

基于细胞周期进程精确识别的同步诱导提高多倍体诱导效率

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摘要:【目的】细胞周期的不同步性是限制多倍体诱导效率的关键因素。为快速实现有效的细胞周期同步化预处理并基于此提高多倍体诱导效率, 有必要建立一套系统的方法精准识别细胞周期进程来为之助力。【方法】本研究以银腺杨 84K 为材料, 选取 5-氨基尿嘧啶 (5-AU) 为同步化诱导剂, 秋水仙碱为加倍诱导剂, 利用流式细胞术和 EdU 荧光标记精确追踪了离体培养过程中外植体分化的细胞周期进程, 并使用彗星实验确定了外植体对诱导剂的耐受性, 最终在合适的范围内确定了有效的处理体系。【结果】结果表明银腺杨 84K 叶片分化过程中 S 期细胞的生长始于分化两天之后, 而 G2/M 期细胞则在分化四天后开始出现。彗星实验等方法, 确定了材料对 5-AU 的耐受浓度 (1.1-1.7 mM) 和处理时长 (12-24 小时), 缩小了后续正交实验的范围。最终将同步化有效激发处理优化为在叶片分化的两天后使用 1.5mM 的 5-AU 处理 24h, 随后脱药恢复 24h 后进行染色体加倍。其中 S 期细胞同步效率从 28.48% 提高至 38.13%, G2/M 期细胞同步效率从 1.59% 提高至 7.47%, 并将四倍体诱导率的平均水平提高至 40.90%。【结论】研究表明通过精确识别关键的细胞周期阶段, 可以快速有效的优化同步化技术, 从而提高多倍体诱导效率。我们的研究提供了一种系统化的方法, 有助于开发更高效的多倍体诱导系统, 能够提高木本植物的倍性育种效率。

关键词: 5-氨基尿嘧啶, 细胞周期鉴定, 染色体加倍, EdU 荧光标记, 流式细胞术, 四倍体诱导, 同步化

Synchronization Induction Based on Precise Identification of Cell Cycle Progression to Enhancing Polyploid Induction Efficiency

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Abstract: 【Objective】Asynchrony in the cell cycle is a key limiting factor in the efficiency of polyploid induction. To rapidly achieve effective cell cycle synchronization pretreatment and improve polyploid induction efficiency, it is necessary to establish a systematic method for precise identification of cell cycle progression. 【Methods】In this study, *Populus alba* × *P. glandulosa* ('84K' poplar) was used as the research material, with 5-aminouracil (5-AU) as the synchronization inducer and colchicine as the chromosome doubling agent. Flow cytometry and EdU fluorescence labeling were used to precisely track the cell cycle progression of explants during in vitro culture, while comet assays were used to determine the tolerance of explants to the inducers. Based on this,

an effective treatment system within the appropriate range was established. **【Results】** The results showed that during leaf differentiation in 84K poplar, the increase in S-phase cells began two days after differentiation, while G2/M phase cells began to appear after four days. Comet assays and other methods identified the tolerance concentration range of 5-AU (1.1-1.7 mM) and treatment duration (12-24 hours), which narrowed the range of subsequent orthogonal experiments. The optimized synchronization treatment was determined to be 1.5 mM 5-AU for 24 hours, applied two days after leaf differentiation, followed by a 24-hour recovery before colchicine treatment for chromosome doubling. As a result, S-phase cell synchronization efficiency increased from 28.48% to 38.13%, and G2/M-phase synchronization efficiency increased from 1.59% to 7.47%, while the average tetraploid induction rate was raised to 40.90%. **【Conclusion】** The study demonstrates that precise identification of key cell cycle phases can rapidly and effectively optimize synchronization techniques, thereby improving polyploid induction efficiency. Our research provides a systematic approach to improving breeding efficiency in woody plants and contributes to the development of more efficient polyploid induction systems.

Key words: 5-aminouracil, cell cycle identification, chromosome doubling, EdU fluorescence labeling, flow cytometry, tetraploid induction, synchronization.