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# 獐茅*HAK*基因表达调控区的分离及其在水稻中的功能分析

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摘要 獐茅高亲和性K+转运蛋白基因(*AlHAK1*)是从单子叶禾本科盐生植物獐茅(*Aeluropus littoralis* (Gouan) Parl)中克隆, 对于细胞营养和离子渗透调节起关键作用。为了进一步了解*AlHAK1*基因的表达调控机制, 文章采用基因组步移法分离了*AlHAK1*基因转录起始位点上游长度约1.3 kb的启动子区域。启动子顺式元件分析显示该序列具有典型的TATA和CAAT盒, 以及一些与植物生长发育和环境响应相关的顺式元件。为了明确*AlHAK1*启动子的功能, 将其与*GUS*基因融合构建到植物表达载体pCAMBIA1301上, 通过农杆菌介导转化法导入水稻中。对转基因植株进行GUS组织化学染色, 结果显示在转化*AlHAK1*启动子水稻的根、茎、叶、花药和内外稃部位均检测到GUS活性。GUS荧光定量分析显示*AlHAK1*启动子调节*GUS*表达活性低于组成型启动子CaMV35S和Ubiquitin, 但其根部和茎部的GUS活性相对较高。对转化植株进行不同胁迫处理后检测GUS活性, 结果表明受到ABA、干旱、高温的诱导后其茎部和根部GUS活性有所提高, 推测位于该启动子-682 bp的HSE元件和-1 268 bp的MybBS元件可能在高温、ABA和干旱诱导的表达调控中起作用。

关键词： 獐茅 高亲和性K+转运蛋白基因(*HAK1*) 启动子克隆 顺式元件分析 GUS组织化学染色

Abstract： The *AlHAK1* gene encoding a high-affinity K+ transporter was isolated from *Aeluropus littoralis* (Gouan) Parl, a graminaceous halophyte, and plays a crucial role in nutrition and ion homeostasis in plant cell. To investigate the regulation role of *AlHAK1* on the transcriptional level, an about 1.3 kb 5'-flanking region of the *AlHAK1* gene containing a putative promoter was cloned by genome walking method. *Cis*-regulatory elements analysis showed *AlHAK1*-promoter region contained typical TATA and CAAT boxes, and some growth and development relative motifs, as well as environmental responsive elements. To reveal the function and regulating role, the *Al-HAK1* promoter was fused to the *β-glucuronidase (GUS)* reporter gene in the pCAMBIA1301 vector and introduced into rice via *Agrobacterium*-mediated transformation. Histochemical staining indicated that the GUS expression directed by *AlHAK1* promoter was observed in leaves, stems, roots, anther, lemma, and palea. GUS quantitative fluorometric analysis indicated that GUS activity directed by *AlHAK1* promoter was lower than CaMV35S and Ubiquitin constitutive promoters; however, in the roots and stems the GUS activity was relatively high and displayed a tissue-specific expression pattern. Under ABA, high temperature or drought stress, the GUS activity directed by *AlHAK1* promoter was inducible in the roots and stems, suggesting the elements of HSE (-682 bp) and MybBS (-1 268 bp) might play a role in the inducible regulation.

Keywords： *Aeluropus littoralis*, high-affinity K+ transporter gene (*HAK1*), promoter isolation, Cis-regulatory elements analysis, GUS assays

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