

‘库尔勒香梨’*kfpMYB* 基因表达高低与萼片脱落和宿存相关性分析

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摘要:【目的】明确‘库尔勒香梨’*kfpMYB*基因表达高低与萼片脱落和宿存的相关性。【方法】在新疆库尔勒巴格吉格代村梨园于盛花期选取强势树和弱势树各3株,各采30朵花的萼片,共180朵。同时每株树上各标记100朵花,落花7d后进行调查,记录萼片的宿存与脱落情况。参照艾德莱植物RNA快速提取试剂盒的说明书提取总RNA。以总RNA为模板,按照TakaRa PrimescriptTMRT reagent Kit试剂盒的说明书合成‘库尔勒香梨’cDNA第一条链。根据cDNA序列信息设计引物,通过实时荧光定量PCR明确*kfpMYB*基因在强势树与弱势树中的相对表达量,并用SPSS软件分析田间调查的数据。【结果】*kfpMYB*基因在强势树2~5序位萼片中的表达量均高于弱势树。*kfpMYB*基因在强势树和弱势树第2序位的表达量显著高于其他序位的表达量。强势树和弱势树的第2、3序位的宿萼率均显著高于第4、5序位,第5序位的脱萼率显著高于其他序位。不同树势中‘库尔勒香梨’萼片*kfpMYB*基因相对表达量高低与萼片脱落和宿存有相关性,但不显著;在不同序位中,第4序位中‘库尔勒香梨’萼片*kfpMYB*基因的相对表达量与萼片宿存呈显著负相关,第5序位中‘库尔勒香梨’萼片*kfpMYB*基因的相对表达量与萼片脱落呈显著负相关。【结论】‘库尔勒香梨’萼片*kfpMYB*基因的表达量与萼片的脱落和宿存存在相关性,不同树势中的相关性不显著,但在不同序位中存在显著相关。

关键词: ‘库尔勒香梨’;萼片;*kfpMYB*;相关性

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Expression of the *kfpMYB* gene and its relationship with calyx persistence in the fruits of ‘Kuerlexiangli’ pear

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Abstract:【Objective】‘Kuerlexiangli’ pear is one of the most important agricultural crops in Xinjiang. The calyx of ‘Kuerlexiangli’ pear may be either persistent or deciduous. A persistent calyx can negatively affect fruit shape, reducing its appeal to customers. Therefore, it is of great importance to reveal the mechanism controlling the calyx persistence. The purpose of this study was to determine the correlation between *kfpMYB* gene expression and calyx persistence.【Methods】Flowers of ‘Kuerlexiangli’ pear were collected in an orchard at Bagejigedai village, Korla city, Xinjiang province. The flowers were collected at the full flowering stage. In the flowering period, three ‘Kuerlexiangli’ pear trees with strong tree potential and weak tree potential were selected, and 30 flowers of 2–5 in the strong tree and the weak tree were collected respectively. Thirty flowers were collected from each tree. After collection, the flowers were immediately frozen in liquid N and then taken to the laboratory where they were

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stored at $-80\text{ }^{\circ}\text{C}$ in an ultra low temperature refrigerator. These samples were used to measure gene expression. We also marked 100 flowers on each tree (the second, third, fourth and fifth opened flowers in the clusters). Calyx persistence was determined a week after petal drop. Total RNA was extracted using an Aidelai kit for rapid extraction of plant RNA according to the manufacturer's directions. The concentration of total RNA was detected with a nucleic acid analyzer. The integrity of the total RNA was detected by 1% agarose gel electrophoresis. Total RNA was used as a template to synthesize the first chain of cDNA with a TakaRa Prime Script TMRT Reagent Kit according to the manufacturer's instructions. This step also included the removal of genomic DNA. The primers were designed according to cDNA sequence information of 'Kuerlexiangli' pear *kfpMYB* gene. The relative expression of the *kfpMYB* gene in the high vigor trees and the low vigor trees was determined by real-time fluorescence quantitative PCR. The data was analyzed using SPSS software. 【Results】The results of real-time quantitative PCR showed that the expression of *kfpMYB* expression in the 2-5 opened flowers in the clusters on high vigor tree was higher than in the 2-5 opened flowers in the clusters on low vigor trees. The average relative expression quantity of *kfpMYB* was 1.80 on high vigor trees. The *kfpMYB* expressions on high vigor tree number 1, 2, and 3 were all greater than 1.80 (67, 75, and 67% respectively). The average relative expression quantity of *kfpMYB* was 1.22 on low vigor trees. The expressions on low vigor trees number 1, 2, and 3 were all less than 1.80 (67, 75, and 83% respectively). The field survey results showed that calyx persistence was significantly greater in the second, third and fourth opened flowers in the clusters than fifth opened flowers in the clusters on high vigor trees, the second, third opened flowers in the clusters than the fourth and fifth opened flowers in the clusters on low vigor trees. The rates of calyx persistence on high vigor tree numbers 1, 2 and 3 were 70, 80, and 67%, respectively. The rates of calyx persistence on low vigor tree numbers 1, 2, and 3 were 60, 63, and 75% respectively. The relative expression of the MYB gene in the high vigor trees was positively correlated with calyx persistence, whereas the relative expression of the MYB gene in low vigor trees was negatively correlated with calyx abscission. 【Conclusion】The calyx of 'Kuerlexiangli' pear had greater persistence when *kfpMYB* expression was high and less persistence when *kfpMYB* gene expression was low. The relative expression of *kfpMYB* in 4-5 opened flowers in the clusters on vigor trees was significantly higher than that in 1-2 opened flowers in the clusters on weak tree. The relative expression of *kfpMYB* in 'Kuerlexiangli' pear at flowering stage was closely related to the abscission and persistence of calyx.

Key words: 'Kuerlexiangli' pear; Calyx; *kfpMYB*; Relationship

'库尔勒香梨' (*Pyrus sinkiangensis* Yu) 同一树上同一花序不同序位花的萼片有的自动脱落, 有的则宿存, 产生脱萼、宿萼及突萼等多种果形, 造成果形不规则, 特别是大量的宿萼果影响了果实品质和外观, 直接影响其产值。前人从形态^[1]、植物生长调节剂^[2-3]、砧木类型^[4]、授粉^[5]、修剪^[6]以及光照^[7]等方面对'库尔勒香梨'萼片脱落与宿存进行了研究, 并对脱萼组和宿萼组进行高通量测序, 发现有 12 054 个差异基因, 与花萼脱落相关的差异表达基因主要涉及光合作用、植物激素信号转导、细胞壁修饰、转录调控和糖代谢^[8]。笔者课题组研究了'库尔勒香梨'萼片发育规律^[1]和萼片脱落过程中内源激素的变

化^[9]。采用 DDRT-PCR 分离获得 2 条与苹果有很高同源性的转录因子 *SPL*^[10] 和 *MYB*^[11]。利用 RACE 技术克隆到 *MYB* 全长 cDNA 序列, 并利用实时荧光定量 PCR 对 *kfpMYB* 的表达进行分析, 初步认为 *kfpMYB* 转录因子表达高低与萼片宿存和脱落相关^[12], 不同生长调节物质处理对 *kfpMYB* 转录水平的影响^[13], 比较'库尔勒香梨'脱萼组和宿萼组花器官的转录组, 筛选 2 者之间的差异表达基因, 获得了与萼片发育相关的基因及其对应的功能注释^[14], 但前期的研究没有将 *kfpMYB* 基因表达量的变化与萼片脱落和宿存的相关性了解足够清楚, 因此开展此项研究具有一定的理论及应用价值。

*MYB*类转录因子在植物生长发育过程中起着非常关键的作用,参与对激素的应答,不仅参与赤霉素途径控制开花,而且还参与了依赖于ABA的基因表达,启动一些受ABA诱导基因的表达^[15]。杜海等^[16]从大豆中克隆了2个*MYB*新基因*GmMYBJ6*和*GmMYBJ7*,其中*GmMYBJ6*受ABA、GA₃和NAA的诱导,而*GmMYBJ7*则受ABA和NAA的诱导。拟南芥中的*AtMYB106/NOK*、*AtMYB16/MIXTA*和*AtMYB17*分别参与了毛状体分支、花瓣表皮细胞形态建成和花序的早期发育的调控^[17-19],*AtMYB88*和*AtMYB124/FLP*通过调控细胞周期相关的基因,诱导气孔后期的正常分化^[20-21]。拟南芥*AtMYBL5*基因是由于干旱和高盐环境诱导表达的R2R3-MYB基因,受ABA信号诱导表达^[22]。香梨盛花期喷施赤霉素,突顶果由同株对照枝的24.3%增加到96.9%,果形指数由1.17增加到1.39,说明了赤霉素的作用与宿萼有关^[23]。邵月霞等^[24]发现内源激素GA₃在萼端的分布相对高于果肉,可能致使果实萼端细胞分裂增快,因而出现萼端突起现象。调查研究表明‘库尔勒香梨’第2序位的花,其萼片多数不会自行脱落,而第4序位的花,其萼片多数会自行脱落。而二者表达量之间存在明显差异。因此,*MYB*类转录因子的表达很可能与萼片宿存相关^[11]。

为了进一步验证盛花期萼片中*kfpMYB*表达量高低是否与萼片脱落和宿存相关,笔者主要利用实时荧光定量PCR检测盛花期‘库尔勒香梨’萼片中*kfpMYB*基因的相对表达量,并调查萼片脱落和宿存的情况,分析二者的相关性。

1 材料和方法

1.1 材料

2016年4月中旬在新疆库尔勒市巴格吉格代村梨园盛花期选取树势较强和树势较弱的‘库尔勒香梨’树各3株,分别采集强势树与弱势树2~5序位的花各30朵,将萼片和子房分开保存,迅速投入液氮,回实验室后放入-80℃超低温冰箱中保存备用,用于表达量的检测。同时每株树上各标记100朵花,落花7d后进行调查,记录萼片的宿存与脱落情况。

1.2 方法

1.2.1 ‘库尔勒香梨’总RNA的提取及cDNA的合成 盛花期采集‘库尔勒香梨’萼片,采用植物RNA快速提取试剂盒(艾德莱,北京)进行总RNA的提

取。总RNA的浓度用核酸浓度测定仪(NANO-Drop 2000)测定,其完整性用1%(w,后同)琼脂糖凝胶电泳检测。以总RNA为模板,具体操作按照TaKaRa Primescript™ RT reagent Kit试剂盒的说明书(TaKaRa, Japan)进行,合成‘库尔勒香梨’cDNA第1条链,包括基因组DNA的去除。

1.2.2 引物设计 根据笔者课题组前期所获得的‘库尔勒香梨’*kfpMYB*基因^[12]的cDNA全长(Accession No.:KT236444.1),利用Primer 5.0软件设计实时荧光定量PCR的引物M1(5'-TCCTCCTTCTGATGGTCTTGTC-3')和M2(5'-CAGTTGATGTCCTCGTCCTCTT-3')。以*Actin*作为内参基因,其引物为A1(5'-GCAAGGTCAGACGAAGG-3')和A2(5'-CCATCCAGGCTGTTCTCTC-3')。

1.2.3 实时荧光定量分析 以各个取样点的cDNA为模板,用CFX manager(Bio-Rad USA)实时定量PCR仪进行*MYB*基因的实时定量PCR分析。加入20 μL体系的反应液:SYBR Green Realtime PCR Master Mix 12.5 μL, ddH₂O 4.5 μL,上游引物0.5 μL,下游引物0.5 μL, cDNA 2 μL, 试验共设3个重复。反应程序为95℃预变性3 s, 95℃变性5 s, 59.5℃退火5 s, 72℃延伸20 s, 40个循环后于72℃延伸1 min。反应结束后分析荧光值变化曲线和融解曲线,记录Ct值。采用比较Ct法的相对定量法分析表达,计算出基因相对表达量,借助SPSS软件进行差异显著性分析。

1.3 ‘库尔勒香梨’*kfpMYB*表达量与萼片脱落和宿存相关性分析

把调查的数据导入Excel表,依据试验目的进行相应整理,通过SPSS软件进行相关性分析。

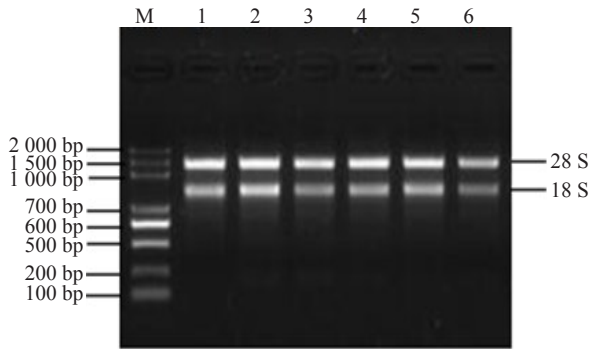
2 结果与分析

2.1 总RNA的提取

‘库尔勒香梨’萼片总RNA电泳结果显示,所提取的RNA 28S和18S条带清晰,28S条带的亮度明显比18S条带高,且没有杂带(图1),说明所提取的RNA完整性高。OD_{260/280}为1.85~2.10,表明RNA质量较好,可用于后续试验。

2.2 ‘库尔勒香梨’*kfpMYB*基因表达量检测

实时荧光定量PCR检测结果(图2)表明,*kfpMYB*基因在强势树2~5序位萼片中的表达量均高于弱势树2~5序位。*kfpMYB*基因在强势树第2序

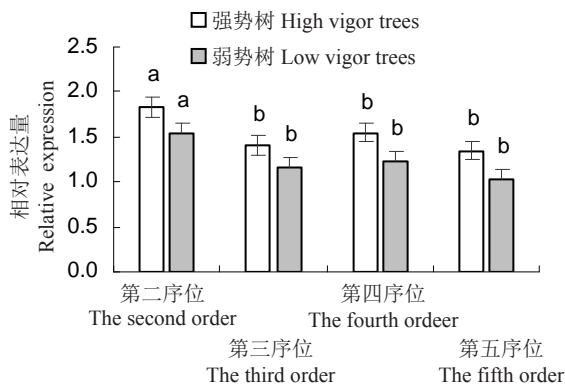


M. DL2000; 1~3 为强势树; 4~6 为弱势树。

M. DL2000; 1-3 are high vigor trees; 4-6 are low vigor trees.

图 1 ‘库尔勒香梨’萼片总 RNA 电泳分析

Fig. 1 Agro-gel electrophoresis of the flower of ‘Kuerlexiangli’ pear calyx RNA



不同小写字母表示差异显著 ($p \leq 0.05$)。下同。

Different small letters indicate significant difference at $p \leq 0.05$. The same below.

图 2 不同树势中‘库尔勒香梨’萼片 *kfpMYB* 基因的相对表达量

Fig. 2 The relative expression of *kfpMYB* gene of ‘Kuerlexiangli’ pear calyx in different trees

位的表达量显著高于其他序位的表达量,为 1.83,其他序位之间没有差异,强势树中 *kfpMYB* 基因的表达量呈先减后增的趋势;*kfpMYB* 基因在弱势树第 2 序位的表达量显著高于其他序位的表达量,为 1.51,其他序位之间没有差异,弱势树中 *kfpMYB* 基因的表达量呈递减趋势。

2.3 不同树势‘库尔勒香梨’脱萼率与宿萼率

2.3.1 强势树中‘库尔勒香梨’脱萼率与宿萼率 田间数据调查结果(图 3)表明,强势树第 2、3、4 序位的宿萼率均显著高于第 5 序位,第 2 序位的宿萼率最高(58%),且宿萼率呈递减趋势;第 4 和第 5 序位的脱萼率显著高于第 2 序位,第 5 序位的脱萼率最高,为 33%,且脱萼率呈递增趋势。

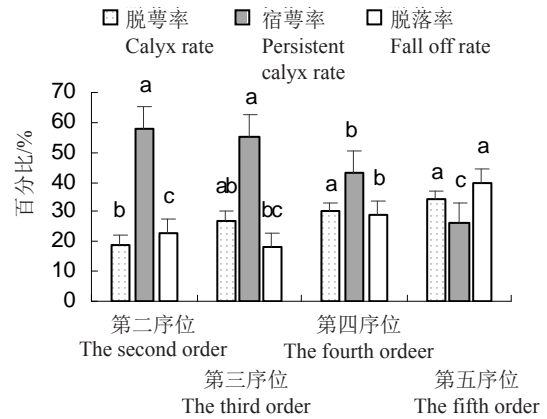


图 3 强势树中‘库尔勒香梨’的宿萼率与脱萼率

Fig. 3 Persistent calyx rate and calyx rate of ‘Kuerlexiangli’ pear in high vigor trees

2.3.2 弱势树中‘库尔勒香梨’宿萼率与脱萼率 田间数据调查结果(图 4)表明,弱势树第 2、3 序位的宿萼率均显著高于第 4、5 序位,第 2 序位的宿萼率最高,为 47%,且宿萼率呈递减趋势;第 5 序位的脱萼率显著高于其他序位,为 43%,且脱萼率呈递增趋势。

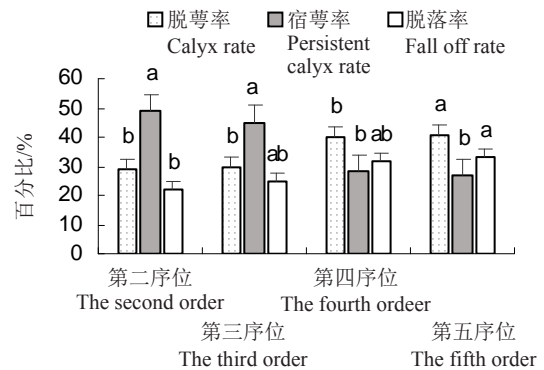


图 4 弱势树中‘库尔勒香梨’的宿萼率与脱萼率

Fig. 4 Persistent calyx rate and calyx rate of ‘Kuerlexiangli’ pear in low vigor trees

2.4 ‘库尔勒香梨’萼片 *kfpMYB* 基因的表达量与萼片脱落及宿存的相关性

不同树势中‘库尔勒香梨’萼片 *kfpMYB* 基因相对表达量与萼片脱落和宿存的相关性分析见表 1。在强势树中‘库尔勒香梨’萼片 *kfpMYB* 基因的相对表达量与萼片宿存呈正相关,但不显著,与萼片脱落呈显著负相关。

不同序位中‘库尔勒香梨’萼片 *kfpMYB* 基因的相对表达量与萼片脱落宿存的相关性分析见表 2。在第 2、3 序位中‘库尔勒香梨’萼片 *kfpMYB* 基因的

表1 不同树势中 *kfpMYB* 基因相对表达量与萼片脱落及宿存的相关系数

Table 1 The relative expression of *kfpMYB* gene and the correlation coefficient of calyx and persistent in different tree vigors

	强势树 High vigor trees	弱势树 Low vigor trees
宿萼率 Persistent calyx rate	0.101	0.073
脱萼率 Calyx rate	-0.220*	-0.128

注:*表示在 0.05 水平上显著相关。下同。

Note: * indicates the significant correlation at 0.05 level. The same below.

表2 不同序位中 *kfpMYB* 基因相对表达量与萼片脱落宿存的相关系数

Table 2 The relative expression of *kfpMYB* gene and the correlation coefficient of calyx and persistent in different orders

	第二序位 The second order	第三序位 The third order	第四序位 The fourth order	第五序位 The fifth order
宿萼率 Persistent calyx rate	0.101	0.086	-0.003*	0.213
脱萼率 Calyx rate	-0.220	-0.185	-0.036	-0.439*

相对表达量与萼片宿存呈正相关,与萼片脱落呈负相关,但均不显著;在第4序位中,‘库尔勒香梨’萼片 *kfpMYB* 基因的相对表达量与萼片宿存呈显著负相关,与萼片脱落呈负相关,但不显著;第5序位中‘库尔勒香梨’萼片 *kfpMYB* 基因的相对表达量与萼片宿存呈正相关,但不显著,与萼片脱落呈显著负相关。

由此可以得出,在不同树势中‘库尔勒香梨’萼片 *kfpMYB* 基因的相对表达量与萼片脱落和宿存有相关性,但不显著;在不同序位中,第4序位中‘库尔勒香梨’萼片 *kfpMYB* 基因的相对表达量与萼片宿存呈显著负相关,第5序位中‘库尔勒香梨’萼片 *kfpMYB* 基因的相对表达量与萼片脱落呈显著负相关。

3 讨论

‘库尔勒香梨’果实萼片存在脱落和宿存两种特性,大量调查结果表明,长势较强的‘库尔勒香梨’第2序位花均生成宿萼果,长势较弱的‘库尔勒香梨’第4序位均生成脱萼果。‘库尔勒香梨’在不同环境条件及管理方式,其对果实萼片脱落与宿存均产生不同影响。在此方面已有较多报道,选择特定授粉

品种对香梨进行人工授粉可在一定程度上提高‘库尔勒香梨’脱萼果的比例,改善果实品质^[5]。对‘库尔勒香梨’来说,光照条件好的部位脱萼果比例较高,光照条件差的宿萼果比例较高^[25]。花期是决定果形变化的主要时期^[26],而且决定‘库尔勒香梨’脱萼果形成的时期主要在初花至盛花阶段,末花以后,作用逐渐递减^[27]。在初花期和盛花期分别喷施 NAA(10 mg·L⁻¹)能够显著提高‘库尔勒香梨’萼片脱落率^[28-29]。盛花期喷施复合试剂 M1,能够显著提高‘库尔勒香梨’脱萼率、坐果率,提升果实品质^[30],在‘库尔勒香梨’高产优质生产中具有推广价值。

本试验根据前人已获得的‘库尔勒香梨’*kfpMYB* 基因的 cDNA 序列设计引物,通过实时荧光定量 PCR 明确 *kfpMYB* 基因在强势树与弱势树中的相对表达量。本试验所用到的 *MYB* 基因为典型的 R2R3-*MYB* 结构域,此基因编码的蛋白于第 45~49 位和 98~146 位之间是高度保守的 *MYB* 结构功能域——SANT。此蛋白没有信号肽区域,在 C 端位置有一个典型的疏水性区域。本试验结果表明,‘库尔勒香梨’在盛花期强树势花序中 2~5 序位萼片的 *kfpMYB* 相对表达量显著高于弱势树花序中 2~5 序位萼片的表达量,第 2 序位的表达量显著高于其他序位。强势树第 2、3、4 序位的宿萼率均显著高于第 5 序位;第 4 和第 5 序位的脱萼率显著高于第 2 序位。由此可以推断,第 2 序位中萼片的 *kfpMYB* 基因表达量越高,其宿萼率也会相应的增高。不同树势中‘库尔勒香梨’萼片 *kfpMYB* 基因相对表达量与萼片脱落和宿存有相关性,但不显著;在不同序位中,第 4 序位中‘库尔勒香梨’萼片 *kfpMYB* 基因的相对表达量与萼片宿存呈显著负相关,第 5 序位中‘库尔勒香梨’萼片 *kfpMYB* 基因的相对表达量与萼片脱落呈显著负相关。

4 结论

不同树势中‘库尔勒香梨’在盛花期 *kfpMYB* 的相对表达量与萼片脱落和宿存有相关性,但不显著;不同序位中‘库尔勒香梨’在盛花期 *kfpMYB* 的相对表达量与萼片脱落和宿存有相关性,与第 4、5 序位呈显著负相关;‘库尔勒香梨’萼片中 *kfpMYB* 基因相对表达量高,导致萼片宿存,相对表达量低,导致萼片脱落。

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