

拟南芥 HAM 同源基因在杨树中的初步研究

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摘 要:植物干细胞是植物体内具有自我更新和多向分化潜能的细胞群体, 主要位于植物体茎尖分生组织、根尖分生组织和维管形成层的干细胞龛中。其中维管形成层的干细胞能够调控植物的次生长, 是林木生长和木材形成之源。GRAS 家族中的转录因子 HAIRYMERISTEM (HAM) 基因是控制分生组织发育和干细胞稳态的关键调节因子。miRNA 是广泛存在于真核生物中的一类非编码小分子 RNA, 通过切割或者抑制翻译调控靶基因的表达, 在植物生长发育和响应逆境胁迫等方面发挥着重要的调控作用。在拟南芥中已鉴定出 HAM 亚家族的 4 个成员 (AtHAM1~AtHAM4), 其中 AtHAM1、AtHAM2 和 AtHAM3 属于同一组, 都含有保守的 microRNA170/171 结合位点, 受 21-nt miR170/171 家族 microRNAs 的靶向负调控, 而 AtHAM4 属于不同的组, 缺乏保守的 miR170 / 171 靶位点。在拟南芥中过表达 miR171 导致茎尖干细胞起始和维持缺陷, 分生组织结构紊乱, 茎尖分支减少, 这些表型与 *ham1ham2ham3* 三重或 *ham1ham2* 双重功能缺失突变体的表型类似。AtHAM 基因的 miR171 结合位点的沉默突变导致其不能被 miR171 切割。这些突变的 HAM 基因产生的 mRNA 不能被 miR171 识别, 但其中编码蛋白的氨基酸序列不变。当 miR171 不敏感的 AtHAM1 在表皮层特异性表达时, 会导致表皮中 CLV3 表达降低, 分生组织发育异常。本研究根据已有文献中 8 个杨树 HAM 基因的氨基酸序列信息, 经 NCBI 数据库序列比对, 结果显示 8 个杨树 HAM 蛋白的 N 端不保守, 而 C 端均比较保守, 均含有 GRAS 家族特有的结构域。经过与拟南芥 HAM 亚家族氨基酸序列进行比对, 选出与 AtHAM1 同源性最高的杨树 PtHAM 基因, 以‘84K’杨 cDNA 为模板, 参考杨树基因组序列信息, 进行 PCR 扩增获得目的基因片段。通过 Cell-PLoc2.0 在线软件进行预测, 结果显示 PtHAM 定位在细胞核。本研究以绿色荧光蛋白作为报告基因, 通过 Gateway 技术构建融合表达载体, 经过农杆菌 GV3101 介导转化将表达载体导入本氏烟草叶肉原生质体进行亚细胞定位。此外, 本研究利用在线工具 psRNATarget 预测 PtHAM 是否存在 ptc-miR171 的结合位点。其结果显示, ptc-miR171c、ptc-miR171d、ptc-miR171k 和 PtHAM 匹配最优, 在其第 2 个~第 21 个核苷酸的互补区域内, 与 PtHAM 完全互补配对, 而在第 1 个碱基处则出现错配。综合分析表明, PtHAM 存在 ptc-miR171 的结合位点, 推测其受到 ptc-miR171 的调控。

Preliminary study of Arabidopsis HAM homologous gene in poplar

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Abstract: Plant stem cells are cell populations with self-renewal and multi-directional differentiation potential in plants, which are mainly located in stem cell niches of shoot apical meristem, root apical meristem and vascular

cambium. Stem cells in the vascular cambium can regulate the secondary growth of plants and are the source of tree growth and wood formation. The transcription factor HAIRYMERISTEM (HAM) gene in the GRAS family is a key regulator of meristem development and stem cell homeostasis. MicroRNAs (miRNAs) are a class of non-coding small RNAs that are widely present in eukaryotes. They regulate the expression of target genes by cutting or inhibiting translation, and play an important regulatory role in plant growth and development and response to stress. Four members of the HAM subfamily (AtHAM1 ~ AtHAM4) have been identified in *Arabidopsis thaliana*. Among them, AtHAM1, AtHAM2 and AtHAM3 belong to the same group, all of which contain conserved microRNA170 / 171 binding sites and are negatively regulated by 21-nt miR170 / 171 family microRNAs, while AtHAM4 belongs to different groups and lacks conserved miR170 / 171 target sites. Overexpression of miR171 in *Arabidopsis* resulted in defects in the initiation and maintenance of stem tip stem cells, disordered meristem structure, and reduced shoot tip branches. These phenotypes are similar to the phenotypes of *ham1ham2ham3* triple or *ham1ham2* double function deletion mutants. The silencing mutation of miR171 binding site in AtHAM gene resulted in its inability to be cleaved by miR171. The mRNA produced by these mutant HAM genes could not be recognized by miR171, but the amino acid sequence of the encoded protein was unchanged. When miR171-insensitive AtHAM1 is specifically expressed in the epidermis, it will lead to a decrease in CLV3 expression in the epidermis and abnormal meristem development. In this study, according to the amino acid sequence information of 8 poplar HAM genes in the existing literature, the NCBI database sequence alignment showed that the N-terminus of the 8 poplar HAM proteins was not conserved, while the C-terminus was relatively conserved, and all contained GRAS family-specific domains. After comparing with the amino acid sequence of *Arabidopsis thaliana* HAM subfamily, the PtHAM gene of poplar with the highest homology with AtHAM1 was selected. The cDNA of '84K' poplar was used as a template, and the target gene fragment was obtained by PCR amplification with reference to the genome sequence information of poplar. PtHAM was predicted by Cell-PLoc2.0 online software and the results showed that PtHAM was located in the nucleus. In this study, green fluorescent protein was used as a reporter gene, and a fusion expression vector was constructed by Gateway technology. The expression vector was introduced into *N. benthamiana* mesophyll protoplasts by *Agrobacterium* GV3101-mediated transformation for subcellular localization. In addition, this study used the online tool psRNATarget to predict whether PtHAM has a binding site for ptc-miR171. The results showed that ptc-miR171 c, ptc-miR171 d, ptc-miR171 k and PtHAM matched best. In the complementary region of the second to twenty-first nucleotides, they were completely complementary to PtHAM, and mismatched at the first base. Comprehensive analysis showed that PtHAM has a binding site for ptc-miR171, and it is speculated that it is regulated by ptc-miR171.