

枣果实成熟过程中类黄酮的积累 及相关基因的表达分析

李 希, 石倩倩, 祝恒均, 杜江涛, 李新岗*

(西北农林科技大学林学院·陕西省林业综合重点实验室, 陕西杨凌 712100)

摘要:【目的】探究枣果实成熟过程中类黄酮的积累及相关基因的表达模式, 为枣功能成分类黄酮的开发利用提供依据。【方法】以‘稷山板枣’和‘骏枣’为材料, 采用分光光度法测定两个品种果实成熟过程中果皮和果肉的总黄酮和总黄烷醇含量, 利用HPLC检测类黄酮组分的含量变化, qRT-PCR分析类黄酮合成相关基因的表达。【结果】‘稷山板枣’和‘骏枣’成熟过程中, 果皮和果肉的总黄酮和总黄烷醇含量逐渐下降, 干枣时期有所增加, 果皮中含量高于果肉, ‘稷山板枣’含量高于‘骏枣’; 两个品种果皮和果肉中类黄酮组分基本相同, 黄烷醇和黄酮醇含量从白熟期逐渐下降, 部分黄酮醇组分含量在成熟后期(半红/全红)含量有所上升; 类黄酮合成相关基因在两种枣果皮中的表达量从白熟期到完熟期呈下调趋势, 但品种间及不同成熟时期的表达有所差异。相关性分析结果显示, 下游基因与类黄酮及其组分含量呈显著或极显著相关。【结论】两个枣品种成熟过程中, 类黄酮积累模式基本一致, 枣果皮较果肉含量高, 且均在果实成熟早期积累最高, 随果实成熟而下降; 结构基因 $F3H$ 、 $F3'H$ 可能对类黄酮的合成积累具有关键促进作用, LAR 可能是黄烷醇途径中的关键调控基因。

关键词:枣; 果实; 类黄酮; 积累模式; 基因表达

中图分类号:S665.1

文献标志码:A

文章编号:1009-9980(2020)10-1464-11

The patterns of flavonoids accumulation and the expression of biosynthesis related genes during the course of maturation of the Chinese jujube fruit

LI Xi, SHI Qianqian, ZHU Dajun, DU Jiangtao, LI Xingang*

(College of Forestry, Northwest Agriculture & Forestry University/Key Comprehensive Laboratory of Forestry, Shaanxi Province, Yangling 712100, Shaanxi, China)

Abstract: 【Objective】Chinese jujube (*Ziziphus jujuba* Mill.) is an economic forest fruit tree in the Rhamnaceae. The jujube production in China accounts for more than 90% in the world. Jujube fruits are rich in flavonoids. The study aimed to explore the accumulation pattern of the flavonoids and the expression of related genes during the ripening of jujube fruit, and provide a basis for the development and utilization of functional components in jujube fruits. 【Methods】The experimental materials ‘Jishanbanzao’ and ‘Junzao’ were obtained from the Jujube Experimental Station of Northwest A & F University in Qingjian, Shaanxi, China. The jujube fruits at different stages (white maturity, beginning-red, half-red, red maturity, full maturity, dry date period) were separated into peel and pulp. The samples were immediately frozen in liquid nitrogen and stored in the ultra low temperature refrigerator at -80 °C. The contents of total flavonoids and total flavanols in the peel and the pulp of two jujube varieties at different degree of maturation were determined by spectrophotometry, and the change of each flavonoid component content was detected by HPLC. qRT-PCR was used to analyze the expression of

收稿日期:2020-05-19 接受日期:2020-06-08

基金项目:国家基础研发课题(2018YFD1000607);甘肃省高等学校科研项目(2017A-046)

作者简介:李希,女,在读硕士研究生,研究方向为经济林学。Tel:18829354698, E-mail:lixi@nwafu.edu.cn

*通信作者 Author for correspondence. Tel:18220683122, E-mail:xingangle@nwafu.edu.cn

flavonoid synthesis-related genes. 【Results】 During the whole maturation process of ‘Jishanbanzao’ and ‘Junzao’, the content of total flavonoids in the peel and the pulp showed a continuous downward trend as the fruit matured, and it increased slightly in the dry date period. The content of total flavonoids of ‘Jishanbanzao’ was higher than that of ‘Junzao’ in all stages. The total flavonoids content in the pulp of the two varieties was much lower than that of the peel (the difference was 3.27 to 36.90 times). The total flavanols content in the peel of the two varieties was the highest in the white maturity period, and decreased slowly at the beginning red stage, and reduced greatly in the half-red stage and full red stage. The lowest content was found in the dry date period, only about 1.5% of the initial ripening content. During the ripening process, the total flavanol content in the pulp was always lower than that of the peel but it increased in the dry date period which was higher than that in the peel. Four kinds of flavanols and five kinds of flavonols were detected in the peel and pulp of ‘Jishanbanzao’ and ‘Junzao’ by HPLC. Flavanols were catechin, epicatechin, procyanidin B1 and procyanidin B2. The contents of catechin in the two varieties were the highest in the peels and pulps, the content of proanthocyanidin B2 in the peel was the lowest, and the contents of epicatechin and proanthocyanidin B2 in the pulp were lower. The four flavanol substances in the peels gradually decreased with the ripeness of the fruits and the contents decreased to the minimum value at the stage of dry date. The change trends of the four flavanol components in the pulps from white maturity stage to the full maturity stage were consistent with the peels, showing a continuous decline, but it increased significantly in the dry date period. Quercetin and its glycoside derivatives were the main flavonols. Quercetin and its glycosides showed a downward trend as a whole from the white maturity stage to the full maturity stage in the peels of the two jujube varieties, but the contents of some substances increased slightly when they were half-red or full-red. The contents of all flavonol components in the pulps were significantly lower than that in the peel, and gradually decreased from the white maturity stage, but the content increased in the dry date period. qRT-PCR analysis found that the changes in the expression of flavonoid synthesis-related genes in the peels of the two jujubes were basically the same. There was a downward trend from the white maturity to the full maturity stage, but the expression varied with the varieties and the different maturity periods. The correlation analysis results showed that the downstream genes (*F3H*, *F3'H*, *FLS*, *ANR*, *LAR*, *UFGT*) were significantly or extremely significantly related to the content of flavonoids and the components. 【Conclusion】 The accumulation patterns of flavonoids in the two jujube varieties during maturation were basically the same with slight differences. The contents of the total flavonoids in the peel were higher than that in the pulp (the difference was about 20 times). The accumulation of flavonoids was the highest in the early stage of fruit ripening, and it decreased with the ripening of the fruit. Structural genes showed a downward trend as the fruit ripening, and downstream genes were significantly correlated with the flavonoid content. The structural genes *F3H* and *F3'H* might play a key role in promoting the synthesis and accumulation of flavonoids, and *LAR* might be the key regulatory gene in the flavanol pathway.

Key words: Chinese jujube (*Ziziphus jujuba* Mill.); Fruits; Flavonoids; Accumulation pattern; Gene expression

枣(*Ziziphus jujuba* Mill.)又名中国枣、红枣、大枣,为鼠李科枣属植物,是原产我国的重要经济树种,栽培利用历史悠久,品种资源丰富^[1]。我国一直是世界枣生产大国,栽培面积和产量均居世界首位^[2]。枣果实美味可口,因集营养和药用于一体而

备受推崇,即具“药食同源”的功效。枣果除了含有糖、酸、维生素等营养成分外,还含有酚、生物碱、多糖等多种生物活性物质,这些活性成分使枣果实具有抗氧化、抗过敏、增强免疫力、抗辐射、抗癌和抑制血管舒张等功能^[3-4]。

类黄酮是植物中一类重要的多酚类次生代谢物,具有很强的生物活性,除了在影响植物色泽、提高植物抗逆性等方面有重要作用外,对人体也具有极强的抗病毒、提高免疫力等生理功效^[5-6]。目前,果实的类黄酮研究主要集中在苹果、柑橘及葡萄等^[7-9]树种,相较之下,关于枣黄酮类物质的研究内容较为单一。近年来,国内外学者利用溶剂提取法、超声波辅助法、酶解法、大孔树脂吸附法等方法研究了枣中类黄酮的提取效率并进行优化^[10-11]。关于不同品种和不同部位的总黄酮含量比较、枣果实类黄酮的组分鉴定和抗氧化活性的研究较多^[10,12-13],而其合成调控机制报道较少,且主要集中在与着色相关的花青素上^[14]。

笔者以制干品种‘骏枣’和‘稷山板枣’为材料,探究枣果实成熟过程中类黄酮含量的动态变化,以

及代谢途径中相关结构基因的表达差异,以明确枣果实类黄酮的积累模式及其分子合成机制,为进一步研究枣果实类黄酮的生物学功能奠定基础,也为枣果实功能成分类黄酮的深度开发和综合利用提供依据。

1 材料和方法

1.1 材料

试验材料‘稷山板枣’和‘骏枣’采自西北农林科技大学清涧红枣试验站。采集成熟过程中不同时期的枣果实,即白熟期、初红期、半红期、全红期、完熟期以及干枣(图1)。取样时,将果皮与果肉分离,分别将果皮与果肉迅速置于液氮中处理,每个样品3个生物学重复,每个重复用果10~15个,所有样品保存于-80℃超低温冰箱。



WM. 白熟期; BR. 初红期; HR. 半红期; RM. 全红期; FM. 完熟期; DP. 干枣。

WM. White maturity; BR. Beginning-red; HR. Half-red; RM. Red maturity; FM. Full maturity; DP. Dry date period.

图1 ‘稷山板枣’和‘骏枣’不同成熟阶段的果实

Fig. 1 The fruits of ‘Jishanbanzao’ and ‘Junzao’ at different stages of maturity

1.2 方法

1.2.1 类黄酮物质提取 类黄酮物质提取参考Shi等^[15]的方法,稍有改动。取-80℃保存的枣果皮和果肉进行冷冻干燥后,分别取样品0.5 g加入4 mL 1%盐酸-甲醇提取液,超声15 min,置于4℃冰箱浸提24 h(避光),12 000 r·min⁻¹离心15 min,吸取上清液,真空浓缩后加提取液至1 mL复溶,用0.22 μm有机滤膜过滤后备用。

1.2.2 总黄酮和总黄烷醇含量测定 总黄酮含量测定参照Wang等^[16]的方法,稍有改动。枣果皮、果肉提取液20、200 μL,分别加1.98、1.8 mL甲醇稀释,然后加入150 μL NaNO₂(0.5 mol·L⁻¹)和150 μL AlCl₃(0.3 mol·L⁻¹)后混匀。5 min之后,加入750 μL

NaOH(1 mol·L⁻¹),在510 nm波长下测定吸光值,以芦丁为标准(10~250 mg·L⁻¹),结果以芦丁等价值表示。

总黄烷醇含量测定参照Li等^[17]的方法。将25 μL 枣果皮/果肉提取液和1 mL p-DMACA溶液混匀。10 min后,在640 nm波长下测定吸光值,儿茶素为标准品(5~250 mg·L⁻¹),结果以儿茶素等价值表示。

1.2.3 利用HPLC测定类黄酮组分 用安捷伦1260高效液相色谱仪对提取液进行类黄酮组分检测。检测参考Shi等^[15]的方法。在280、320、360、520 nm波段下检测黄酮醇、黄烷醇等组分的含量。

1.2.4 利用qRT-PCR分析类黄酮合成相关基因的表达 按照天根多糖多酚植物总RNA提取试剂盒

说明书, 分别提取‘稷山板枣’和‘骏枣’不同成熟阶段枣果皮的总RNA。cDNA的合成参照TaKaRa反转录试剂盒说明书。通过‘骏枣’转录组数据筛选类黄酮合成相关的差异基因(*PAL*、*CHS*、*CHI*、*F3H*、*F3'H*、*FLS*、*ANR*、*LAR*、*UFGT*), 根据‘骏枣’基因组数据, 利用Primer 5.0设计荧光定量PCR特异性引

物, 内参基因选择*UBQ1*和*UBQ2*^[18], 引物序列如表1所示。以‘稷山板枣’和‘骏枣’不同成熟阶段枣果皮的cDNA为模板, 使用TaKaRa公司的SYBR Premix Ex Taq™ II试剂盒, 在LightCycler 96荧光定量PCR仪上进行荧光定量表达分析, 采用 $2^{\Delta\Delta Ct}$ 方法进行数据分析。

表1 qRT-PCR引物序列

Table 1 Primer sequences for qRT-PCR

基因 Gene	上游引物 Forward primer(5'-3')	下游引物 Reverse primer(5'-3')
<i>PAL</i>	CCCAATGGCGAATACCTCA	GACAAGATTCCGACAGCACAA
<i>4CL</i>	GTGGGTCGGATCAAGGAGC	TCGCCATACTTATCATCAGGGAA
<i>CHS</i>	ATCACCGCCGTCACTTCCG	CCGTCTATGGCTCCATCCGAGT
<i>CHI</i>	AATCCCTTCCCTCCTCA	GCTCACTTCCCTGTTACCT
<i>F3H</i>	TTGGGTTCTATGACACCG	TCTCAATTGGGAGGGTAC
<i>F3'H</i>	GGAAGATCAGCTCCGTACACCT	GACCTAGCATCACCCCTCCCTAAA
<i>LAR</i>	AATGGCAGTGTCTTGTGTT	CTTGGAGTGCTTTAATGGTGG
<i>ANR</i>	GAAGGGCTATGCCGTCAAT	CCTCAGTTAGATCAGCACCAA
<i>FLS</i>	CAAAGAAACCATCCCAGAAGA	ATGGACTCGAGATTCTACCTCCAT
<i>UFGT</i>	CAGAACGGATCGGAGGGAA	CCAACCACAATGGGATACAAA
<i>UBQ1</i>	TGGATGATTCTGGCAAAG	GTAATGGCGGTCAAAGTG
<i>UBQ2</i>	CACCCGTTACTTGCTTTC	CTCTTCCCATTGTCCTCC

1.3 数据分析

运用Excel 2010和SPSS 19.0进行数据的统计整理和差异性分析。

2 结果与分析

2.1 枣果实成熟过程中总黄酮、总黄烷醇含量的变化

图2-A~B显示了‘稷山板枣’和‘骏枣’果实成熟过程中果皮和果肉总黄酮含量的变化。两个品种枣果皮中总黄酮含量均随着果实的成熟呈现持续下降趋势, 干枣有所上升, 且‘稷山板枣’在各个阶段均高于‘骏枣’。‘稷山板枣’和‘骏枣’总黄酮含量(w, 后同)在白熟期最高, 分别达到85.41、73.07 mg·g⁻¹; 在完熟期下降至最低, 仅有4.1、3.17 mg·g⁻¹, 两个成熟阶段分别相差20.78、23.05倍。两个品种果肉中总黄酮含量远低于果皮(相差3.27~36.90倍), 变化趋势和果皮相同, 从白熟期到完熟期两个品种的含量分别为1.01~2.73 mg·g⁻¹和0.86~1.98 mg·g⁻¹, 干枣果皮和果肉中含量均有所增加。

图2-C~D为两个枣品种的果皮和果肉中总黄烷醇含量, 其变化趋势与总黄酮相似。‘稷山板枣’和‘骏枣’果皮中的总黄烷醇含量同样在白熟期最高,

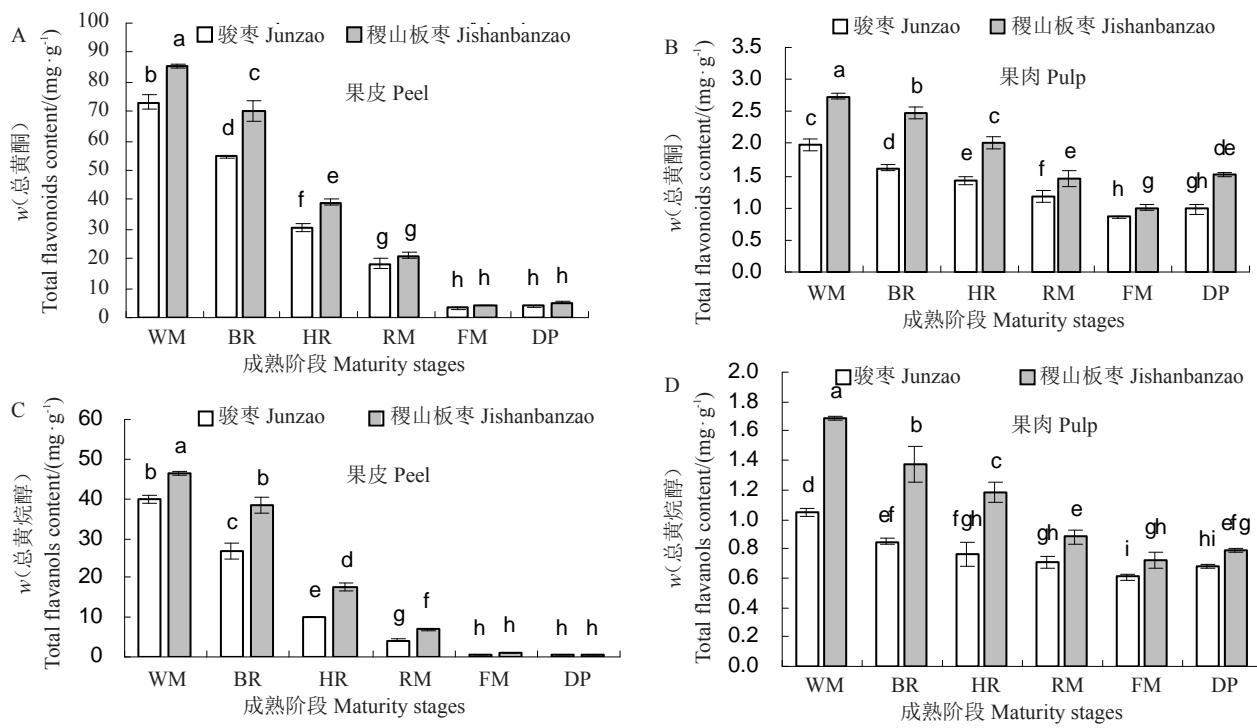
分别为46.48、39.70 mg·g⁻¹, 在初红阶段下降较为缓慢, 而半红和全红时则有大幅度降低, 直至干枣时达到最小值, 仅有成熟初期含量的1.5%左右。在成熟过程中果肉的总黄烷醇含量始终处于很低的水平, ‘稷山板枣’果肉中含量变化范围为0.72~1.69 mg·g⁻¹, 而‘骏枣’含量更低, 最高仅有1.05 mg·g⁻¹, 和果皮有所不同的是, 干枣果肉中总黄烷醇含量增加, 且高于果皮中的含量。

2.2 成熟过程中枣果皮和果肉黄烷醇类物质含量的变化

利用高效液相色谱(HPLC)检测了‘稷山板枣’和‘骏枣’果皮和果肉中的类黄酮组分, 共检测到4种黄烷醇和5种黄酮醇物质。

儿茶素、表儿茶素、原花青素B1和原花青素B2是‘稷山板枣’和‘骏枣’果皮和果肉中主要的黄烷醇物质, 两个品种果皮和果肉中儿茶素含量均最高, 果皮中原花青素B2含量最低, 果肉中表儿茶素和原花青素B2含量较低。

如图3所示, 枣果皮中除了‘骏枣’表儿茶素含量在完熟期、原花青素B1含量在干枣阶段比‘稷山板枣’略高之外, 其他各个成熟阶段中‘稷山板枣’的4类黄烷醇物质含量均比‘骏枣’高。两个枣品种果



不同小写字母表示差异显著($p < 0.05$)。下同。

Different small letters indicate significant differences ($p < 0.05$). The same below.

图2 枣果实成熟过程中果皮和果肉总黄酮(A、B)、总黄烷醇(C、D)含量的变化

Fig. 2 The content of total flavonoids (A, B) and total flavanols (C, D) in the peel and pulp of jujube fruit during ripening

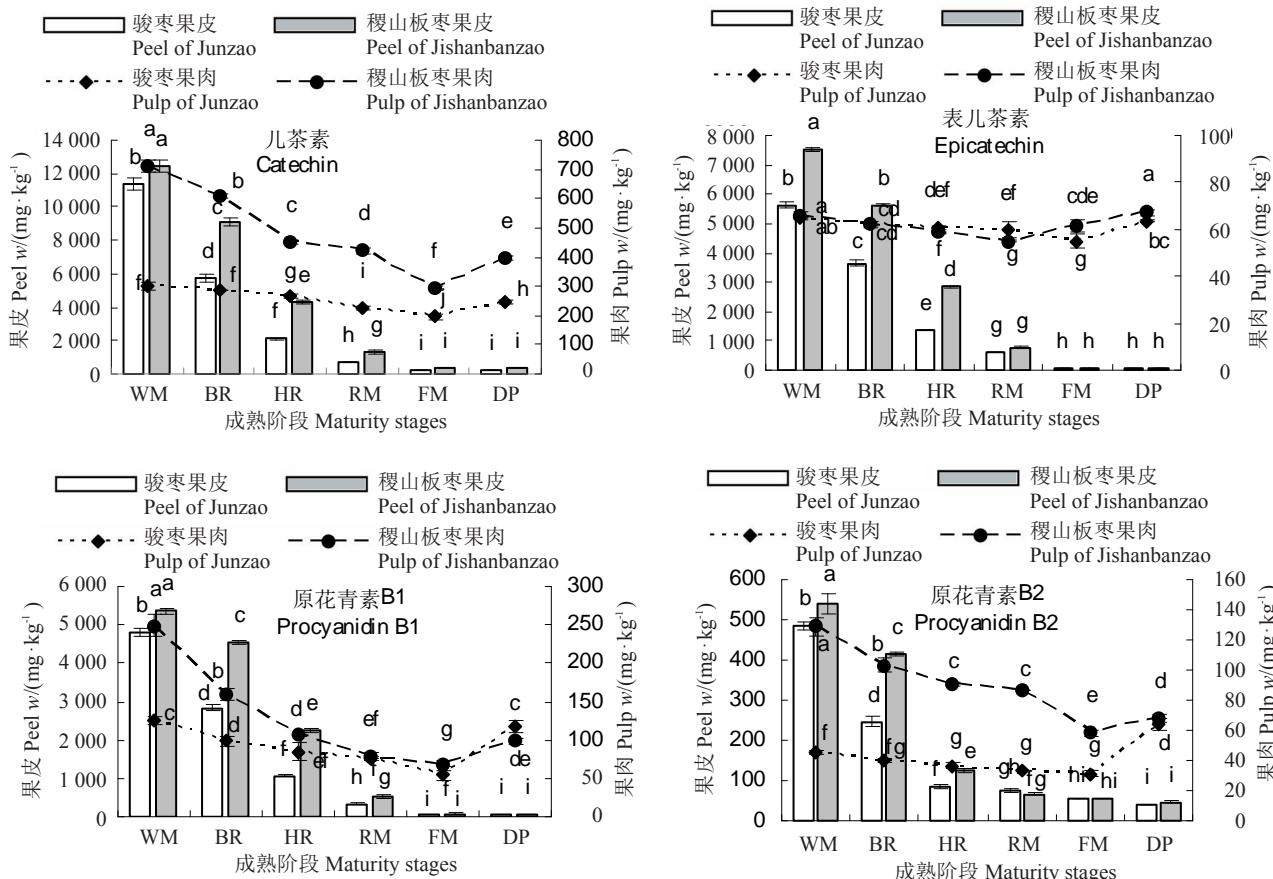


图3 ‘稷山板枣’和‘骏枣’果皮、果肉中黄烷醇组分含量的变化

Fig. 3 The content of flavanol components in the peel and pulp of ‘Jishanbanzao’ and ‘Junzao’

皮中4种物质含量与总黄烷醇含量的变化趋势相似, 均在白熟期积累水平最高, ‘稷山板枣’和‘骏枣’儿茶素含量分别高达 $12\ 438.53$ 、 $11\ 357.45\text{ mg}\cdot\text{kg}^{-1}$, 两个品种这一时期果皮中4种物质总含量均占到总黄烷醇含量的56%左右, 然后随着果实的成熟显著下降, 干枣中含量较完熟期降低, 但并不显著。

干枣阶段除外, 两个枣品种果肉中4种黄烷醇组分的含量均低于果皮, 白熟期到完熟期的变化趋

势和果皮一致, 呈现持续下降趋势, 但果肉中干枣的4种黄烷醇组分均显著增加。

2.3 成熟过程中枣果皮和果肉黄酮醇类物质含量的变化

槲皮素及其糖苷类衍生物是枣果实中检测到的主要黄酮醇类物质。图4显示了‘稷山板枣’和‘骏枣’成熟过程中果皮和果肉中黄酮醇物质含量的变化。槲皮素在枣果皮中的变化趋势与其糖苷类衍生

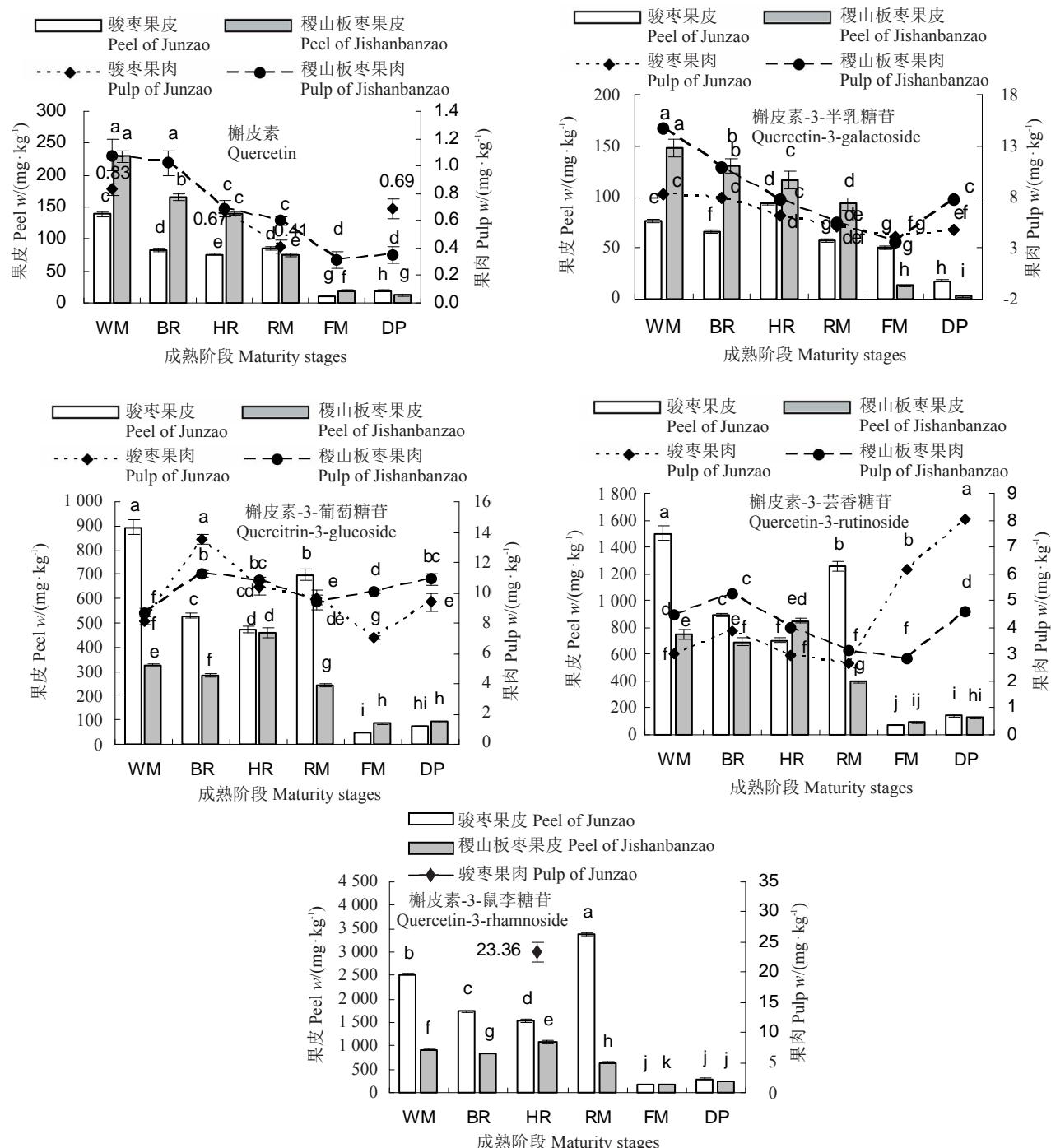


图4 ‘稷山板枣’和‘骏枣’果皮、果肉中黄酮醇组分的含量变化

Fig. 4 The content of flavonol components in the peel and pulp of ‘Jishanbanzao’ and ‘Junzao’

物不同,且两个品种的积累模式也不完全相同。*‘稷山板枣’*槲皮素在白熟期含量最高($229.85 \text{ mg} \cdot \text{kg}^{-1}$),随着果实成熟含量逐渐减少,干枣为 $12.15 \text{ mg} \cdot \text{kg}^{-1}$;‘骏枣’在白熟到半红期槲皮素呈现不断下降的趋势,且含量均低于‘稷山板枣’,而在全红期含量上升,后又下降,干枣含量略有上升。果肉中槲皮素含量在‘骏枣’初红和完熟期时没有检测到,但在其他时期两个品种中含量持续下降,干枣时略微上升。

枣果实成熟过程中,4种槲皮素糖苷类衍生物中的槲皮素3-鼠李糖苷和槲皮素3-芸香糖苷(芦丁)在果皮中的含量较高,槲皮素3-半乳糖苷含量最少。槲皮素3-半乳糖苷在‘稷山板枣’果皮中从白熟到干枣含量呈持续下降趋势,而在‘骏枣’果皮中从白熟期开始缓慢下降,在半红期上升达到峰值($93.11 \text{ mg} \cdot \text{kg}^{-1}$)。除槲皮素3-半乳糖苷外,其他三种槲皮素糖苷类衍生物含量在‘骏枣’果皮中高于‘稷山板枣’,在整体上均呈现下降趋势,果实成熟早期白熟期含量较高,随着果实成熟含量逐渐降低,‘稷山板枣’在半红期,‘骏枣’在全红期又大量积累,尤其是全红时槲皮素3-鼠李糖苷在‘骏枣’果皮中含量达到顶峰($3378.69 \text{ mg} \cdot \text{kg}^{-1}$),随后降低,干枣中含量又显著增加。

4种槲皮素糖苷类衍生物在果肉中的含量均较低,其中槲皮素3-鼠李糖苷仅在‘骏枣’半红期检测到,含量也仅有 $23.36 \text{ mg} \cdot \text{kg}^{-1}$;槲皮素3-半乳糖苷在两个品种中均是逐渐下降,干枣中含量显著增加;而槲皮素3-葡萄糖苷和槲皮素3-芸香糖苷含量从白熟期到初红期上升,随后下降,同样干枣中含量增加。

2.4 ‘稷山板枣’和‘骏枣’成熟过程中类黄酮相关基因的表达

由于果肉中物质种类和果皮相同,且含量变化趋势相似,因此,荧光定量表达分析仅对两个品种枣果皮中类黄酮合成相关基因进行比较。从图5可以看出,类黄酮合成相关基因在两种枣果皮中表达量的变化趋势基本相同,但在不同成熟阶段和品种之间存在差异。两个品种中*PAL*、*CHS*、*CHI*三个基因变化趋势相同,其表达量在白熟期最高,初红期下降,在半红期又上升,其后随着果实成熟又逐渐降低,且在‘稷山板枣’中的表达量均高于‘骏枣’。*4CL*基因在‘稷山板枣’中的表达模式与*PAL*等3个基因类似,在‘骏枣’中随着果实成熟在初红期最大,

后持续降低。*F3H*、*F3'H*、*LAR*、*ANR*、*FLS*在两个品种中的表达相似,均是白熟期表达量最大,随着果实成熟不断下降至完熟期,表达量最小,但有所区别的是‘骏枣’中*F3H*、*F3'H*在半红期有所上升。*UFGT*在两个品种中表达有差异,‘稷山板枣’从白熟期到初红期表达量下降,但在半红期上升到最高,后逐渐下降,在‘骏枣’中白熟期到初红期下降,在半红期开始上升,在完熟期表达量下降。

2.5 类黄酮含量与相关基因表达量的相关性分析

对枣果皮类黄酮合成相关基因的表达量和总黄酮、总黄烷醇以及类黄酮组分含量进行相关性分析。结果如表2所示,两个品种枣果实中,总黄酮和总黄烷醇含量和上游基因*PAL*、*4CL*、*CHI*表达没有显著的相关性,‘稷山板枣’总黄酮、总黄烷醇含量和*F3H*、*FLS*表达呈极显著相关,与*F3'H*、*LAR*表达呈显著相关,而‘骏枣’总黄酮、总黄烷醇含量与*ANR*、*LAR*的表达呈极显著正相关,其中总黄酮含量还与*F3H*、*F3'H*、*FLS*表达呈显著相关。

儿茶素、表儿茶素等4种黄烷醇组分含量均与*LAR*的表达呈显著或极显著正相关,还与*F3H*、*F3'H*和*ANR*表达呈显著相关,槲皮素在两个枣品种中均与*FLS*、*UFGT*表达呈显著相关,4类槲皮素糖苷物质含量主要与*UFGT*表达呈显著相关。

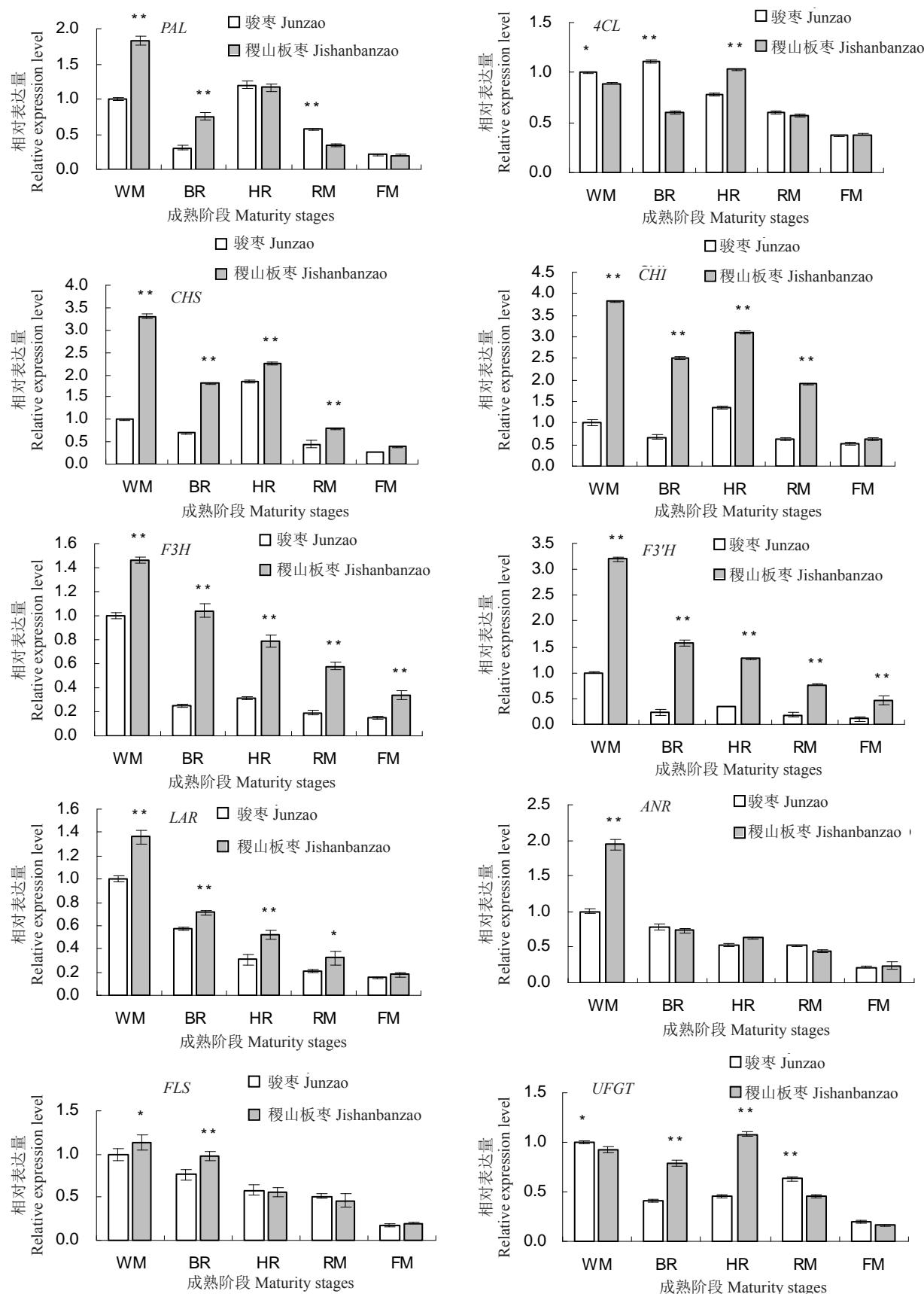
3 讨 论

3.1 枣果实成熟过程中总黄酮和总黄烷醇含量的变化

国内外学者研究发现,枣果实中含有大量的类黄酮。本研究发现,枣品种间总黄酮含量差异较大,这和前人的结果一致^[19]。许多水果果皮中的黄酮含量高于果肉,如苹果、柑橘和香蕉等^[8,20-21]。Wang等^[22]发现,枣果皮中的总黄酮含量比果肉中高约10倍,与本研究结果一致,且相差倍数更高,可能是由于品种间的差异或者提取方法不同所致。两个枣品种果皮和果肉的总黄酮、总黄烷醇含量均在成熟初期(白熟期)最高,随果实的成熟呈逐渐下降趋势,这与张琼等^[23]在‘冬枣’中的结果一致,在猕猴桃、李等水果成熟期间也有类似的趋势^[24-25]。在干枣中,枣果皮和果肉中的总黄酮含量都有所增加,这可能是由于枣果干制失水干物质浓缩以及成熟后物质转化所致。

3.2 枣果实成熟过程中类黄酮组分含量的变化

枣果实中已报道具有多种类黄酮物质,包括原



*和**分别表示差异显著($p < 0.05$)和极显著差异($p < 0.01$)。

* and ** indicate significant difference ($p < 0.05$) and extremely significant difference ($p < 0.01$), respectively.

图 5 不同成熟阶段枣果皮中类黄酮合成相关基因的表达

Fig. 5 Expression of flavonoid synthesis-related genes in jujube peel at different stages of maturity

表2 类黄酮与相关基因表达量的相关性分析

Table 2 Correlation analysis of flavonoids and related gene expression levels

		PAL	4CL	CHS	CHI	F3H	F3'H	LAR	ANR	FLS	UFGT
TFC	JS	0.833	0.514	0.889*	0.857	0.982**	0.914*	0.935*	0.857	0.994**	0.794
	JZ	0.515	0.851	0.412	0.471	0.889*	0.903*	0.961**	0.963**	0.895*	0.730
TFAC	JS	0.813	0.458	0.868	0.815	0.973**	0.909*	0.931*	0.852	0.990**	0.753
	JZ	0.310	0.867	0.232	0.283	0.841	0.841	0.980**	0.955**	0.838	0.713
C	JS	0.846	0.472	0.890*	0.822	0.982**	0.939*	0.957*	0.891*	0.981**	0.742
	JZ	0.354	0.774	0.214	0.290	0.917*	0.912*	0.999**	0.930*	0.808	0.772
EC	JS	0.854	0.499	0.899*	0.837	0.983**	0.935*	0.953*	0.883*	0.982**	0.767
	JZ	0.316	0.856	0.226	0.280	0.852	0.852	0.988**	0.958*	0.841	0.728
PB1	JS	0.822	0.488	0.879*	0.820	0.965**	0.899*	0.920*	0.835	0.980**	0.778
	JZ	0.335	0.827	0.230	0.293	0.880*	0.878	0.995**	0.946*	0.823	0.739
PB2	JS	0.765	0.319	0.809	0.721	0.950*	0.911*	0.932*	0.871	0.967**	0.621
	JZ	0.295	0.725	0.119	0.203	0.921*	0.908*	0.993**	0.914*	0.798	0.791
Q	JS	0.919*	0.687	0.960**	0.949*	0.983**	0.934*	0.944*	0.882*	0.956*	0.888*
	JZ	0.537	0.610	0.264	0.318	0.682	0.696	0.693	0.839	0.950*	0.930*
Q-3-G	JS	0.783	0.703	0.848	0.937*	0.881*	0.780	0.796	0.719	0.889*	0.903*
	JZ	0.904*	0.525	0.985**	0.984**	0.416	0.489	0.384	0.452	0.458	0.361
Q-3-Glu	JS	0.689	0.948*	0.728	0.842	0.544	0.467	0.462	0.397	0.484	0.934*
	JZ	0.535	0.632	0.268	0.323	0.697	0.711	0.715	0.857	0.959*	0.932*
Q-3-R	JS	0.798	0.880*	0.856	0.928*	0.759	0.662	0.669	0.578	0.732	0.995**
	JZ	0.460	0.588	0.173	0.228	0.659	0.667	0.680	0.827	0.941*	0.922*
Q-3-Rha	JS	0.745	0.897*	0.801	0.911*	0.690	0.592	0.597	0.516	0.660	0.974**
	JZ	0.341	0.331	0.055	0.081	0.327	0.336	0.322	0.548	0.755	0.742

注: JS. 稷山板枣; JZ. 骏枣; TFC. 总黄酮; TFAC. 总黄烷醇; C. 儿茶素; EC. 表儿茶素; PB1. 原花青素 B1; PB2. 原花青素 B2; Q. 槲皮素; Q-3-G. 槲皮素-3-半乳糖苷; Q-3-Glu. 槲皮素-3-葡萄糖苷; Q-3-R. 槲皮素-3-芸香糖苷; Q-3-Rha. 槲皮素-3-鼠李糖苷。*表示显著相关($p < 0.05$), **表示极显著相关($p < 0.01$)。

Note: JS. Jishanbanzao; JZ. Junzao; TFC. Total flavonoids content; TFAC. Total flavanols content; C. Catechin; EC. Epicatechin; PB1. Procyanidin B1; PB2. Procyanidin B2; Q. Quercetin; Q-3-G. Quercetin-3-galactoside; Q-3-Glu. Quercitrin-3-glucoside; Q-3-R. Quercetin-3-rutinoside; Q-3-Rha. Quercetin-3-rhamnoside. * indicates significant correlation ($p < 0.05$), ** indicates extremely significant correlation ($p < 0.01$).

花青素B1~B4、儿茶素、表儿茶素等黄烷醇,槲皮素、山奈酚、芦丁、槲皮素-3-洋槐糖苷等黄酮醇,还含有根皮素衍生物及花青素类物质等^[15,26-27]。本研究鉴定出了4种黄烷醇和5种黄酮醇,均在枣中已有报道。

研究发现,‘梨枣’中儿茶素和表儿茶素含量很高,且未成熟果实中的含量远高于成熟果实^[28]。本研究中,黄烷醇物质中儿茶素含量最高,其变化趋势与表儿茶素、原花青素B1、B2一致,从白熟期逐渐下降到完熟期含量最少,这与Wu等^[29]在‘梨枣’研究中儿茶素和表儿茶素的变化趋势一致。Choi等^[30]研究发现,表儿茶素始终是枣果实中最普遍的黄酮类化合物,在半红期含量达 $61.3 \text{ g} \cdot \text{kg}^{-1}$,其含量远高于本研究结果,可能是品种间的差异导致。儿茶素等具有较强的抗氧化、抗衰老、抑菌等功能^[31],若考虑枣果实功能作用,白熟期可能是较好的采收时期。

两个枣品种黄酮醇的积累模式略有差异。芦丁

是枣中最常见成分,本研究中各阶段果皮中均有,与槲皮苷、异槲皮苷含量一样均是从白熟期开始下降,‘稷山板枣’在半红期,‘骏枣’在全红期又大量积累,在‘骏枣’中的变化趋势与Wu等^[29]在‘梨枣’中报道的结果一致。无论是果肉还是果皮,黄酮醇物质含量在干枣中增加,这和Pu等^[32]研究结果一致,因此干枣各种功能较完熟期可能更强。

3.3 类黄酮合成相关基因的调控

大量研究表明,CHS、CHI、LAR等结构基因对植物类黄酮的合成具有重要调控作用^[33]。本研究发现,‘稷山板枣’和‘骏枣’果皮中随着果实的成熟,F3H、F3'H、LAR、ANR、FLS基因持续下调,PAL、4CL、CHS、CHI、UFGT整体也是下调趋势,但在半红期或全红期会有所上调,而这些基因的表达差异导致了类黄酮积累模式的变化。

类黄酮含量与其合成相关基因表达的相关性研

究发现,*PAL*、*C4H*等基因与烟叶中类黄酮含量呈显著正相关^[34];石榴在果实发育期间,*CHS*和*ANS*基因的表达与花色苷含量呈显著正相关^[35]。本研究的两个枣品种,上游基因*PAL*、*4CL*、*CHI*与总黄酮、总黄烷醇含量没有显著相关性,*CHS*基因也只是与‘稷山板枣’总黄酮含量呈显著正相关,这说明这些上游基因可能不是类黄酮积累的关键限速酶。两个品种总黄酮、总黄烷醇含量和*F3H*、*F3'H*、*FLS*、*ANR*、*LAR*的表达呈显著或极显著正相关,说明下游基因对类黄酮的调控作用较强。其中*F3H*、*F3'H*调控二氢黄酮醇的积累,为后期物质合成积累底物,且与类黄酮单体组分含量也有显著正相关性,可能是类黄酮合成中的关键调控基因。另外,4种黄烷醇组分的积累和*LAR*、*ANR*的表达模式基本一致,且均与*LAR*的表达呈显著或极显著正相关,与*ANR*有显著相关性,说明黄烷醇含量可能是由*LAR*和*ANR*的表达直接决定,且*LAR*是关键调控基因。

4 结 论

对‘稷山板枣’和‘骏枣’果实的研究明确了枣果实成熟期类黄酮含量的变化规律及相关基因的表达特征。枣品种类黄酮积累模式基本一致,果皮较果肉含量高(相差约20倍),且果实成熟早期积累最高,随果实成熟含量下降,干枣期含量略有增加;结构基因随果实成熟呈下调趋势,下游基因与类黄酮含量具有显著相关性,其中*F3H*、*F3'H*可能是类黄酮合成中的关键调控基因,*LAR*可能是黄烷醇途径中的关键调控基因。

参考文献 References:

- [1] 李新岗. 中国枣产业[M]. 北京:中国林业出版社,2015.
LI Xingang. Chinese jujube industry[M]. Beijing: China Forestry Publishing House, 2015.
- [2] 鲁周民,刘坤,闫忠心,李新岗. 枣果实营养成分及保健作用研究进展[J]. 园艺学报,2010,37(12): 2017-2024.
LU Zhoumin, LIU Kun, YAN Zhongxin, LI Xingang. Research status of nutrient component and health functions of *Ziziphus jujuba* Mill.[J]. Acta Horticulturae Sinica, 2010, 37(12): 2017-2024.
- [3] 樊保国. 枣果的功能因子与保健食品的研究进展[J]. 食品科学,2005,26(9): 569-573.
FAN Baoguo. Research status of functions factors and health food of *Ziziphus jujuba*[J]. Food Science, 2005, 26(9): 569-573.
- [4] GAO Q H, WU C S, WANG M. The jujube (*Ziziphus jujuba* Mill.) fruit: a review of current knowledge of fruit composition and health benefits[J]. Journal of Agricultural & Food Chemistry, 2013, 61(14): 3351-3363.
- [5] 张德权,台建祥,付勤. 生物类黄酮的研究及应用概况[J]. 食品与发酵工业,1999,25(6): 52-57.
ZHANG Dequan, TAI Jianxiang, FU Qin. Survey on research and application of bioflavonoids[J]. Food and Fermentation Industries, 1999, 25(6): 52-57.
- [6] 邹凤莲,寿森炎,叶纨芝,卢钢. 类黄酮化合物在植物胁迫反应中作用的研究进展[J]. 细胞生物学杂志,2004,26(1): 39-44.
ZOU Fenglian, SHOU Senyan, YE Wanzh, LU Gang. Advances in the research on flavonoid biosynthesis and plant stress response[J]. Chinese Journal of Cell Biology, 2004, 26 (1): 39-44.
- [7] 聂继云,吕德国,李静,刘凤之,李萍. 苹果果实中类黄酮化合物的研究进展[J]. 园艺学报,2009,36(9): 1390-1397.
NIE Jiyun, LÜ Deguo, LI Jing, LIU Fengzhi, LI Ping. Advances in studies on flavonoids in apple fruit[J]. Acta Horticulturae Sinica, 2009, 36(9): 1390-1397.
- [8] 张玉,吴慧明,白丽萍,刘晓春. 快速液相色谱法测定柑橘中6种类黄酮化合物含量[J]. 果树学报,2008,25(4): 615-617.
ZHANG Yu, WU Huiming, BAI Liping, LIU Fengzhi, LI Ping. Advances in studies on flavonoids in citrus fruit by rapid resolution liquid chromatography[J]. Journal of Fruit Science, 2008, 25(4): 615-617.
- [9] MASA A, VILANOVA M, POMAR F. Varietal differences among the flavonoid profiles of white grape cultivars studied by high-performance liquid chromatography[J]. Journal of Chromatography A, 2007, 1164(1/2): 291-297.
- [10] 袁亚娜,张平平,何庆峰,吴海清,樊秀花,张立金. 冬枣黄酮的分析及体外抗氧化活性[J]. 食品科学,2013,34(17): 70-73.
YUAN Yana, ZHANG Pingping, HE Qingfeng, WU Haiqing, FAN Xiuhua, ZHANG Lijin. Determination and antioxidant activity of flavonoids from winter jujube[J]. Food Science, 2013, 34(17): 70-73.
- [11] 刘杰,陈默然,赵志雅,王露露,于雪娜,李玉玺. 枣渣中黄酮提取工艺的优化及抗氧化性研究[J]. 安徽农学通报,2018,24(16): 22-24.
LIU Jie, CHEN Moran, ZHAO Zhiya, WANG Lulu, YU Xuena, LI Yuxi. Study on extraction and antioxidant activity of flavonoids in jujube slag[J]. Anhui Agricultural Science Bulletin, 2018, 24(16): 22-24.
- [12] PAWLOWSKA A M, CAMANGI F, BADER A, BRACA A. Flavonoids of *Zizyphus jujuba* L. and *Zizyphus spina-christi* (L.) Willd (Rhamnaceae) fruits[J]. Food Chemistry, 2009, 112(4): 858-862.
- [13] WANG B N, HUANG Q Y, VENKITASAMY C, CHAI H K, GAO H, CHENG N, PAN Z L. Change in phenolic compounds and their antioxidant capacities in jujube (*Ziziphus jujuba* Mill.) during three edible maturity stages[J]. LWT-Food Science and Technology, 2016, 66: 56-62.
- [14] 石倩倩. 枣果实色泽性状形成的分子机制研究[D]. 杨凌:西北农林科技大学,2019.
SHI Qianqian. Molecular mechanism of the formation of fruit pigment in jujube fruits[D]. Yangling: Northwest A & F University, 2019.
- [15] SHI Q Q, ZHANG Z, SU J J, ZHOU J, LI X G. Comparative analysis of pigments, phenolics, and antioxidant activity of Chi-

- nese jujube (*Ziziphus jujuba* Mill.) during fruit development[J]. *Molecules*, 2018, 23(8):1917-1931.
- [16] WANG X Q, LIC Y, LIANG D, ZOU Y J, LI P M, MA F W. Phenolic compounds and antioxidant activity in red-fleshed apples[J]. *Journal of Functional Foods*, 2015, 18: 1086-1094.
- [17] LI Y G, TANNER G, LARKIN P. The DMACA-HCl protocol and the threshold proanthocyanidin content for bloat safety in forage legumes[J]. *Journal of the Science of Food and Agriculture*, 1996, 70(1): 89-101.
- [18] ZHANG C M, HUANG J, LI X G. Identification of appropriate reference genes for RT-qPCR analysis in *Ziziphus jujuba* Mill. [J]. *Scientia Horticulturae*, 2015, 197: 166-169.
- [19] ZHANG H, JIANG L, YE S, YE Y B, REN F Z. Systematic evaluation of antioxidant capacities of the ethanolic extract of different tissues of jujube (*Ziziphus jujuba* Mill.) from China[J]. *Food and Chemical Toxicology*, 2010, 48(6): 1461-1465.
- [20] 郑洁,赵其阳,张耀海,焦必宁.超高效液相色谱法同时测定柑橘中主要酚酸和类黄酮物质[J].中国农业科学,2014,47(23): 4706-4717.
ZHENG Jie, ZHAO Qiyang, ZHANG Yaohai, JIAO Bining. Simultaneous determination of main flavonoids and phenolic acids in citrus fruit by ultra performance liquid chromatography[J]. *Scientia Agricultura Sinica*, 2014, 47(23): 4706-4717.
- [21] 高鹏钊,苗红霞,张建斌,徐碧玉,刘菊华.3个香蕉品种黄酮含量与果实发育成熟的关系[J].热带作物学报,2016,37(10): 1894-1899.
GAO Pengzhao, MIAO Hongxia, ZHANG Jianbin, XU Biyu, LIU Juhua. The relationship of flavonoids of three banana varieties and fruit development and ripening[J]. *Chinese Journal of Tropical Crops*, 2016, 37(10): 1894-1899.
- [22] WANG C T, CHENG D, CAO J K, JIANG W B. Antioxidant capacity and chemical constituents of Chinese jujube (*Ziziphus jujuba* Mill.) at different ripening stages[J]. *Food Science and Biotechnology*, 2013, 22(3): 639-644.
- [23] 张琼,周广芳,沈广宁,祝恩元,王红清.冬枣果皮着色过程中类黄酮类物质成分及含量的变化[J].园艺学报,2010,37(2): 193-198.
ZHANG Qiong, ZHOU Guangfang, SHEN Guangning, ZHU Enyuan, WANG Hongqing. The flavonoids in the fruit peel of *Ziziphus jujuba* Mill. 'Dongzao' during coloring process[J]. *Acta Horticulturae Sinica*, 2010, 37(2): 193-198.
- [24] 张元慧,关军锋,杨建民,赵树堂.李果实发育过程中果皮色素、糖和总酚含量及多酚氧化酶活性的变化[J].果树学报,2004,21(1): 17-20.
ZHANG Yuanhui, GUAN Junfeng, YANG Jianmin, ZHAO Shutang. Study on the changes of contents of pigments, total phenolics, sugars and polyphenol oxidase (PPO) activity in the fruit skin of plum cultivars during fruit development[J]. *Journal of Fruit Science*, 2004, 21(1): 17-20.
- [25] 黄春辉,廖光联,谢敏,陶俊杰,曲雪艳,陈璐,徐小彪.不同猕猴桃品种果实发育过程中总酚和类黄酮含量及抗氧化活性的动态变化[J].果树学报,2019,36(2): 174-184.
HUANG Chunhui, LIAO Guanglian, XIE Min, TAO Junjie, QU Xueyan, CHEN Lu, XU Xiaobiao. Dynamic changes in total phenols, flavonoids and antioxidant capacity during fruit development of different kiwifruit cultivars[J]. *Journal of Fruit Science*, 2019, 36(2): 174-184.
- [26] CHOI S H, AHN J B, KOZUKUE N, LEVIN C E, FRIEDMAN M. Distribution of free amino acids, flavonoids, total phenolics, and antioxidative activities of jujube (*Ziziphus jujuba*) fruits and seeds harvested from plants grown in Korea[J]. *Journal of Agricultural & Food Chemistry*, 2011, 59(12): 6594-6604.
- [27] HUDINA M, LIU M, VEBERIC R, STAMOAR F, COLARIC M. Phenolic compounds in the fruit of different varieties of Chinese jujube (*Ziziphus jujuba* Mill.) [J]. *Journal of Horticultural Science and Biotechnology*, 2008, 83(3): 305-308.
- [28] 于金刚,王敏,李援农,吴春森,高清菡.不同滴灌制度对梨枣抗氧化活性的影响[J].食品科学,2011,32(1): 39-44.
YU Jingang, WANG Min, LI Yuannong, WU Chunsen, GAO Qinghan. Effect of different drip irrigation regimes on antioxidants in pear-jujube (*Ziziphus jujuba* Mill. cv. Lizao)[J]. *Food Science*, 2011, 32(1): 39-44.
- [29] WU C S, GAO Q H, GUO X D, YU J G, WANG M. Effect of ripening stage on physicochemical properties and antioxidant profiles of a promising table fruit 'Pear-jujube' (*Ziziphus jujuba* Mill.)[J]. *Scientia Horticulturae*, 2012, 148: 177-184.
- [30] CHOI S H, AHN J B, KIM H J, IM N K, KOZUKUE N. Changes in free amino acid, protein, and flavonoid content in Jujube (*Ziziphus jujube*) fruit during eight stages of growth and antioxidative and cancer cell inhibitory effects by extracts[J]. *Journal of Agricultural & Food Chemistry*, 2012, 60(41): 10245-10255.
- [31] 毛清黎,施兆鹏,李玲,刘仲华,朱旗.茶叶儿茶素保健及药理功能研究新进展[J].食品科学,2007,28(8): 584-589.
MAO Qingli, SHI Zhaopeng, LI Ling, LIU Zhonghua, ZHU Qi. Research advances of health and pharmacological functions of tea catechins[J]. *Food Science*, 2007, 28(8): 584-589.
- [32] PU Y F, DING T, WANG W J, XIANG Y J, YE X Q, LIE M, LIU D H. Effect of harvest, drying and storage on the bitterness, moisture, sugars, free amino acids and phenolic compounds of jujube fruit (*Ziziphus jujuba* cv. Junzao)[J]. *Journal of the Science of Food & Agriculture*, 2017, 98(2): 628-634.
- [33] CHENGKE B, JUN X, BO C, XIA L, GUISHUANG L. Transcriptomic analysis and dynamic expression of genes reveal flavonoid synthesis in *Scutellaria viscidula*[J]. *Acta Physiologae Plantarum*, 2018, 40(9): 1-11.
- [34] 高娅北,李成刚,陈二龙,张明刚,孔德辉,孙占伟,史龙飞,宋朝鹏.烤烟烟叶类黄酮合成关键酶活性及其调控基因的表达分析[J].中国烟草学报,2019,25(1): 86-92.
GAO Yabei, LI Chenggang, CHEN Erlong, ZHANG Minggang, KONG Dehui, SUN Zhanwei, SHI Longfei, SONG Zhaopeng. Analysis of key enzyme activities and expression of regulatory genes in synthesis of flavonoids in flue-cured tobacco[J]. *Acta Tabacaria Sinica*, 2019, 25(1): 86-92.
- [35] 招雪晴,苑兆和.2个石榴品种果皮花色苷合成相关基因表达分析[J].西北植物学报,2018,38(5): 823-829.
ZHAO Xueqing, YUAN Zhaohe. Expression profiles of anthocyanin biosynthetic genes in two cultivars of *Punica granatum* L. [J]. *Acta Botanica Boreali-Occidentalis Sinica*, 2018, 38(5): 823-829.