

新疆野苹果膜酵母双杂交文库的构建及 MsPYR1-like 互作蛋白筛选

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摘 要:【目的】新疆野苹果是世界栽培苹果的祖先, 其具有育种研究价值, 但其种子的双重休眠难以解除, 严重影响新疆野苹果的育种进程。为探究新疆野苹果种子 ABA 受体 *MsPYR1-like* 的互作蛋白, 深入解析 *MsPYR1-like* 影响新疆野苹果种子休眠调控过程的分子机制。文库构建可为丰富新疆野苹果种子休眠调控研究提供科学支撑, 能为新疆野苹果的野生资源保护及育种奠定分子理论基础。【方法】本研究以休眠解除过程中 2 个关键时期的新疆野苹果种子为试验材料, 使用分离泛素系统构建新疆野苹果种子休眠解除关键时期的膜酵母双杂交 cDNA 文库, 提取总 RNA 和分离纯化 mRNA, 文库质粒转入酵母菌株 NMY51 感受态细胞, 并用诱饵载体 pBT3-SUC-PYR1 筛选 *MsPYR1-like* 的互作蛋白。【结果】成功构建新疆野苹果种子膜酵母双杂交 cDNA 文库。经检测, 库容为 2.76×10^8 CFU/mL, 重组率大于 95%, 插入片段的平均长度大于 1000 bp, 能符合后续酵母双杂交筛选的标准。pBT3-SUC-PYR1 诱饵蛋白表达载体, 经自激活活性检测, 验证诱饵质粒在膜酵母系统中无自激活活性, 可用于膜酵母文库筛选。利用膜酵母双杂交筛选构建的新疆野苹果种子休眠解除关键时期的膜酵母 cDNA 文库, 挑选 96 个阳性酵母克隆, 经过测序、Blast 比对和酵母回转验证, 获得了 28 个与 *MsPYR1-like* 相互作用的候选蛋白。发现 *MsPYR1-like* 与 LEA、低温诱导蛋白、14-3-3-like 蛋白、SPL、ARF 和 ARP 等蛋白具有互作关系, 上述这些蛋白被报道参与种子的休眠与萌发过程。【结论】构建高质量的新疆野苹果种子膜酵母双杂交文库, 使用酵母双杂交筛库技术鉴定出多个与 *MsPYR1-like* 互作蛋白, 为研究 *MsPYR1-like* 基因及互作蛋白在种子休眠解除过程的分子机制提供重要资源。

关键词: 新疆野苹果; *MsPYR1-like*; 膜酵母双杂交; 蛋白互作; 种子休眠与萌发

Construction of a membrane yeast two-hybrid library and screening of *MsPYR1-like* interacting proteins in *Malus sieversii*

Abstract: 【Objective】*Malus sieversii* is the ancestor of cultivated apples in the world, and it has breeding research value, but the double dormancy of its seeds is difficult to lift, which seriously affects the breeding process of *Malus sieversii*. To explore the interaction protein of *MsPYR1-like* ABA receptor in *Malus sieversii* seeds, the molecular mechanism of *MsPYR1-like* influencing the regulation of seed dormancy in *Malus sieversii* seeds was investigated. The construction of the library can provide scientific support to enrich the research on the regulation of seed dormancy of *Malus sieversii*, and lay a molecular theoretical foundation for the conservation of wild resources and breeding of wild apple in Xinjiang. 【Method】A membrane yeast two-hybrid cDNA library was constructed using the split-ubiquitin system for the two critical periods of dormancy release in *Malus sieversii* seeds,

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total RNA was extracted and mRNA was isolated and purified, and the library plasmid was transferred into the yeast strain NMY51 receptor cells and screened for the presence of the membrane yeast cDNA library using the decoy vector pBT3-SUC-PYR1 with *MsPYR1-like* interacting proteins. **【Result】** Successfully constructed *Malus sieversii* seed membrane yeast two-hybrid cDNA library. The library capacity reached 2.76×10^8 CFU/mL, the recombination rate was greater than 95%, and the average length of the inserted fragments was greater than 1000 bp, which could meet the criteria for subsequent yeast two-hybrid screening. pBT3-SUC-PYR1 bait protein expression vector was detected by the self-activating activity assay, which verified that the bait plasmid did not have self-activating activity in the membrane yeast system, and could be used for membrane yeast library screening. Using the membrane yeast cDNA library constructed by membrane yeast two-hybrid screening for the critical period of seed dormancy is lifted in *Malus sieversii*, 96 positive yeast clones were selected, and 28 candidate proteins interacting with *MsPYR1-like* were obtained after sequencing, blast comparison, and yeast rotary verification. *MsPYR1-like* was found to have interactions with LEA, low-temperature-inducible protein, 14-3-3-like protein, SPL, ARF and ARP, which were reported to be involved in seed dormancy and germination. **【Conclusion】** A high-quality membrane yeast two-hybrid library of *Malus sieversii* was constructed, and a number of proteins interacting with *MsPYR1-like* were identified using yeast two-hybrid sieve library technology, which provides an important resource for the discussion the molecular mechanism of the *MsPYR1-like* gene and the interacting proteins in the process of seed dormancy is lifted.

Keywords: *Malus sieversii*; *MsPYR1-like*; membrane yeast two-hybrid; protein interactions; seed dormancy and germination.