



铁皮石斛种子接菌共生萌发抑制差减杂交文库的构建及序列分析

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摘要 目的 研究铁皮石斛 (*Dendrobium officinale*) 种子接菌共生萌发差异基因表达谱特征。方法 采用抑制差减杂交 (SSH) 技术, 分别以接菌及未接菌培养5周的铁皮石斛种子cDNA作为检测子与驱赶子, 构建铁皮石斛种子接菌共生萌发抑制性消减cDNA文库。结果 对部分克隆产物测序, 经GenBank中BLASTx同源比较后, 进行基因功能注释, 得到100个具有植物同源性的表达序列标签 (EST), 主要涉及细胞与染色体结构、RNA合成、信号转导、能量与代谢、蛋白质合成与降解及细胞防御等。随机挑选5个目标基因, 实时荧光定量PCR分析发现这5个基因在共生萌发的种子中均上调表达。结论 铁皮石斛种子接菌共生萌发涉及多种途径相关基因表达调控, 本实验为兰科植物种子萌发分子机制研究奠定基础。

关键词: 铁皮石斛 表达序列标签 共生萌发 基因表达 抑制差减杂交

Abstract: Objective To investigate differential gene expression profiling of symbiotic germinated seeds of *Dendrobium officinale*. METHODS cDNAs from 5-week symbiotic germinated seeds and 5-week aseptic cultivated seeds, taken as the tester and driver respectively, were used to construct a suppressive subtractive hybridization (SSH) cDNA library. RESULTS By sequencing positive clones and BLASTx analysis against GenBank database, 100 expressed sequence tags (EST) homologous to plant known genes were obtained. Functional annotation revealed that they were grouped into serials of cellular processes including cell and chromosome structure, RNA synthesis, signal transduction, energy metabolism, protein synthesis and degradation, and cell defense, etc. Real-time quantitative RT-PCR analyses showed that the five randomly selected genes were all up-regulated in symbiotic germinated seeds. CONCLUSION The symbiotic seed germination of *D. officinale* is involved in multiple pathways, and the results of this study will lay a foundation for further molecular elucidation of seed germination in Orchidaceae.

Keywords: *Dendrobium officinale*, expressed sequence tags (EST), symbiotic germination, gene expression, suppressive subtractive hybridization (SSH)

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