

‘早酥’梨及其芽变果实品质、酚类组分和色素合成基因表达分析

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摘要:【目的】明确‘早酥’及其2个红色芽变品种采后果实品质、酚类组分及色素合成相关基因表达水平差异,为生产上科学合理推广新品种提供参考依据。【方法】以‘早酥’及其2个芽变品种果实为试材,对不同贮藏时间的果实外观、内在品质、酚类物质含量和色素合成相关基因表达水平进行测定。【结果】与‘早酥’相比,2个红色芽变品种果实单果质量、果形指数均下降;贮藏前后‘早酥’梨果肉硬度和破裂力均显著低于‘红早酥’和‘红早酥’芽变。‘早酥’的可溶性固形物、可滴定酸和维生素C含量最高,其次是‘红早酥’芽变和‘红早酥’。2个红色芽变品种果皮中共检测到25种酚类物质,‘早酥’中共检测到23种酚类物质,缺少矢车菊素-半乳糖苷和芍药色素-半乳糖苷;3种梨果的果肉中共检测到8种酚类物质且种类相同。不同色素合成基因在3种梨果中表达模式不一致。【结论】‘早酥’的红色芽变品种果实质地较硬,可溶性固形物、可滴定酸和维生素C含量均低于‘早酥’,果皮中酚类物质种类和含量均高于‘早酥’,大部分花青苷合成结构基因和调节基因在2个芽变品种中上调表达,而色素合成相关基因表达水平决定了其为全红或条红类型。

关键词:‘早酥’梨;芽变品种;品质;酚类物质;色素合成基因

中图分类号:S661.2

文献标志码:A

文章编号:1009-9980(2020)12-1787-11

Analysis of fruit quality, composition of phenolic compounds and expression of genes related to pigment synthesis in ‘Zaosu’ pear and two sport varieties (strains)

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Abstract: 【Objective】Fruit quality, composition of phenolic compounds and expression level of the genes related to pigment synthesis of ‘Zaosu’ pear (*Pyrus bretschneideri* Rehd.) and the two sport varieties (strains) were evaluated to provide reference for extension of new varieties in production. 【Methods】Using the fruits of ‘Zaosu’ pear and two sport varieties (strains) as materials, the appearance, internal quality, and composition of phenolic compounds expression level of the genes related to pigment synthesis of the fruits with different storage time were measured. 【Results】The values of brightness and color saturation of the fruits of ‘Zaosu’ were higher than those of ‘Zaosu Red’ and its bud sport. After 14 days storage, the values of brightness and color saturation of the fruits of all three varieties (strains) increased, the fruit skins brightened gradually. The content of anthocyanin in the fruits of bud

收稿日期:2020-06-22 接受日期:2020-09-01

基金项目:国家重点研发计划项目(2016YFD0400903-06);国家现代农业产业技术体系建设专项(CARS-29-19);中国农业科学院科技创新工程(CAAS-ASTIP)

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sport of 'Zaosu Red' was significantly higher than that of 'Zaosu Red', while the content of anthocyanin in the fruits of 'Zaosu' was very low. Compared with 'Zaosu' fruits, the single fruit weight and fruit shape index of the two red sport varieties (strains) decreased. The flesh firmness and rupture force of the fruits of three varieties (strains) were significantly different before and after storage, among them 'Zaosu' pear was the lowest and 'Zaosu Red' bud sport was the highest. The flesh firmness and rupture force of the fruits of the three varieties (strains) decreased after 14 days storage. In terms of internal quality, the contents of total soluble solids, titratable acid and vitamin C of 'Zaosu' fruits were the highest, followed by 'Zaosu Red' bud sport and 'Zaosu'. After 14 days storage, the total soluble solids contents, titratable acid contents and vitamin C contents of the fruits of the three varieties (strains) decreased, while the contents of total soluble solids and vitamin C of the fruits of 'Zaosu' were still the highest. While the ratios of solids and acids of the fruits of the two bud varieties (strains) were higher than that of 'Zaosu' before and after storage. 25 kinds of polyphenols were detected in the fruit peels of the two sport varieties (strains), including one simple polyphenol, two phenolic acids, five flavanols, twelve flavonols, three flavonoids and two anthocyanins. The content of arbutin was the highest and peonidin 3-*O*-galactoside was the lowest. respectively. 23 kinds of polyphenols were detected in the in the fruit peels of 'Zaosu' pear, 2 kinds of anthocyanins (cyanidin galactoside and paeoniflorin galactoside) were not detected. The content of arbutin was the highest and polyphenols B1 was the lowest, respectively. 8 kinds of phenols were detected in the fruit pulps of the three varieties, including one simple polyphenol, two phenolic acids, three flavanols. The content of arbutin was the highest and procyanidin B1 was the lowest. On the day of harvest, the expression patterns of the *PpPAL2*, *PpCHS1*, *PpCHS2*, *PpANS*, *PpUFGT2*, *PpbHLH* and *PpWD40* were the same in the fruits of the three varieties (strains), and the expression level of these genes in the fruits of 'Zaosu Red' bud sport was significantly higher than those of 'Zaosu'. The expression level of the *PpCHI1* was the highest in the fruits of 'Zaosu Red' bud sport, followed by 'Zaosu' and 'Zaosu Red'. The expression level of the *PpCHS4* was the highest in the fruits of 'Zaosu Red', significantly higher than those in the fruits of 'Zaosu Red' bud sport and 'Zaosu'. The *PyMYB114* was expressed specifically in the fruits of 'Zaosu Red' bud sport on the harvest day and the level of expression decreased during storage. It was lower in the fruits of other two varieties (strains). The expression level of the *PpPAL2*, *PpCHS1*, *PpCHS2*, *PpCHI1*, *PpANS*, *PpUFGT2*, *PyMYB114* in the fruits of the three varieties (strains) decreased after 14 days storage at 20 °C. The expression patterns of different genes for pigment synthesis in three varieties (strains) were different.【Conclusion】The fruits of the two red bud sport varieties (strains) of 'Zaosu' had a hard texture, and their contents of total soluble solids, titratable acid and vitamin C were all lower than those of 'Zaosu'. Most of the structural genes and regulatory genes were up-regulated in the two sport varieties (strains). The expression level of genes related to pigment synthesis might determine the types of coloration of the fruits of the bud sport varieties (strains) in pear.

Key words: 'Zaosu' pear; Bud sports; Quality; Phenolic compounds; Pigment synthetic gene

‘早酥’梨由中国农业科学院果树研究所育成，母本为‘苹果梨’，父本为‘身不知’。‘早酥’梨的红色芽变现象首次发现于陕西渭南，并由西北农林科技大学将其定名为‘红早酥’，其表现形式有全红和条红两种类型，全红类型为‘红早酥’芽变。红色芽变品种在外观上得到广大种植者重视，并在一定区域

内得到了快速发展，然而，‘早酥’梨及其两个红色芽变品种之间果实品质、酚类物质组分及含量是否有差异、红色芽变果实品质是否优于‘早酥’等方面的研究尚未见报道。目前，关于‘早酥’梨及其芽变品种‘红早酥’的研究主要集中在花青苷合成和类黄酮组分等方面。翟锐等^[1]研究表明‘早酥’果皮中类黄

酮种类要少于‘红早酥’。董晓勤等^[2]研究了‘早酥’和‘红早酥’的光合生理特性,发现‘红早酥’中叶绿素和花色素苷含量显著高于‘早酥’。钱敏杰^[3]发现‘红早酥’各组织中花青苷含量均显著高于‘早酥’,且条纹着色果实中红色条纹花青苷含量高于绿色条纹。‘红早酥’花青苷合成相关结构基因 *PbCHS*、*PbDFR*、*PbUFGT* 的克隆及表达分析已有相关报道^[4-5]。此外,一些转录因子也参与了花青苷合成调控,如 MYB 家族、bHLH 家族和 WD40 家族等^[6-10]。在‘红早酥’中已鉴定到众多 MYB 家族、ERF 家族和 COP 家族成员正/负向调控花青苷合成^[11-14]。胡敏^[15]和仇宗浩^[16]利用差异蛋白质组学方法研究‘早酥’及其红色芽变品种,分别鉴定到了 22 个蛋白质点和 71 个差异蛋白;Liu 等^[17]对‘早酥’及其早熟芽变品种进行蛋白组学研究并鉴定到了 25 个差异表达蛋白;赵莞^[18]利用代谢组学技术对不同处理的‘红早酥’类黄酮化合物积累进行了研究,发现乙烯利能够与茉莉酸甲酯共同作用调控‘红早酥’果皮着色。笔者以‘早酥’及其红色芽变品种‘红早酥’和‘红早酥’芽变为试材,对果实采后品质、酚类物质组分及含量、色素合成相关基因的表达水平进行比较分析,旨在明确‘早酥’梨红色芽变品种品质特点,为示范推广提供依据,也为研究红梨果皮着色机制提供理论支撑。

1 材料和方法

1.1 试验材料

‘早酥’‘红早酥’和‘红早酥’芽变梨果试材均来自中国农业科学院果树研究所梨种质资源圃,具体采收时间见表 1。选取长势良好、树龄 10 a(年)的梨树,以种子尖端变褐作为果实成熟采收标准,在树冠外围和内膛不同方向均匀采收,采收后立即运回实验室。选择大小一致、无病虫害及磕碰伤的果实,每种果实随机分为 2 组,每组 1 个重复,每个重复包含

表 1 ‘早酥’梨及芽变品种采收时间
Table 1 Harvesting date of ‘Zaosu’ pear and its budding pear

品种名称 Cultivar name	采收日期 Harvesting date	盛花后时间 Time after blooming/d
早酥 Zaosu	08-26	123
红早酥 Zaosu Red	09-22	150
红早酥芽变 Zaosu Red bud sport	09-22	150

10 个果实。第 1 组果实于采摘当天进行果实相关指标测定,第 2 组果实在(20±1)℃、相对湿度 85%~90%环境条件下贮藏 14 d 后再进行果实相关指标测定。果实相关指标测定取样时将果皮和果肉分别用液氮速冻后装入密封袋中,于-80℃超低温冰箱中保存,测定时液氮冷冻研磨成粉末,用于基因表达分析。

1.2 果实性状测定

采用游标卡尺测定每个果实的纵横径,分析天平测定每个果实单果质量,TMS-PRO 质构仪(探头直径为 75 mm)测定果实破裂力和硬度,PR-101α 折光仪(日本 ATAGO)测定可溶性固形物含量,CR-400 色差计测定果皮颜色。所用光源为 D65,分别测定果皮的亮度(L^*)和饱和度(C^*)。测定以上指标每次用果 10 个,取平均值。

可滴定酸采用酸碱滴定法测定,所用仪器为瑞士万通 808 电位滴定仪;维生素 C 含量采用 2,6-二氯酚酚滴定法测定,所用仪器为瑞士万通 808 电位滴定仪;酚类物质含量采用超高效液相色谱—二极管阵列—串联四级杆质谱联用法(UPLC-PDA-MS/MS-ESI)测定,对果皮和果肉分别取样进行测定,测定方法参见李静等^[19];花青素含量测定取 0.5 g 梨果皮在液氮中研磨,以 15%盐酸乙醇为提取液,过夜提取 24 h,取上清液于分光光度计 530 nm 波长下检测 OD 值,用 $A_{530} \cdot \text{mg}^{-1}$ (以鲜质量计)作为花青素的相对含量。以上测定指标均为 3 个技术重复。

1.3 RNA 提取和 cDNA 第一条链合成

采用 Trizol 提取试剂盒提取试验梨果果皮的总 RNA。以 3 种试材的 RNA 为模板,使用第一链 cDNA 合成试剂盒(RevertAid Premium Reverse Transcriptase)(Thermo Scientific™ EP0733)将 RNA 反转录成 cDNA,用于后续研究。

1.4 引物设计及 qRT-PCR

采用 StepOne 型荧光定量 PCR 仪(ABI),反应体系为 SybrGreen qPCR Master Mix 10 μL ,cDNA 模板 2 μL ,上下游引物各 0.4 μL ,ddH₂O 7.2 μL ;扩增程序为 95℃预变性 3 min;95℃变性 7 s,57℃退火 10 s,72℃延伸 15 s,共 45 个循环,每个样本 3 次重复。*PpPAL2*、*PpCHS1*、*PpCHS2*、*PpCHS4*、*PpCH11*、*PpANS*、*PpUFGT2*、*PyMYB114*、*PpbHLH*、*PpWD40* 的定量 PCR 特异引物序列参考 Qian 等^[20]的方法(表 2)。

表 2 实时荧光定量 PCR 引物
Table 2 Primer sequence used in qRT-PCR

目的基因 Target gene	引物编号 Primer code	引物序列(5'-3') Primer sequence(5'-3')
<i>PpPAL2</i>	PpPAL2-F	GGATTGCTTGAAGGAGTGGAAATG
	PpPAL2-R	ATTCGTAGAAGATCCAGTACAATGC
<i>PpCHS1</i>	PpCHS1-F	GGGTGTACTCTTCGGATTGG
	PpCHS1-R	AAAGGCGGAAAACAATACATATACG
<i>PpCHS2</i>	PpCHS2-F	GGAGACCGTCGTGCTTCATAG
	PpCHS2-R	CTTCAATTCGTAAATATCATCAAAG-TTCC
<i>PpCHS4</i>	PpCHS4-F	GGAATCGGACCAGGACTCAC
	PpCHS4-R	AGCCCTCCAAATTATACTCTCTCG
<i>PpANS</i>	PpANS-F	AGTTGTTCAGGAAAAGCCAAGAGG
	PpANS-R	ACAAAGCAGGCAGATAGGAGTAGC
<i>PpUFGT2</i>	PpUFGT2-F	GGTCAAACTGGCAGTAGAG
	PpUFGT2-R	TGTCCGCCCGTAGATGGTA
<i>PpbHLH</i>	PpbHLH-F	CTGATAGAGATGAGATGCCCTTACC
	PpbHLH-R	GGATGGATGATTGGACCGAGTG
<i>PpWD40</i>	PpWD40-F	GGCGGCGTCGGTGTATTC
	PpWD40-R	GGTCTCTGCTTGTTCATCC
<i>PpACTIN</i>	PpACTIN-F	CCATCCAGGCTGTTCTCTC
	PpACTIN-R	GCAAGGTCCAGACGAAGG
<i>PpCHI1</i>	PpCHI1-Fn	TTGAGTTCTTCAGGGACATCG
	PpCHI1-Rn	TCCAAAAGGCAACGCAAT
<i>PyMYB114</i>	PyMYB114-F	GCCACATCCGTCATAAGACCTC
	PyMYB114-R	GCCACTCATGTGTAACCCTC

1.5 数据分析

采用 Excel 2010 进行数据处理,采用 SPSS 21.0 进行差异显著性分析。

2 结果与分析

2.1 ‘早酥’及其红色芽变果实成熟期及外观比较

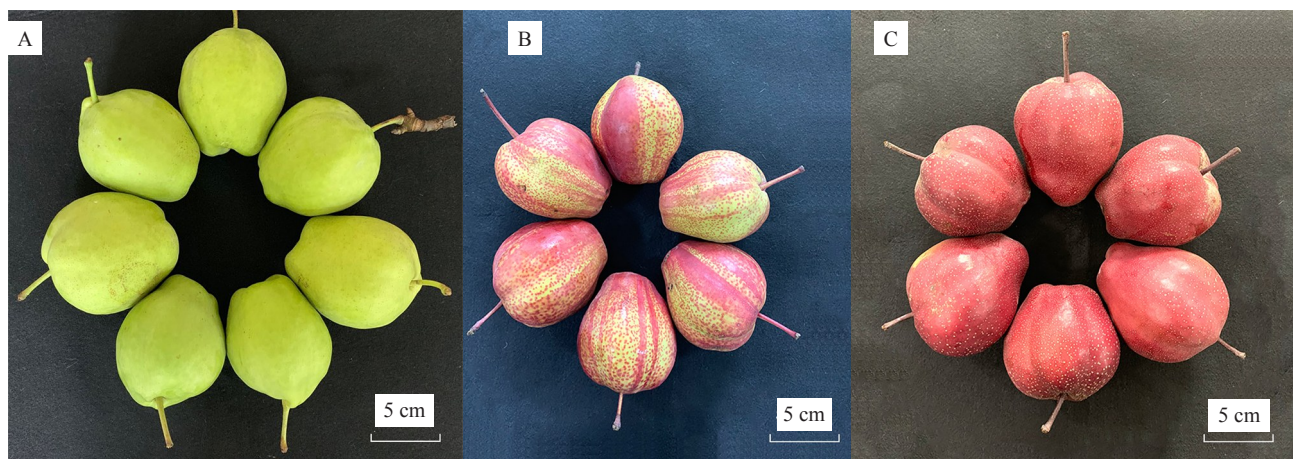
在辽宁兴城地区,‘早酥’果实一般于8月下旬成熟,而‘红早酥’成熟期略晚,在9月下旬成熟。‘早酥’梨果皮呈绿色或黄绿色(图1-A),其芽变品种‘红早酥’果皮为条红(图1-B),而‘红早酥’芽变果皮全红(图1-C)。从图2-A、B可知,3种梨果中‘早酥’果皮亮度最高,显著高于其他2个芽变品种,在贮藏14 d后3种梨果的 L^* 和 C^* 均呈上升趋势,表明果皮颜色正在发生变化,果皮逐渐变亮,果实正在趋于成熟,这与观察到的果皮颜色变化趋势一致。‘红早酥’芽变中花青素含量显著高于‘红早酥’,而‘早酥’中花青素含量极低,几乎检测不到(图2-C)。

由图3可知,3种梨果中以‘早酥’的单果质量最高,为318.8 g,‘红早酥’芽变的单果质量最低,为220.1 g,显著低于‘红早酥’和‘早酥’;果形指数即为纵横径比,三者果形指数均大于1,均呈卵圆形或圆锥形,但芽变品种的果形指数显著低于‘早酥’。

2.2 ‘早酥’及其红色芽变品种果实内在品质比较

2.2.1 ‘早酥’及其红色芽变品种果实质地比较

3种梨果中以‘早酥’破裂力最低,‘红早酥’芽变破裂力最高但下降最快,‘红早酥’贮藏期破裂力下降最慢。3种梨果中‘红早酥’芽变的硬度最高,其次是‘红早酥’,‘早酥’硬度最低;经过14 d贮藏后,果肉硬度全部下降,‘红早酥’芽变硬度下降最快,且2个芽变品种的硬度显著高于‘早酥’(表3)。

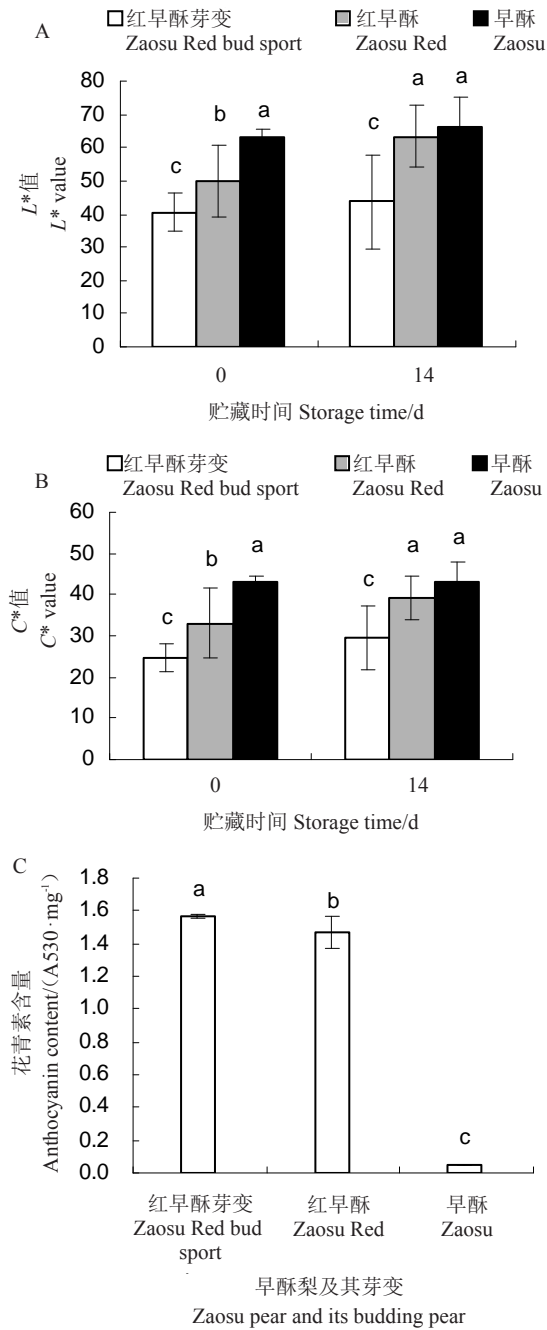


A. 早酥; B. 红早酥; C. 红早酥芽变。

A. Zaosu; B. Zaosu Red; C. Zaosu Red bud sport.

图 1 ‘早酥’及其芽变果实

Fig. 1 ‘Zaosu’ and its budding pear



不同小写字母表示差异显著 ($p < 0.05$)。下同。
Different small letters represent significant difference at $p < 0.05$. The same below.

图 2 ‘早酥’梨及其芽变果实果皮颜色比较
Fig. 2 Peel color comparison of ‘Zaosu’ pear and its budding pear

2.2.2 ‘早酥’及其红色芽变品种果实可溶性固形物、可滴定酸和维生素C含量比较 ‘早酥’可溶性固形物含量显著高于其芽变品种,在贮藏14 d后,3种梨果实可溶性固形物含量均呈下降趋势,‘早酥’可溶性固形物含量仍最高。‘红早酥’芽变和‘红早酥’可滴定酸含量较低,贮藏14 d时,‘红早酥’芽变

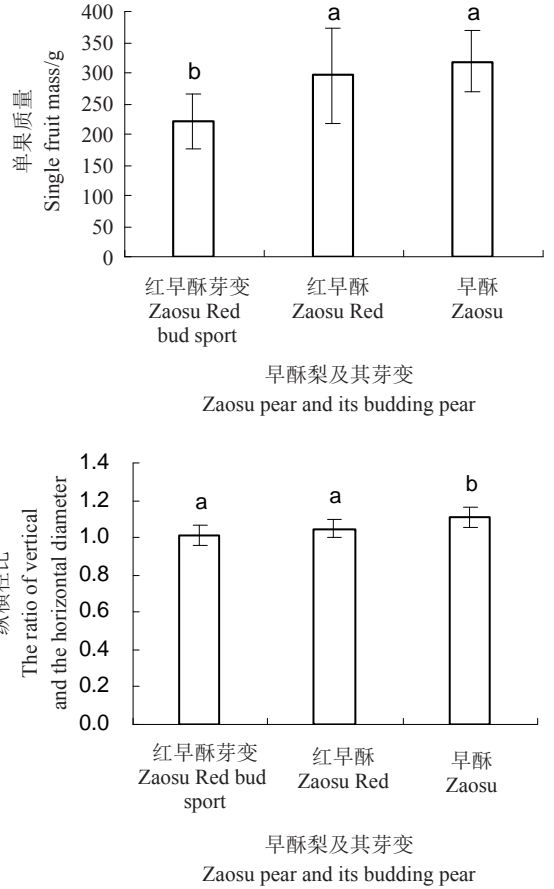


图 3 ‘早酥’梨及其芽变果实单果质量及果形指数比较
Fig. 3 Single fruit mass and fruit shape of ‘Zaosu’ pear and its budding pear

表 3 ‘早酥’梨及其芽变果实质地比较
Table 3 Texture comparison of ‘Zaosu’ pear and its budding pear

品种名称 Cultivar name	0 d		14 d	
	破裂力 Rupture force/N	果肉硬度 Flesh firmness/N	破裂力 Rupture force/N	果肉硬度 Flesh firmness/N
红早酥芽变 Zaosu Red bud sport	19.89 a	16.05 a	16.86 a	13.84 a
红早酥 Zaosu Red	17.71 b	14.77 b	17.59 a	13.45 a
早酥 Zaosu	16.21 c	12.58 c	13.89 b	11.12 b

注:0 d 和 14 d 代表贮藏时间。下同。

Note: 0 d and 14 d represent storage time. The same below.

和‘早酥’可滴定酸含量呈下降趋势,而‘红早酥’则呈现上升趋势。由表4可知,采收当日‘早酥’维生素C含量最高,为35.86 mg·kg⁻¹,其次为‘红早酥’芽变(29.55 mg·kg⁻¹)和‘红早酥’(23.83 mg·kg⁻¹),而贮藏14 d后‘红早酥’芽变和‘红早酥’维生素C含量均有所下降,‘早酥’维生素C含量仍显著高于前两

表 4 ‘早酥’梨及其芽变果实品质比较
Table 4 Quality comparison of ‘Zaosu’ pear and its budding pear

品种名称 Cultivar name	0 d				14 d			
	w(可溶性固形物) Soluble solids content/%	w(可滴定酸) Titratable acid content/%	w(维生素C) Vitamin C content/(mg·kg ⁻¹)	固酸比 Solid acid ratio	w(可溶性固形物) Soluble solids content/%	w(可滴定酸) Titratable acid content/%	w(维生素C) Vitamin C content/(mg·kg ⁻¹)	固酸比 Solid acid ratio
红早酥芽变 Zaosu Red bud sport	11.34 ab	0.092 c	29.55 b	123.21 a	10.63 a	0.086 c	22.51 b	123.60 a
红早酥 Zaosu Red	11.04 b	0.104 b	23.83 c	106.47 b	10.37 a	0.136 a	22.59 b	76.25 c
早酥 Zaosu	11.50 a	0.122 a	35.86 a	94.01 c	11.11 a	0.117 b	37.98 a	94.72 b

注: 0 d 和 14 d 代表贮藏时间。

Note: 0 d and 14 d represent storage time.

者。‘红早酥’芽变和‘红早酥’的固酸比显著高于‘早酥’, 贮藏 14 d 后, ‘红早酥’芽变和‘早酥’固酸比上升, ‘红早酥’固酸比下降。

2.3 ‘早酥’及其红色芽变品种果实酚类物质比较

如表 5 所示, 在果皮中, ‘红早酥’芽变和‘红早酥’共检测到 25 种酚类物质, 含量最高和最低的多

表 5 ‘早酥’及其芽变果实果皮中酚类物质含量比较
Table 5 Comparison of polyphenol contents in pericarp of ‘Zaosu’ and its budding pear (mg·100 g⁻¹)

序号 Number	酚类名称 Polyphenol name	0 d			14 d		
		红早酥芽变 Zaosu Red bud sport	红早酥 Zaosu Red	早酥 Zaosu	红早酥芽变 Zaosu Red bud sport	红早酥 Zaosu Red	早酥 Zaosu
1	熊果苷 Arbutin	172.24±2.63 c	198.39±0.30 a	178.83±0.58 b	165.35±0.77 b	173.58±0.63 a	162.72±0.14 c
2	儿茶素 Cate	26.83±0.24 a	12.03±0.07 b	8.95±0.20 c	21.72±0.65 a	16.45±0.77 b	6.46±0.18 c
3	表儿茶素 Epicate	25.04±0.69 b	12.45±0.60 c	31.89±0.09 a	19.36±1.71 b	22.28±1.32 a	24.47±0.51 a
4	原花青素 B1 Procyanidins B1	0.94±0.11 a	0.44±0.02 b	0.25±0.02 c	1.57±0.10 a	0.79±0.04 b	0.33±0.03 c
5	原花青素 B2 Procyanidins B2	6.97±0.46 a	3.51±0.05 b	3.54±0.12 b	8.23±0.80 a	5.27±0.51 b	3.55±0.17 c
6	原花青素 C1 Pro-cyanidins C1	5.58±0.70 a	3.61±0.16 b	4.04±0.38 b	6.34±0.04 a	5.00±0.10 b	4.49±0.46 b
7	矢车菊素-半乳糖苷 Cyanidin 3-O-galactoside	0.76±0.16 a	0.63±0.11 a	-	1.02±0.02 a	0.65±0.03 b	-
8	芍药色素-半乳糖苷 Peonidin 3-O-galactoside	0.07±0.01 a	0.06±0.01 a	-	0.10±0.01 a	0.07±0.00 b	-
9	绿原酸 Chlorogenic acid	32.84±0.18 a	14.43±0.14 b	10.61±0.15 c	32.50±0.69 a	24.43±1.04 b	11.81±0.22 c
10	Z-咖啡酰奎尼酸 Z-caffeoylquinic acid	0.36±0.03 b	0.24±0.07 c	0.84±0.06 a	0.17±0.01 b	0.15±0.01 b	0.21±0.01 a
11	木樨草素-芸香糖苷 Luteolin 7-O-rutinoside	2.18±0.06 a	2.31±0.06 a	1.52±0.23 b	1.77±0.03 a	1.86±0.01 a	3.58±2.47 a
12	木樨草素-葡萄糖苷 Luteolin-7-O-glucoside	6.09±0.24 b	9.09±0.19 a	4.37±0.31 c	5.04±0.02 b	7.02±0.02 a	4.17±1.16 b
13	槲皮素-阿拉伯糖酰葡萄糖苷 Quercetin 3-O-arabinosylglucoside	3.67±0.04 a	2.81±0.01 b	1.05±0.01 c	2.81±0.02 a	2.62±0.05 b	0.84±0.02 c
14	芦丁 Rutin	2.77±0.11 b	3.06±0.02 a	1.50±0.01 c	2.10±0.02 b	2.39±0.01 a	1.04±0.05 c
15	槲皮素-半乳糖苷 Quercetin-galactoside	20.39±0.30 b	21.92±0.12 a	0.94±0.10 c	17.65±0.13 a	16.76±0.13 b	0.73±0.07 c
16	槲皮素-葡萄糖苷 quercetin 3-O-glucoside	10.69±0.07 b	13.06±0.03 a	4.62±0.16 c	9.51±0.22 a	9.95±0.23 a	3.37±0.34 b
17	槲皮素-3-O-6-丙二酰-葡萄糖苷 Quercetin-3-O-6- malonyl -glucoside	19.56±1.00 b	25.22±0.52 a	6.70±0.43 c	16.17±0.41 a	16.75±0.26 a	3.13±2.09 b
18	异鼠李素-刺槐糖苷 Isorhamnetin 3-O-robinioside	6.86±0.15 a	5.87±0.09 b	1.81±0.08 c	5.14±0.04 a	4.42±0.01 ab	1.12±0.36 b
19	异鼠李素-芸香糖苷 Isorhamnetin 3-O-rutinoside	7.08±0.19 b	7.46±0.19 a	5.18±0.22 c	5.51±0.05 a	5.45±0.09 a	3.67±0.45 b
20	异鼠李素-半乳糖苷 Isorhamnetin 3-O-galactoside	54.26±0.14 a	54.96±0.25 a	2.76±0.04 b	50.20±1.54 a	43.20±1.23 b	1.65±0.92 c
21	异鼠李素-葡萄糖苷 Isorhamnetin 3-O-glucoside	27.57±0.11 b	31.66±0.17 a	13.25±0.83 c	24.94±0.98 a	24.55±0.97 a	11.53±0.80 b
22	异鼠李素 3-O-6"-丙二酰-半乳糖苷 Isorhamnetin 3-O-6"-malonylglactoside	45.76±1.80 a	46.22±0.57 a	2.14±0.11 b	37.73±1.36 a	31.57±0.95 b	1.64±0.05 c
23	异鼠李素-3-O-6"-丙二酰-葡萄糖苷 Isorhamnetin 3-O-6"-malonylglucoside	46.32±2.49 b	52.98±1.42 a	22.55±1.47 c	37.81±0.93 a	35.51±0.52 b	16.85±0.47 c
24	异鼠李素 3-O-丙二酰-半乳糖苷 Isorhamnetin 3-O-malonylglactoside	2.55±0.24 a	2.83±0.25 a	1.21±0.19 b	2.15±0.14 a	2.02±0.09 a	0.91±0.05 b
25	柯伊利素-葡萄糖苷 Chrysoeriol 7-O-glucoside	3.76±0.15 c	5.44±0.04 a	5.13±0.16 b	3.03±0.00 c	4.76±0.10 a	4.13±0.00 b

酚物质为熊果苷和芍药色素-半乳糖苷;‘早酥’共检测到23种酚类物质,含量最高和最低的多酚物质为熊果苷和原花青素B1。简单酚有熊果苷1种,‘红早酥’中含量最高,显著高于‘红早酥’芽变和‘早酥’,在贮藏14 d后含量呈下降趋势;酚酸类物质2种,分别为绿原酸和Z-咖啡酰奎尼酸,‘红早酥’芽变中绿原酸含量最高,显著高于‘红早酥’和‘早酥’,3个品种中绿原酸含量均远高于Z-咖啡酰奎尼酸,说明绿原酸为主要酚酸成分;黄烷醇类物质5种,包括儿茶素、表儿茶素、原花青素B1、原花青素B2和原花青素C1,儿茶素、表儿茶素含量较高,是主要的黄烷醇类物质,原花青素B1、原花青素B2和原花青素C1在贮藏期含量均有所升高;共检测到12种黄酮醇类物质(表5,13-24),包括槲皮素糖苷类物质5种、异鼠李素糖苷类物质7种,‘早酥’中各黄酮醇类物质含量低于2个芽变品种,在贮藏14 d后三者的黄酮醇类物质含量均降低;共检测到黄酮类物质3

种,分别为木犀草素-葡萄糖苷、木犀草素-芸香糖苷和柯伊利素-葡萄糖苷,‘红早酥’的木犀草素-葡萄糖苷和柯伊利素-葡萄糖苷含量显著高于其他品种;2个芽变品种中共检测到花色苷类物质2种,分别为矢车菊素-半乳糖苷和芍药色素-半乳糖苷,矢车菊素-半乳糖苷的含量是芍药色素-半乳糖苷的10倍,是主要的花色苷组成成分,在贮藏14 d时二者均呈上升趋势。而在‘早酥’中,并未检测到花色苷类物质,与花青苷含量变化趋势一致。

在3种梨果果肉中共检测到共计3类8种酚类物质,分别为熊果苷、儿茶素、表儿茶素、原花青素B1、原花青素B2、原花青素C1、绿原酸和Z-咖啡酰奎尼酸,其中熊果苷含量最高(4.73~4.93 mg·100 g⁻¹),其次为绿原酸,原花青素B1含量最低(0.01~0.02 mg·100 g⁻¹)(表6)。果肉中没有检测到黄酮醇类和黄酮类物质,而检测到的酚类物质种类和含量远低于果皮。

表6 ‘早酥’梨及其芽变果实果肉中酚类物质含量比较

Table 6 Comparison of polyphenol content in pulp of ‘Zaosu’ pear and its budding pear (mg·100 g⁻¹)

序号 Number	酚类名称 Polyphenol name	0 d			14 d		
		早酥芽变 Zaosu Red bud sport	红早酥 Zaosu Red	早酥 Zaosu	早酥芽变 Zaosu Red bud sport	红早酥 Zaosu Red	早酥 Zaosu
1	熊果苷 Arbutin	4.93±0.06 a	4.91±0.01 a	4.73±0.03 b	5.11±0.09 c	5.26±0.01 b	5.99±0.02 a
2	儿茶素 Cate	0.05±0.01 b	0.08±0.01 a	0.09±0.01 a	0.04±0.01 b	0.03±0.00 b	0.08±0.00 a
3	表儿茶素 Epicate	0.42±0.01 c	0.47±0.02 b	0.54±0.01 a	0.23±0.02 b	0.11±0.00 c	0.37±0.00 a
4	原花青素B1 Procyanidins B1	0.01±0.00 a	0.02±0.00 a	0.01±0.01 a	0.01±0.00 a	0.008±0.00 a	0.01±0.00 a
5	原花青素B2 Procyanidins B2	0.10±0.01 c	0.26±0.01 b	0.29±0.00 a	0.18±0.01 b	0.17±0.01 b	0.27±0.01 a
6	原花青素C1 Procyanidins C1	0.03±0.00 b	0.08±0.01 a	0.09±0.00 a	0.06±0.01 b	0.05±0.01 b	0.10±0.01 a
7	绿原酸 Chlorogenic acid	3.70±0.13 a	2.77±0.10 c	3.14±0.08 b	2.03±0.05 a	0.75±0.01 c	1.70±0.01 b
8	Z-咖啡酰奎尼酸 Z-caffeoylquinic acid	0.51±0.02 a	0.39±0.02 b	0.52±0.01 a	0.32±0.03 a	0.16±0.01 b	0.31±0.00 a

2.4 ‘早酥’及其红色芽变品种果实花青苷合成途径相关基因表达量比较

由图4可知,在采收当日,*PpPAL2*、*PpCHS1*、*PpCHS2*、*PpANS*、*PpUFGT2*、*PpbHLH*和*PpWD40*在3种梨果中的表达模式一致,表达量均为在‘红早酥’芽变中最高,在‘早酥’中最低,且‘红早酥’芽变与‘早酥’间差异显著;*PpCH11*在‘红早酥’芽变中表达量最高,其次是‘早酥’和‘红早酥’;*PpCHS4*在‘红早酥’中表达量最高,显著高于‘红早酥’芽变和‘早酥’;*PpMYB114*在采收当日‘红早酥’芽变中特异性表达,在其他果实以及贮藏期表达量均较低。贮藏14 d时,*PpPAL2*、*PpCHS1*、*PpCHS2*、*PpCH11*、*PpANS*、*PpUFGT2*、*PpMYB114*表达量均呈下降趋

势;*PpCHS4*、*PpbHLH*和*PpWD40*表达量在‘红早酥’芽变和‘红早酥’中呈下降趋势,但在‘早酥’中呈上升趋势。

3 讨论

3.1 ‘早酥’及其红色芽变品种果实外观品质差异

红皮梨由于艳丽的果皮颜色和潜在的营养价值受到了消费者的喜爱,并得到了广泛种植^[21]。‘早酥’梨果皮呈绿色或黄绿色,其芽变品种出现了全红和条红两种类型。从外观角度来看,‘早酥’的两个红色芽变品种更能引起消费者以及种植者的兴趣。质地是评价果实内在品质的重要指标之一,本研究结果表明,‘早酥’的破裂力和果肉硬度均显著低于其

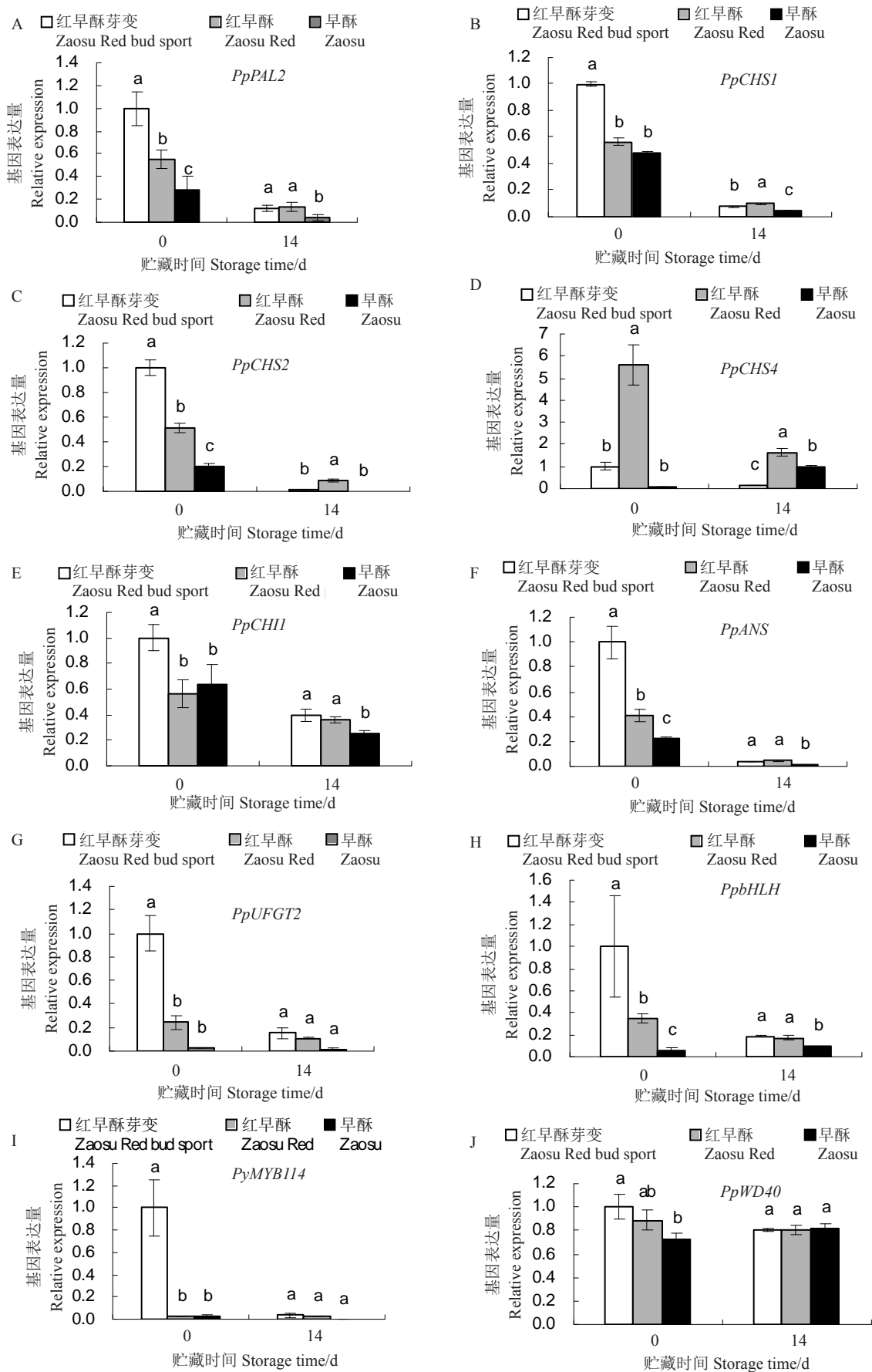


图 4 花青苷合成途径 10 个关键基因在‘早酥’梨及其红色芽变品种中表达量比较

Fig. 4 Comparison of expression levels of 10 key genes in anthocyanin biosynthesis pathway in ‘Zaosu’ pear and its budding pear

红色芽变品种,果肉质地类型更趋向于消费者喜爱的酥脆型。可溶性固形物和可滴定酸含量决定了果实的营养和风味特点,本研究中,‘早酥’的可溶性固形物、可滴定酸和维生素C含量最高,因此,综合内在品质与外观品质,‘早酥’的品质在一定程度上优于其芽变品种。

3.2 ‘早酥’及其红色芽变品种果实酚类物质差异

多酚是植物体内重要的次生代谢产物,主要存在于植物的根、叶和果实中,具有重要的抗氧化作用,是评价果实品质的重要指标^[22]。本研究中‘早酥’与2个芽变品种在酚类物质的组成和含量上存在差异。前人研究表明,红梨果皮表面的红色成分主要为矢车菊素-3-半乳糖苷和芍药素-3-半乳糖苷^[23-24]。本研究发现,与芽变品种相比,‘早酥’果皮中缺少矢车菊素-半乳糖苷和芍药素-半乳糖苷,与前人研究结果一致。在‘早酥’梨果皮中大部分酚类物质含量要低于2个‘芽变品种’。3种梨果的果肉中只检测到8种酚类物质,种类和含量远低于果皮,这与Salta等^[25]对6个梨栽培品种的研究结果一致。张小双等^[26]研究发现,在‘早酥’果皮和果肉中含量最高的均为熊果苷,Cui等^[27]和Lin等^[28]分别对鸭梨和16个梨品种进行研究发现,果皮中含量最高的为熊果苷和绿原酸。本文中,无论是‘早酥’还是其芽变品种,果皮和果肉中含量最高的均为熊果苷。酚类物质能够对果实的色泽和风味产生影响,进而影响果实品质,植物多酚的种类和含量也可以作为评价果实品质的重要指标^[29]。多酚物质中的绿原酸、儿茶素具有苦味和涩味^[30-31]。‘早酥’的绿原酸和儿茶素含量显著低于芽变品种,果实风味更佳。

3.3 ‘早酥’及其红色芽变品种果实色素合成基因表达差异

红梨果皮颜色主要由花青苷含量及比例决定,目前关于色素合成基因在不同红皮梨果实、不同环境中的表达模式研究已见诸多报道,但是关于‘早酥’、‘红早酥’和‘红早酥’芽变之间的基因表达差异研究较少^[32-33]。本研究选取了10个已被报道与花青苷合成相关的基因,其中*PpPAL2*、*PpCHS1*、*PpCHS2*、*PpCHS4*、*PpCHI1*、*PpANS*、*PpUFGT2*为结构基因,*PyMYB114*、*PpbHLH*、*PpWD40*为调节基因。结果表明,大部分结构基因和调节基因在2个芽变品种中上调表达且差异明显。除*PpCHS4*和*PpWD40*外,其余基因在‘红早酥’芽变中的表达量

均显著高于‘红早酥’。贮藏14 d后,*PpUFGT2*、*PyMYB114*和*PpWD40*的表达水平在组内差异不显著,表明这3个基因可能主要在果实发育期或贮藏前期发挥功能。

‘红早酥’芽变品种果面全红,与‘红早酥’在外观上存在一定区别。翟锐^[34]认为‘红早酥’红色条纹果实着色模式与维管束依赖型花青苷积累相关,并鉴定到*PbGA2ox8*能够诱导*PbUFGT1*等关键基因表达上调从而在维管束区域调控花青苷合成;Qian等^[20]认为‘红早酥’出现全红和条红类型与MYB家族转录因子*PyMYB10*的表达水平有关(*PyMYB10*能够调控*PpUFGT2*表达);Yao等^[35]利用QTL定位鉴定到*PyMYB114*并证实其能够调控梨花青苷合成,但并未对其在‘早酥’中的表达量进行研究。笔者在前人研究基础上,对*PyMYB114*、*PpbHLH*和*PpWD40*在3种试材中进行表达量分析,结果表明*PyMYB114*在贮藏前期的‘红早酥’芽变中高表达,*PpbHLH*在‘红早酥’芽变中表达量显著高于‘红早酥’。Yang等^[36]对‘早红考密斯’及其绿色芽变品种中的*WD40*基因进行研究,认为其并不是调控花青苷合成的关键转录因子。而在本文中,贮藏前后*PpWD40*在3个品种中的表达量没有差异,因此*PpWD40*可能不是调控‘早酥’芽变品种花青苷合成的关键转录因子。综合分析,2个红色芽变品种着色差异主要由花青苷合成关键基因表达水平决定,且*PpbHLH*和*PyMYB114*可能是关键调控因子,但具体作用机制还需进一步验证。

4 结 论

‘红早酥’和‘红早酥’芽变作为‘早酥’的红色芽变品种,对我国红梨品种选育具有重要意义,但其果肉硬度高,可溶性固形物、可滴定酸以及维生素C含量均显著低于‘早酥’。‘早酥’及其红色芽变品种中含量最高的酚类物质均为熊果苷,果皮酚类物质种类和含量比果肉丰富;与其红色芽变品种相比,‘早酥’中缺少主要的花青苷成分——矢车菊素-半乳糖苷和芍药素-半乳糖苷。大部分花青苷合成途径结构基因和调节基因在2个芽变品种中上调表达且差异明显;红色芽变品种中全红和条红类型的产生与花青苷合成关键基因差异表达相关。

参考文献 References:

[1] 翟锐,房晨,范二婷,蔡猛,王志刚,徐凌飞.‘早酥’梨及其红

- 皮芽变‘红早酥’梨类黄酮组分变化与合成模式研究[J]. 果树学报, 2016, 33(S1): 75-82.
- ZHAI Rui, FANG Chen, FAN Erting, CAI Meng, WANG Zhigang, XU Lingfei. The dynamic concentration changes and biosynthesis pattern of flavonoid compounds in ‘Zaosu’ pear and its red bud mutant ‘Red zaosu’ [J]. Journal of Fruit Science, 2016, 33(S1): 75-82.
- [2] 董晓勤, 周鹏, 仇宗浩, 翟锐, 徐凌飞. 两个品种绿皮梨及其红色芽变品种光合生理特性的比较[J]. 北方园艺, 2013(20): 1-5.
- DONG Xiaoqin, ZHOU Peng, QIU Zonghao, ZHAI Rui, XU Lingfei. Comparison of photosynthetic physiological characteristics between two varieties of green pear and red bud variety[J]. Northern Horticulture, 2013(20): 1-5.
- [3] 钱敏杰. DNA 甲基化和 microRNA 调控红梨果皮着色的机制研究[D]. 杭州: 浙江大学, 2017.
- QIAN Minjie. Mechanism of DNA methylation and MicroRNA reginating peel coloration in red pear[D]. Hangzhou: Zhejiang University, 2017.
- [4] 冯文亭, 翟锐, 王志刚, 朱春琴, 徐凌飞. ‘红早酥’梨花青苷合成相关基因的克隆及表达分析[J]. 西北农业学报, 2015, 24(6): 75-83.
- FENG Wenting, ZHAI Rui, WANG Zhigang, ZHU Chunqin, XU Lingfei. Cloning and expression analysis of anthocyanin biosynthesis related genes in ‘Hongzaosu’ pear[J]. Acta Agriculturae Boreali-Occidentalis Sinica, 2015, 24(6): 75-83.
- [5] 张士伟. ‘红早酥’梨 PbUFGT1 基因的调控研究[D]. 杨凌: 西北农林科技大学, 2015.
- ZHANG Shiwei. The regulation of PbUFGT1 gene in ‘Redzaosu’ pear[D]. Yangling: Northwest Agriculture and Forestry University, 2015.
- [6] NI J, BAI S, ZHAO Y, QIAN M, TAO R, YIN L, GAO L, TENG Y. Ethylene response factors Pp4ERF24 and Pp12ERF96 regulate blue light-induced anthocyanin biosynthesis in ‘Red Zaosu’ pear fruits by interacting with MYB114[J]. Plant Molecular Biology, 2019, 99(1/2): 67-78.
- [7] BAN Y, HONDA C, HATSUYAMA Y, IGARASHI M, BESH-HO H, MORIGUCHI T. Isolation and functional analysis of a MYB transcription factor gene that is a key regulator for the development of red coloration in apple skin[J]. Plant & Cell Physiology, 2007, 48(7): 958-970.
- [8] PIERANTONI L, DONDINI L, DE FRANCESCHI P, MUSACCHI S, WINKEL B S, SANSVINI S. Mapping of an anthocyanin-regulating MYB transcription factor and its expression in red and green pear, *Pyrus communis*[J]. Plant Physiology Biochemistry, 2010, 48(12): 1020-1026.
- [9] YUAN K, WANG C, WANG J, XIN L, ZHOU G, LI L, SHEN G. Analysis of the MdMYB1 gene sequence and development of new molecular markers related to apple skin color and fruit-bearing traits[J]. Molecular Genetics and Genomics, 2014, 289(6): 1257-1265.
- [10] AGUILAR-BARRAGÁN A, OCHOA-ALEJO N. Virus-induced silencing of MYB and WD40 transcription factor genes affects the accumulation of anthocyanins in chilli pepper fruit[J]. Biologia Plantarum, 2014, 58(3): 567-574.
- [11] 赵莹晓. PbMYB12 转录因子参与调控梨类黄酮合成的功能研究[D]. 杨凌: 西北农林科技大学, 2018.
- ZHAO Yingxiao. The biological function of PbMYB12 in regulation of flavonoid biosynthesis in pear fruit[D]. Yangling: Northwest Agriculture and Forestry University, 2018.
- [12] 王智敏. 红梨果皮着色相关 R2R3 型 MYB 转录因子基因功能分析[D]. 杨凌: 西北农林科技大学, 2016.
- WANG Zhimin. Functional analysis of R2R3 MYB transcription factors about coloration in red pear[D]. Yangling: Northwest Agriculture and Forestry University, 2016.
- [13] 杜艳民. ‘早酥’红色芽变果实 PbMYBR 及相关基因的克隆及其表达分析[D]. 杨凌: 西北农林科技大学, 2012.
- DU Yanmin. Cloning and expression analysis of PbMYBR and related genes in red skin mutant of ‘Zaosu’ pear[D]. Yangling: Northwest Agriculture and Forestry University, 2012.
- [14] WU M, SI M, LI X, SONG L, LIU J, ZHAI R, CONG L, YUE R, YANG C, MA F, XU L, WANG Z. PbCOPI.1 contributes to the negative regulation of anthocyanin biosynthesis in pear[J]. Plants, 2019, 8(2): 39.
- [15] 胡敏. 早酥梨及其红皮芽变差异蛋白质组学研究[D]. 杨凌: 西北农林科技大学, 2011.
- HU Min. Differential proteomics analysis of the Zaosu pear and its redskin bud mutation[D]. Yangling: Northwest Agriculture and Forestry University, 2011.
- [16] 仇宗浩. 早酥梨及其红色芽变果实的差异蛋白质组学分析[D]. 杨凌: 西北农林科技大学, 2014.
- QIU Zonghao. Proteomics analysis of ‘Zaosu’ pear (*Pyrus bretschneideri* Rehd.) and its red bud mutation after fruit bagging[D]. Yangling: Northwest Agriculture and Forestry University, 2014.
- [17] LIU Y J, DU Y M, XU L F, HU M, LI Z H. A suppression subtractive hybridization library construction for red skin mutant of ‘Zaosu’ pear and MYBR gene analysis[J]. New Zealand Journal of Crop and Horticultural Science, 2012, 40(2): 87-101.
- [18] 赵芫. 乙烯利协同茉莉酸甲酯调控‘红早酥’梨果皮着色的代谢组学研究[D]. 杭州: 浙江大学, 2019.
- ZHAO Yuan. Metabonomics study on ethephon and methyl jasmonate synergistically regulating peel coloration of ‘Hongzaosu’ pear[D]. Hangzhou: Zhejiang University, 2019.
- [19] 李静, 聂继云, 曹玉芬, 李志霞, 闫震, 毋永龙. 砀山酥梨和秋白梨酚类物质 UPLC - PDA - MS/MS - ESI 分析[J]. 园艺学报, 2016, 43(4): 752-762.
- LI Jing, NIE Jiyun, CAO Yufen, LI Zhixia, YAN Zhen, WU Yonglong. UPLC- PDA- MS/MS- ESI analysis of phenolic compounds in fruits of Dangshan suli and Qiubaili pears (*Pyrus bretschneideri*)[J]. Acta Horticulturae Sinica, 2016, 43(4): 752-

- 762.
- [20] QIAN M, SUN Y, ALLAN A C, TENG Y, ZHANG D. The red sport of ‘Zaosu’ pear and its red-striped pigmentation pattern are associated with demethylation of the PyMYB10 promoter [J]. *Phytochemistry*, 2014, 107: 16-23.
- [21] ZHANG D, QIAN M, YU B, TENG Y. Effect of fruit maturity on UV-B-induced post-harvest anthocyanin accumulation in red Chinese sand pear[J]. *Acta Physiologiae Plantarum*, 2013, 35(9): 2857-2866.
- [22] 江丽芳. 雪花梨果皮多酚含量测定[J]. *化学工程与装备*, 2011 (12): 168-172.
- JIANG Lifang. Determination of polyphenols content in Snowflake pear’s peel[J]. *Chemical Engineering & Equipment*, 2011 (12): 168-172.
- [23] 肖长城, 李甲明, 姚改芳, 刘军, 胡红菊, 曹玉芬, 张绍铃, 吴俊. 不同红梨品种果皮中花色苷组分及含量特征分析[J]. *南京农业大学学报*, 2014, 37(4): 60-66.
- XIAO Changcheng, LI Jiaming, YAO Gaifang, LIU Jun, HU Hongju, CAO Yufen, ZHANG Shaoling, WU Jun. Characteristics of components and contents of anthocyanin in peel of red-skinned pear fruits from different species[J]. *Journal of Nanjing Agricultural University*, 2014, 37(4): 60-66.
- [24] DUSSI M C, SUGAR D, WROLSTAD R E. Characterizing and quantifying anthocyanins in red pears and the effect of light quality on fruit color[J]. *Journal of the American Society for Horticultural Science*, 1995, 120(5): 785-789.
- [25] SALTA J, MARTINS A, SANTOS R G, NENG N R, NOGUEIRA J M F, JUSTINO J, RAUTER A P. Phenolic composition and antioxidant activity of Rocha pear and other pear cultivars: A comparative study[J]. *Journal of Functional Foods*, 2010, 2 (2): 153-157.
- [26] 张小双, 郑迎春, 曹玉芬, 田路明, 董星光, 张莹, 齐丹, 霍宏亮. ‘早酥’和‘南果梨’16个部位多酚物质组成及含量分析[J]. *中国农业科学*, 2017, 50(3): 545-555.
- ZHANG Xiaoshuang, ZHENG Yingchun, CAO Yufeng, TIAN Luming, DONG Xingguang, ZHANG Ying, QI Dan, HUO Hongliang. The composition and content of polyphenols in 16 parts of ‘Zaosu’ and ‘Nanguoli’ [J]. *Scientia Agricultura Sinica*, 2017, 50(3): 545-555.
- [27] CUI T, NAKAMURA K, MA L, LI J Z, KAYAHARA H. Analyses of arbutin and chlorogenic acid, the major phenolic constituents in oriental pear[J]. *Journal of Agricultural & Food Chemistry*, 2005, 53(10): 3882-3887.
- [28] LIN L, HARNLY J M. Phenolic compounds and chromatographic profiles of pear skins (*Pyrus* spp.)[J]. *Journal of Agricultural and Food Chemistry*, 2008, 56(19): 9094-9101.
- [29] 王贤萍, 段泽敏, 戴桂林, 杨晓华, 聂国伟, 韩彦龙. 甜樱桃主要栽培品种多酚含量的测定与品质分析[J]. *中国农学通报*, 2011, 27(13): 173-176.
- WANG Xianping, DUAN Zemin, DAI Guilin, YANG Xiaohua, NEI Guowei, HAN Yanlong. Polyphenol quantitative analysis and quality evaluated in fruit of sweet cherry cultivars[J]. *Chinese Agricultural Science Bulletin*, 2011, 27(13): 173-176.
- [30] KALLITHRAKA S, BAKKER J, CLIFFORD M N. Evaluation of bitterness and astringency of (+)-catechin and (-)-epicatechin in red wine and in model solution[J]. *Journal of Sensory Studies*, 1997, 12(1): 25-37.
- [31] TAO R, BAI S, NI J, YANG Q, ZHAO Y, TENG Y. The blue light signal transduction pathway is involved in anthocyanin accumulation in ‘Red Zaosu’ pear[J]. *Planta*, 2018, 248(1): 37-48.
- [32] QIAN M, YU B, LI X, SUN Y, ZHANG D, TENG Y. Isolation and expression analysis of anthocyanin biosynthesis genes from the red Chinese sand pear, *Pyrus pyrifolia* Nakai cv. Mantianhong, in response to methyl jasmonate treatment and UV-B/VIS conditions[J]. *Plant Molecular Biology Reporter*, 2014, 32(2): 428-437.
- [33] YU B, ZHANG D, HUANG C, QIAN M, ZHENG X, TENG Y, SU J, SHU Q. Isolation of anthocyanin biosynthetic genes in red Chinese sand pear (*Pyrus pyrifolia* Nakai) and their expression as affected by organ/tissue, cultivar, bagging and fruit side[J]. *Scientia Horticulturae*, 2012, 136: 29-37.
- [34] 翟锐. 芽变‘红早酥’梨的花青苷与类黄酮合成机理解析[D]. 杨凌: 西北农林科技大学, 2019.
- ZHAI Rui. The biosynthesis pattern of anthocyanin and flavonoid in bud sport ‘Red Zaosu’ pear[D]. Yangling: Northwest Agriculture and Forestry University, 2019.
- [35] YAO G, MING M, ALLAN A C, GU C, LI L, WU X, WANG R, CHANG Y, QI K, ZHANG S, WU J. Map-based cloning of the pear gene MYB114 identifies an interaction with other transcription factors to coordinately regulate fruit anthocyanin biosynthesis[J]. *The Plant Journal*, 2017, 92(3): 437-451.
- [36] YANG Y, ZHAO G, YUE W, ZHANG S, GU C, WU J. Molecular cloning and gene expression differences of the anthocyanin biosynthesis-related genes in the red/green skin color mutant of pear (*Pyrus communis* L.)[J]. *Tree Genetics & Genomes*, 2013, 9 (5): 1351-1360.