成因子[1]。李属（*Prunus* L.）果实大部分以苹果酸为主要有机酸，而在欧洲李（*P. domestica* L.）果实中奎宁酸含量最高[2]，苹果酸含量（ω，后同）仅为27.46%。还有一部分李品种虽然也是以苹果酸为主，但其他有机酸也占有很大比例，比如在‘樱桃李’（*Prunus cerasifera* Enrhart）、‘加拿大李’（*P. nigra* Ait.）、‘黑刺李’（*P. spinosa* L.）等[2]果实中还含有较高奎宁酸，‘Amber Jewel’和‘Angeleno’等[3]一些日本李（*P. salicina* Lindll.）中琥珀酸含量仅次于苹果酸。早期赵密珍等[4]、赵树堂等[5]分别以不同品种的李果实为材料，分析了可滴定酸含量的动态变化，并未研究有机酸各组分含量的变化趋势。据报道，日本李‘Amber jewel’果实中苹果酸含量先增加后降低、而‘Angeleno’与之相反，表现为先降低后增加，‘Blackamber’果实中苹果酸含量在果实发育过程中呈“降-升-降”的变化趋势[3]，可见不同李果实中有机酸代谢存在较大差异。果实中苹果酸代谢主要在细胞质中进行，己糖通过糖酵解途径生成磷酸烯醇式丙酮酸（PEP），PEP在磷酸烯醇式丙酮酸羧化酶（PEPC）、苹果酸脱氢酶（NAD-MDH）的作用下经过一系列反应生成苹果酸，最终在苹果酸酶（NADP-ME）作用下降解为丙酮酸[6]。PEPC、NAD-MDH和NADP-ME是果实中参与苹果酸代谢的关键酶[7]，已在杏[8]、桃[9-10]、苹果[11-12]等多种果实中证实其活性是影响苹果酸积累速率的决定因素。‘蜂糖李’是贵州省地方特色李品种，据笔者课题组前期检测分析，‘蜂糖李’果实具有高糖低酸的特点，品质优良，市场供不应求。‘蜂糖李’果实成熟时总酸含量极低，但其果实中有机酸组分及含量特点及不同发育阶段的变化规律尚不明确。笔者以贵州地方李‘蜂糖李’为试材、‘四月李’为对照，研究其果实全发育时期有机酸含量及其代谢相关酶活性的变化规律，分析2者之间的关系，并进一步探讨‘蜂糖李’和‘四月李’果实全发育时期有机酸合成与降解差异的生理基础，为调控李果实酸度提供参考，也为进一步研究‘蜂糖李’果实有机酸代谢的分子机制奠定基础。

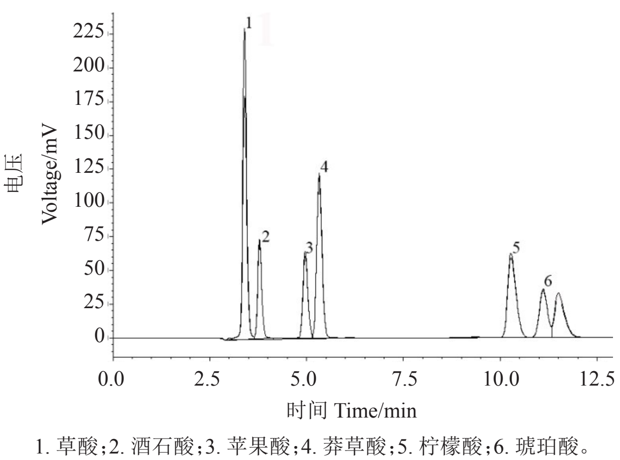
1　材料和方法

1.1　材料

以贵州省镇宁县六马镇‘蜂糖李’以及对照品种‘四月李’为供试材料，分别选取生长势一致且长势良好的9株李树进行样品采集和分析。‘蜂糖李’在花后20 d（2017年4月15日）开始采集样品，‘四月李’在花后25 d（2017年4月15日）开始取样，以后每10 d取1次样，直至果实达到生理成熟（‘蜂糖李’花后90 d左右，‘四月李’花后85 d左右），‘蜂糖李’一共取8次样，‘四月李’7次样。幼果期至成熟期果实大小差异较大，因此每次取果量应根据果实大小而定。样品前处理：果实带皮去核切碎，迅速用液氮冷冻（-196 ℃）后混匀，分2份置于超低温冰箱（-70 ℃）备用，1份用于检测有机酸组分及含量，另1份用于分析酶活性。

1.2　方法

1.2.1　果实中有机酸含量的测定　果实中有机酸提取参照刘雅兰等[13]的方法。提取液经0.22 μm微孔滤膜过滤，滤液置于1.5 mL进样瓶中备用。高效液相色谱（HPLC）条件：色谱柱为Shim-pack VP-ODSC18柱（150 mm×4.6 mm，5 μm），柱温：33 ℃，流动相：0.2%偏磷酸，流速：1.0 mL·min^{-1}，检测器：岛津SPD-10A紫外检测器，检测波长：210 nm。草酸、酒石酸、苹果酸、莽草酸、柠檬酸和琥珀酸标准品均为色谱纯，各有机酸标准品的液相色谱检测结果见图1。6种有机酸均能在12 min内被完全分离，且分离效果良好，各物质分离的先后顺序依次为草酸、酒石酸、苹果酸、莽草酸、柠檬酸和琥珀酸。根据样品的保留时间确定有机酸组分，各有机酸标准曲线回归方程的R^2为0.996 9~1.000 0，线性关系良好，回收



1. 草酸；2. 酒石酸；3. 苹果酸；4. 莽草酸；5. 柠檬酸；6. 琥珀酸。

1. Oxalic acid; 2. Tartaric acid; 3. Malic acid; 4. Shikimic acid; 5. Citric acid; 6. Succinic acid.

图1　标准品中有机酸的高效液相色谱检测

Fig. 1 HPLC spectrum of organic acids in standard samples

率为99.5%~102.7%，精密度较高，符合分析方法的要求。果实中有机酸含量的测定3次重复，平行2次，采用峰面积归一法计算含量，总酸含量由各有机酸相加之和，最终含量(ω)用mg· g^{-1}表示。

1.2.2 果实中苹果酸代谢相关酶活性的测定 酶提取液制备参照Hirai等[14]和Sadka等[15]的方法。将0.5 g果肉用3 mL的提取缓冲液[0.2 mol· L^{-1} Tris-HCl缓冲液(pH=8.2)、0.6 mol· L^{-1}蔗糖、10 mmol· L^{-1}异抗坏血酸]在冰上匀浆，8 000 *g* 4 ℃离心10 min，取上清液置冰上，用于NAD-MDH和NADP-ME活力的测定。PEPC酶提取液的制备前处理同上，上清液在大量透析液(即提取缓冲液)中4 ℃透析过夜，用于PEPC活力的测定。按照酶试剂盒说明书分别测定各个酶活性，3次重复，平行3次。NADP-ME活力的计算：根据NADP-ME催化NADP$^+$还原成NADPH，在340 nm下测定NADPH增加速率，以每g组织每min生成1 nmol NADPH定义为1个酶活力单位(nmol· g^{-1}· min^{-1})。NAD-MDH活力的计算：根据NAD-MDH催化NADH还原草酰乙酸生成苹果酸，导致340 nm处光吸收值下降，以每g组织在反应体系中每min消耗1 nmol的NADH定义为1个酶活力单位(U· g^{-1}· min^{-1})。PEPC活力的计算：根据PEPC催化磷酸烯醇式丙酮酸和CO_2生成草酰乙酸和HPO_4^{2-}，苹果酸脱氢酶进一步催化草酰乙酸和NADP生成苹果酸和NAD$^+$，在340 nm测定NADH减少速率。以每g组织每min消耗1 nmol的NADH定义为1个酶活力单位(nmol· g^{-1}· min^{-1})。

1.2.3 数据统计分析 用Excel 2010软件进行数据整理和绘图，测定结果以平均值(means)±相对标准偏差(standard deviation)表示，应用DPS7.05进行差异显著性分析(Duncan新复极差法)和相关性分析。

2 结果与分析

2.1 生理成熟时期果实中有机酸组分及含量

‘蜂糖李’果实生理成熟期在6月下旬(花后90 d左右)，比‘四月李’晚熟7 d左右(花后85 d左右)。‘蜂糖李’和‘四月李’成熟时均以苹果酸为主要有机酸，分别占总酸的88%、93%，而柠檬酸、草酸和酒石酸含量较低，莽草酸和琥珀酸含量极低(表1)。‘蜂糖李’总酸和苹果酸含量显著低于‘四月李’，除琥珀酸以外，其余有机酸含量与‘四月李’无显著差异。

表1 生理成熟期李果实中有机酸组分及含量

Table 1 The contents of organic acids in plum fruits at physiological mature stage

品种 Cultivar	ω(草酸) Oxalic acid content/(mg· g^{-1})	ω(酒石酸) Tartaric acid content/(mg· g^{-1})	ω(苹果酸) Malic acid content/(mg· g^{-1})	ω(莽草酸) Shikimic acid content/(mg· g^{-1})	ω(柠檬酸) Citric acid content/(mg· g^{-1})	ω(琥珀酸) Succinic acid content/(mg· g^{-1})	ω(总有机酸) Total acid content/(mg· g^{-1})
蜂糖李 Fengtang plum	0.13±0.02 a	0.28±0.07 a	5.23±0.20 b	0.02±0.01 a	0.24±0.17 a	0.04±0.01 a	5.94±0.22 b
四月李 Siyue plum	0.15±0.02 a	0.34±0.04 a	8.02±0.68 a	0.02±0.01 a	0.07±0.01 a	0.02±0.01 b	8.61±0.43 a

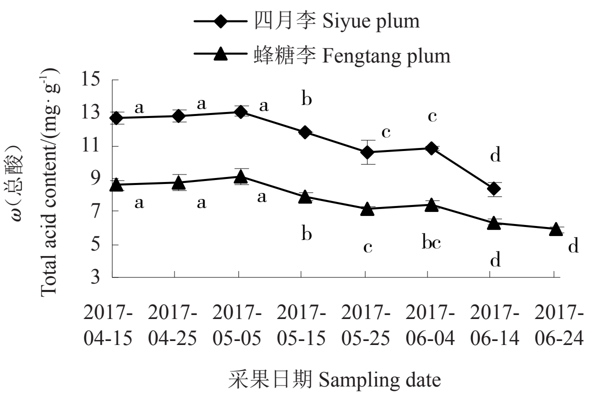
注：多重比较采用 Duncan 新复极差法测验，同列不同小写字母表示在 $P < 0.05$ 水平上有显著性差异。

Note: Statistical multiple comparisons were conducted according to the Duncan multiple tests, different small letters in the same column indicate significant difference at $P < 0.05$.

2.2 果实中有机酸含量变化

2.2.1 总酸 ‘蜂糖李’果实总酸含量随着果实生长发育的动态变化，趋势与‘四月李’基本一致(图2)，整体呈现降低的趋势。在幼果膨大期和硬核期缓慢升高，硬核期结束后开始降低，至5月25日果实二次快速生长，总酸含量又开始缓慢增加，接近果实成熟时总酸含量开始下降，至果实成熟时降到最低。在果实整个生长发育期内，‘蜂糖李’总酸含量始终低于‘四月李’，且差异较为显著。在果实成熟前20 d，‘蜂糖李’总酸含量缓慢降低，而‘四月李’呈先增加后降低的趋势。果实成熟前20 d总酸含量积累的差异是引起成熟果实酸度差异的重要因素。

2.2.2 苹果酸 苹果酸含量在‘蜂糖李’和‘四月李’的整个生长发育过程中占总酸含量的比例均较高，是这2个品种果实中最主要的有机酸。2个品种果实中苹果酸含量的大量积累主要在果实发育前期(图3)，降低主要发生在果实发育后期。2个李品种苹果酸含量均有2次增加的趋势，不同的是‘蜂糖李’第2次苹果酸积累的时期早于‘四月李’，但‘蜂糖李’在成熟前20 d一直降低，而‘四月李’在这个时期却先升高后降低。果实发育初期，‘蜂糖李’苹果酸含量(ω)为4.72 mg· g^{-1}，占总酸的55.08%，果实成



多重比较采用 Duncan 新复极差法测验，不同小写字母表示同一品种不同时期果实在 $P < 0.05$ 水平上有显著性差异。下同。

Statistical multiple comparisons were done according to the Duncan multiple tests, different small letters indicate significant difference at 0.05 level among the same cultivar in the different stages ($P < 0.05$). The same below.

图 2　李果实发育过程中总酸含量的变化

Fig. 2 Changes in total acid content in the two plum cultivars during fruit development

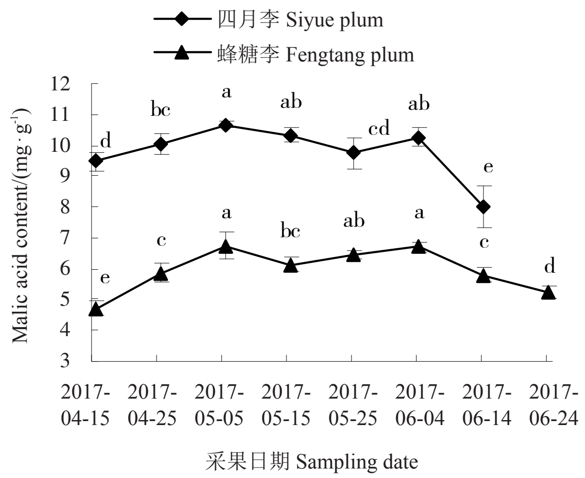


图 3　李果实发育过程中苹果酸含量的变化

Fig. 3 Changes in malic acid content in the two plum cultivars during fruit development

熟时占总酸的88.04%。在整个发育过程中，‘蜂糖李’苹果酸含量始终显著低于‘四月李’，这也是果实成熟时‘蜂糖李’总酸含量显著低于‘四月李’的主要原因。

2.2.3　柠檬酸　‘蜂糖李’和‘四月李’果实中柠檬酸含量均较低。在果实整个发育过程中，‘蜂糖李’果实中柠檬酸含量整体呈增加趋势（图4），而‘四月李’与其相反，这是‘蜂糖李’和‘四月李’果实有机酸代谢最明显的不同之处。在发育前期，‘蜂糖李’果实中柠檬酸含量显著低于‘四月李’，随着果实的生长发育，‘蜂糖李’中柠檬酸含量开始逐渐高于‘四月李’，至果实成熟时含量最高，此时占总酸的4.04%，是成熟时期‘四月李’的3.26倍，而‘四月李’在果实成熟时柠檬酸含量降到最低，占总酸的0.76%。

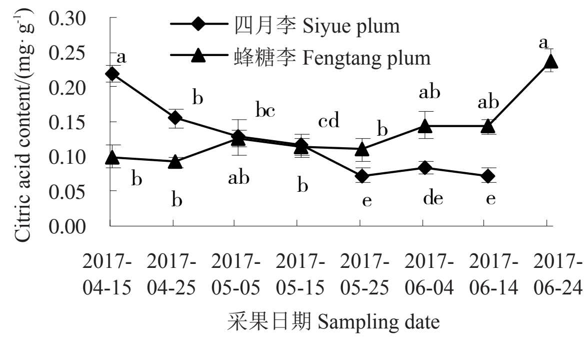


图 4　李果实发育过程中柠檬酸含量的变化

Fig. 4 Changes in citric acid content in the two plum cultivars during fruit development

2.2.4　酒石酸　‘蜂糖李’和‘四月李’果实中酒石酸含量及其占总酸含量的比例从果实开始生长发育至果实成熟均一直降低（图5），‘四月李’酒石酸含量（ω）从1.90 mg·g^{-1}降低至0.34 mg·g^{-1}，而‘蜂糖李’从发育初期开始，酒石酸含量高于‘四月李’，直至‘蜂糖李’生长速率达到最高时，酒石酸含量才迅速降至最低，此时仅占总酸含量的1.11%，且显著低于‘四月李’，之后有所增加，至果实成熟保持平稳。

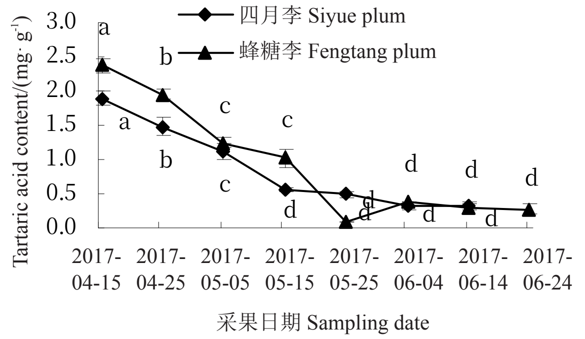


图 5　李果实发育过程中酒石酸含量的变化

Fig. 5 Changes in tartaric acid content in the two plum cultivars during fruit development

2.2.5　草酸　‘蜂糖李’及‘四月李’果实中草酸含量以及占总酸含量的比例在果实整个生长发育内呈现降低的趋势（图6）。‘蜂糖李’果实中草酸含量在果实发育初期占总酸的14.78%，随着果实的生长发育迅速下降，接近果实成熟时占总酸含量的2.28%。2个品种果实中草酸含量均在硬核期迅速下降，直至果实硬核期转向果实第2次快速生长时，‘蜂糖李’果实草酸含量显著高于‘四月李’，其余时期均低于

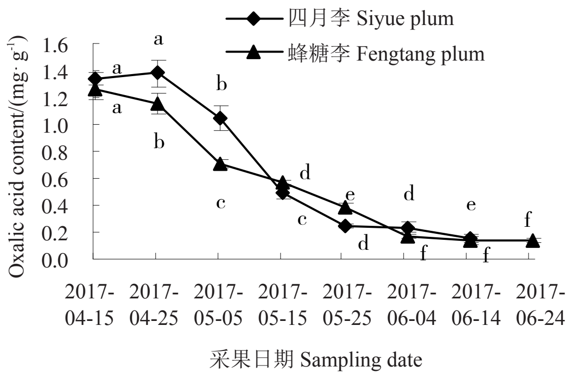


图 6　李果实发育过程中草酸含量的变化

Fig. 6 Changes in oxalic acid content in the two plum cultivars during fruit development

或显著低于‘四月李’。

2.3 苹果酸代谢相关酶活性变化

2.3.1　NAD-苹果酸脱氢酶(NAD-MDH)　从图7可知，在果实生长发育的不同阶段，‘蜂糖李’和‘四月李’果实中NAD-MDH活性差异较大。‘蜂糖李’果实中NAD-MDH活性在82.24~169.12 U·mg^{-1}范围内波动，在整个阶段变化比较平稳。‘四月李’果实中NAD-MDH活性整体呈现“M”型，在果实发育初期和果实成熟时呈现剧增和急降2次较大波动，NAD-MDH在花后45 d活性最高，是同时期‘蜂糖李’6.12倍，而此时也是‘四月李’果实苹果酸含量大量积累的时期，果实成熟时NAD-MDH活性也随之降到最

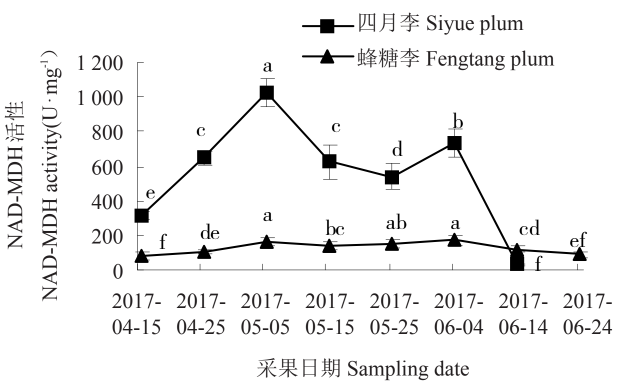


图 7　李果实发育过程中 NAD-MDH 活性的变化

Fig. 7 Changes in NAD-MDH activity in the two plum cultivars during fruit development

低。相关性分析表明，‘蜂糖李’和‘四月李’果实中苹果酸含量与NAD-MDH活性均呈极显著正相关，相关系数分别为0.97**、0.95**。‘四月李’果实中NAD-MDH活性整体高于‘蜂糖李’，与后者苹果酸含量低于前者有着密切的联系(表2)。

表 2　李果实中苹果酸含量与其代谢酶活性的相关性

Table 2 Correlation between malic acids content and some enzymes activities in plum fruits

品种 Cultivar	NAD- MDH	NADP- ME	PEPC
蜂糖李 Fengtang plum	0.97**	-0.86**	0.75*
四月李 Siyue plum	0.95**	-0.87**	0.73*

注：“*”表示相关性在0.05水平显著，“**”表示相关性在0.01水平显著。

Note: “*” indicates correlation is significant at 0.05 level, “**” indicates correlation at 0.01 level, respectively.

2.3.2　NADP-苹果酸酶(NADP-ME)　由图8可以看出，‘蜂糖李’和‘四月李’果实中NADP-ME活性的变化趋势整体相近，均表现为幼果膨大期活性降低，成熟时升高，但不同的是‘蜂糖李’果实中NADP-ME活性始终显著低于‘四月李’。‘蜂糖李’果实NADP-ME活性在硬核期结束至果实成熟前期变化不大，而此期间‘四月李’先迅速增加，后又迅速降低，这与果实中苹果酸含量变化趋势相反。相关性分析表明，‘蜂糖李’和‘四月李’果实中NADP-ME活性的高低与苹果酸含量的变化呈极显著负相关，相关系数r分别为−0.86**、−0.87**(表2)。

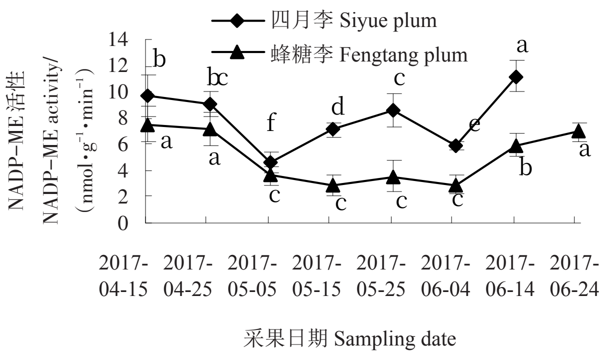


图 8　李果实发育过程中 NADP-ME 活性的变化

Fig. 8 Changes in NADP-ME activity in the two plum cultivars during fruit development

2.3.3　磷酸烯醇式丙酮酸羧化酶(PEPC)　‘蜂糖李’果实中PEPC活性整体呈现“M”型变化(图9)，除了果实成熟期，其余时期均显著高于‘四月李’。在幼果膨大期，PEPC活性逐渐升高，花后40 d左右开始降低，随着果实二次迅速生长酶活性又迅速升高，花后80 d左右迅速降到最低，至果实成熟不再大幅度波动。‘四月李’在果实发育前期和中期表现为“升-降-升”趋势，果实成熟时缓慢降低，整体变化幅度不大。相关性分析表明，‘蜂糖李’和‘四月李’果实中PEPC活性与果实中苹果酸含量呈显著正相

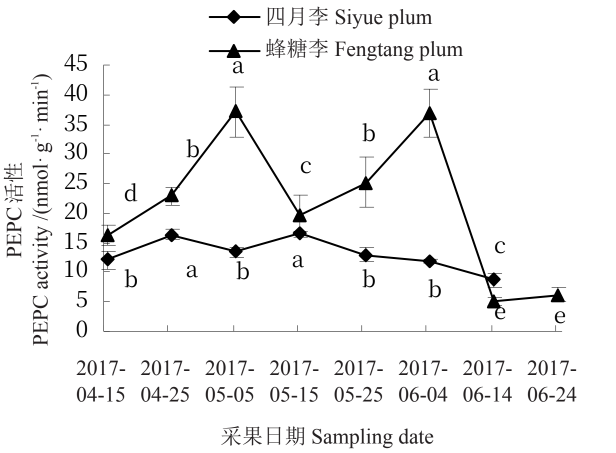


图9　李果实发育过程中PEPC活性的变化

Fig. 9 Changes in PEPC activity in the two plum cultivars during fruit development

关，相关系数r分别为0.75*、0.73*（表2）。

3　讨　论

果实中有机酸组分及含量受果实发育的调控[16]，是遗传特性、自然环境和栽培措施等多种因子共同作用的结果[17]。本研究表明，'蜂糖李'果实中有机酸组分有苹果酸、酒石酸、柠檬酸、草酸、琥珀酸和莽草酸6种，其中苹果酸含量占总酸含量的88%，是果实中主要的有机酸。据报道，李属的8个主要种、野生欧洲李（wild type of *P. domestica* L.）等57份样品[2]总酸含量为11.82~66.38 mg·g^{-1}，而'蜂糖李'果实中总酸含量仅有5.94 mg·g^{-1}，远低于其最小值。因此，'蜂糖李'属于低酸型李品种，低酸是其果实具有独特风味的重要因素之一。

'蜂糖李'果实中总酸含量在果实的生长发育过程中不断降低，与'四月李'基本一致。前者果实在不同发育阶段苹果酸含量占总酸含量的55.08%~88.04%，后者为74.61%~96.14%，可知2者总酸含量的差异主要是由苹果酸含量的差异所致。'蜂糖李'果实中苹果酸含量在果实整个发育过程先增加后降低，且始终显著低于'四月李'，其变化趋势与杏果实极为相近[8]。'蜂糖李'苹果酸在果实成熟前20 d迅速降低，而'四月李'则表现为先升后降，由此可知2者苹果酸含量的差异主要发生在果实发育后期。研究还发现，与'四月李'相比，'蜂糖李'果实中柠檬酸含量的变化趋势与之相反，呈不断增加的趋势，这种差异有待进一步研究。此外，'蜂糖李'在果实发育初期还含有一定比例的草酸和酒石酸，2者均随着果实的生长发育而逐渐降低。

果实中有机酸含量的高低由果实生长发育过程中有机酸合成与降解共同决定[18-19]。在果实发育前期，在'蜂糖李'果实中NADP-ME活性逐渐降低，但PEPC活性急剧升高，而NAD-MDH活性比较稳定，苹果酸含量大量积累，相关性分析表明，'蜂糖李'果实中NAD-MDH、PEPC活性和苹果酸含量存在显著或极显著正相关关系，这表明苹果酸含量的大量积累是受PEPC、NAD-MDH活性共同调控的，但NAD-MDH作用较小，而'四月李'果实中NAD-MDH急剧升高，NADP-ME活性也逐渐降低，而PEPC活性保持稳定，使得苹果酸含量合成远大于降解，表明NAD-MDH是苹果酸大量合成的关键酶。在果实发育后期，'蜂糖李'和'四月李'NADP-ME活性均迅速升高，前者PEPC活性逐渐降低，后者NAD-MDH活性下降，使得2者苹果酸均大量降解，且NADP-ME活性与苹果酸含量存在极显著负相关关系，表明2者果实中苹果酸降解的关键酶是NADP-ME，并再次验证了'蜂糖李'果实中苹果酸含量大量积累的关键酶是PEPC，而在'四月李'中是NAD-MDH。

4　结　论

'蜂糖李'果实中有机酸组分有苹果酸、酒石酸、柠檬酸、草酸、琥珀酸和莽草酸6种，以苹果酸为主要有机酸，属于低酸型果实。'蜂糖李'苹果酸积累的关键时期在果实发育前期，苹果酸含量的变化由PEPC和NADP-ME协同调控，NAD-MDH作用不大。'四月李'果实中影响苹果酸含量的关键酶是NAD-MDH与NADP-ME。

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