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‘翠冠’梨大果型芽变的细胞学及相关基因表达研究

舒莎珊[1],李 鑫[1],骆 军[2],白松龄[1],滕元文[1*]

([1]浙江大学园艺系·农业部园艺植物生长发育与品质调控重点开放实验室,杭州 310058;
[2]上海市农业科学院林木果树研究所·上海市设施园艺技术重点实验室,上海 201403)

摘 要:【目的】对造成大果芽变的原因进行探讨。**【方法】**以‘翠冠’和表现出大果性状的芽变‘潘庄大翠冠’为材料,利用荧光AFLP分子标记进行基因组DNA序列差异分析;用流式细胞仪检测‘潘庄大翠冠’的染色体倍性,用石蜡切片技术进行细胞学观察,用实时荧光定量PCR检测相关基因表达分析。**【结果】**AFLP分析证实‘潘庄大翠冠’为‘翠冠’的大果芽变,果实内在品质无明显差异。流式细胞检测结果显示,‘潘庄大翠冠’为二倍体,与‘翠冠’相同。‘潘庄大翠冠’梨的细胞分裂期从盛花期开始一直到花后24 d,较‘翠冠’长4 d,花后28 d时,果肉细胞层数显著多于‘翠冠’。细胞周期蛋白D3(cyclin D3,*CYCD3*)在‘潘庄大翠冠’中的表达量显著高于‘翠冠’。细胞周期蛋白A2(cyclin A2,*CYCA2*)、细胞周期蛋白依赖性激酶A1(cyclin-dependent kinase A1,*CDKA1*)和细胞周期蛋白依赖性激酶B2(cyclin-dependent kinase B2,*CDKB2*)等基因也有表达差异。**【结论】**‘潘庄大翠冠’确为‘翠冠’的大果芽变,其果实增大的机制并非染色体加倍,而是果实发育过程的细胞分裂期细胞的活跃增殖导致细胞分裂期的延长。

关键词: ‘翠冠’梨;大果型芽变;细胞数目;细胞大小;AFLP

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Studies on cytology and related gene expression pattern of a large-fruited bud mutant from ‘Cuiguan’ pear (*Pyrus pyrifolia*)

SHU Shashan[1], LI Xin[1], LUO Jun[2], BAI Songling[1], TENG Yuanwen[1*]

([1]Department of Horticulture, Zhejiang University · State Agricultural Ministry Key Laboratory of Horticultural Plant Growth, Development & Quality Improvement, Hangzhou 310058, Zhejiang, China; [2]Forestry and Fruit Tree Research Institute, SAAS · Shanghai Key Laboratory of Protected Horticultural Technology, Shanghai 201403, China)

Abstract:【Objective】Fruit size at harvest is determined by both cell number and cell size of flesh which result from cell division and cell expansion processes, respectively. Cell division is regulated by cell cycle. Some important regulatory proteins of cell cycle have been studied, such as cyclins (CYCs) and cyclin-dependent kinases (CDKs). CDKs control the key transitions in the plant cell cycle. CDKA plays a pivotal role in both the G1-to-S and the G2-to-M transitions, while a reduction in CDKB1 activity results in a block at the G2-to-M transition. Plants contain much more CYCs than previously described in other organisms, some of them have been found to associate with CDKs. D-type cyclins (CYCD) are thought to regulate the G1-to-S transition and function at the G2-to-M transition. A-type cyclins regulate the S-to-M phase, and B-type cyclins control both the G2-to-M transition and the intra-M-phase. To investigate cellular and molecular mechanism related to fruit size in pear, ‘Cuiguan’ pear (*Pyrus pyrifolia* Nakai.) and its spontaneous mutant with larger fruit size (named ‘Panzhuang Dacuiguan’) were used as materials in this study.【Methods】AFLP (amplified fragment length polymorphism) was used to analyze the genomic differences between ‘Cuiguan’ and ‘Panzhuang Dacuiguan’. 128 *Eco*R I/*Mse* I selective primer pairs were adopted in AFLP analysis. To minimize the effect of low-intensity background peaks

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作者简介: 舒莎珊,女,在读硕士研究生,研究方向为果实发育生理。E-mail:sss1231tmd@126.com
*通信作者 Author for correspondence. Tel:0571-88982803,E-mail:ywteng@zju.edu.cn

(noise), threshold value for fragment selection were set towards average signal intensity and fragment frequency. FCM (flow cytometry) analysis was carried out to record the chromosome ploidy. Genome size was measured using the Otto method. The filtered fluid was then centrifuged at a speed of 5 000 $r \cdot min^{-1}$ for 2 min. The system's light source of FCM was 488 nm argon lasers. Seasonal changes in longitudinal and transverse diameters were measured at regular intervals during the course of fruit development. Fresh weight, longitudinal diameter and transverse diameter of both 'Cuiguan' and 'Panzhuang Dacuiguan' fruits were measured at harvest. The flesh width was calculated from the difference between the largest width of the transverse section of the fruits and core diameter. Fruit (flower) samples were collected for cytology and gene expression analysis during 0-28 d after full bloom (DAFB). Paraffin section was used for microscopy observation of cell number and size of the fruits. Cell number was determined by counting the number of cell layers. Cell size was determined by measuring the average diameter of seven contiguous cells. Ten observation zones per paraffin section were measured. Quantitative real-time PCR (Q-PCR) was performed to test the gene expression. Each Q-PCR reaction mixture contained SYBR Premix Ex *Taq*™ (10.0 μL), both primer (0.4 μL, 10 $\mu mol \cdot L^{-1}$), cDNA (2 μL), and RNase-free H_2O (7.2 μL) in a total volume of 20 μL. The reaction started with a preliminary step of 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 20 s. The Q-PCR primers were designed using Primier 3 according to obtained sequences of cDNA fragments.【Results】A total of 116 polymorphic primer pairs were chosen from 128 selective primer pairs for AFLP analysis which amplified 8 620 DNA fragments. The polymorphic rate and Neips association coefficient between them were 8.18% and 0.957 4, respectively, confirming that 'Panzhuang Dacuiguan' was a sport of 'Cuiguan'. FCM analysis showed that both 'Cuiguan' and 'Panzhuang Dacuiguan' were diploid. Therefor, the larger fruit size in 'Panzhuang Dacuiguan' was not the result of the chromosome doubling. At maturity stage, 'Panzhuang Dacuiguan' had a 17% larger fruit diameter and a 64% heavier fruit weight than those of 'Cuiguan', while no difference in internal quality was observed between two cultivars. Cell division started at 0 DAFB (days after full bloom) and continued till 24 DAFB in 'Panzhuang Dacuiguan', longer than that of 'Cuiguan' by 4 d. Cell number (cell layers) in the floral-tube tissue was nine cell layers more in 'Panzhuang Dacuiguan' than in 'Cuiguan' at 28 DAFB. The average areas of the cell and cell nucleus at full bloom stage were larger in 'Panzhuang Dacuiguan' than those in 'Cuiguan'. Q-PCR analysis indicated *CYCD3* expression of 'Panzhuang Dacuiguan' fruit during early fruit development was 1.2 times as high as that of 'Cuiguan'. In other cyclin-related genes including *CYCA2*、*CDKA1* and *CDKB2*, different expressions were also observed between 'Panzhuang Dacuiguan' and 'Cuiguan'.【Conclusion】The larger fruit size of 'Panzhuang Dacuiguan' pear, the spontaneous mutant of 'Cuiguan' pear, could be contributed to the increased cell number in the fruit flesh which was related to extended period of cell division. Our work verified the important role of cell division in regulating pear fruit size.

Key words: 'Cuiguan' pear; Large-fruited mutant; Cell number; Cell size; AFLP

芽变是芽分生组织细胞发生的遗传物质的突变，是体细胞突变的一种。芽变是果树产生新变异的丰富源泉，为新品种的选育奠定了基础[1]。了解芽变发生的机制有助于我们更深入和高效地开展芽变选种工作。本研究所用‘翠冠’梨（*Pyrus pyrifolia* ‘Cuiguan’）的大果芽变材料是2004年在上海市奉贤区潘庄农户梨园中发现。同一株砂梨品种‘翠冠’的一部分枝上花果叶均为正常大小，而另一部分枝条枝叶大小正常，但果实在发育过程中明显大于普通‘翠冠’果实。大果枝条经嫁接后，经过多年观察，依然表现出稳定的大果性状。我们推测其为‘翠冠’的大果型芽变，现在暂命名为‘潘庄大翠冠’。

果实大小是其外观品质的重要组成部分，是决定果树经济价值的重要因素之一。梨果实大小由果实生长发育过程中的细胞数目和细胞大小共同决定，但细胞数目对果实最终大小的影响更大[2]。果实