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杜梨类钙调磷酸酶B亚基蛋白基因*PbCBL2*的克隆和功能初探

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Isolation of a Calcineurin B-like Protein Gene *PbCBL2* from *Pyrus betulaefolia* and Preliminary Study of Gene Function

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- 摘要
- 参考文献
- 相关文章

摘 要 类钙调磷酸酶B亚基蛋白（Calcineurin B-like protein，CBLs）是植物中一类重要的钙离子传感器，参与调控植物生长发育及逆境胁迫响应过程。为了探明杜梨CBLs家族成员*PbCBL2*的序列特征和表达模式，以杜梨（*Pyrus betulaefolia* Bunge）幼苗为试材，运用EST搜索结合RACE技术、染色体步移法对*PbCBL2*的cDNA、DNA和启动子进行克隆，采用半定量RT-PCR和原核表达研究该基因在非生物胁迫下的表达模式。结果表明，*PbCBL2*基因cDNA序列长681 bp，编码一个含有226个氨基酸残基的蛋白。基因组DNA序列长1 927 bp，包括8个外显子和7个内含子，启动子序列包含光反应元件、厌氧诱导必需顺式作用元件、赤霉素反应元件和水杨酸响应顺式作用元件。*PbCBL2*编码的多肽具有植物类钙调磷酸酶B亚基蛋白结合Ca^{2+}所必需的4个EF手型结构和1个典型的植物钙调磷酸酶A亚基结合位点。未经处理的杜梨幼苗（对照）根和叶中未检测到*PbCBL2*的表达，*PbCBL2*的表达受NaCl、PEG6000、甘露醇和ABA诱导上调。*PbCBL2*转入大肠杆菌BL21（DE3）后，能够明显减轻NaCl、甘露醇和PEG6000对该菌株的生长抑制。*PbCBL2*基因具备植物CBLs基因家族的固有特征，对盐碱、干旱、渗透胁迫和ABA处理均存在转录响应，大肠杆菌转入该基因后能够提高对盐胁迫和渗透胁迫的耐受能力。

关键词： 杜梨 类钙调磷酸酶B亚基蛋白 基因克隆 基因表达特点 原核表达 逆境胁迫

Abstract： Calcineurin B-like protein（CBLs），as a plant calcium sensor，plays critical role in the regulation of plant growth and stress response process. However，*CBL2* gene sequence feature，expression characteristic and physiological function in birch-leaf pear（*Pyrus betulaefolia* Bunge）are largely unknown. In this study，we isolated the cDNA，genomic DNA and its responding promoter sequences of *PbCBL2* gene from birch-leaf pear seedlings by EST database mining，rapid amplification of cDNA ends（RACE）and genome walking approaches. The results have showed that *PbCBL2* cDNA sequence contained a 681 bp open reading frame which encoded 226 amino acid residues. The length of genomic DNA sequence was 1 927 bp which consists of 8 exons and 7 introns. The promoter region of *PbCBL2* harbored some specific regulatory elements or motifs，such as light responsive element，cis-acting regulatory element essential for the anaerobic induction，gibberellin-responsive element and cis-acting element involved in salicylic acid responsiveness. The deduced *PbCBL2* polypeptide had four EF-hand structure domains（58－71，95－106，132－143 and 176－187 amino acids）which was necessary for calcium-binding and one calcineurin A subunit binding sites（156－171 amino acids）. Semi-quantitative RT-PCR and prokaryotic expression analyses validated that the mRNA abundance of *PbCBL2* is responsive to different abiotic stresses. However，*PbCBL2* expression was barely detected in roots and leafs of birch-leaf pear seedling without abiotic stresses treatment. The inhibition effects on BL21（DE3）growth causing by NaCl，mannitol or PEG6000 were significantly alleviated after *PbCBL2* gene transformation. Our studies have suggested that *PbCBL2* gene has the inherent characteristics of the CBLs gene family in plants，which transcription level is respond to salt，drought，osmotic stresses and ABA treatment. *E. coli* BL21（DE3）tolerance to salt stress and osmotic stress was enhanced by transferred *PbCBL2*.

Keywords： *Pyrus betulaefolia* Bunge， calcineurin B-like protein， gene cloning， gene expression characteristics， prokaryotic expression；environment stress

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