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豆梨铵转运蛋白基因PcAMT1-1 和PcAMT1-2 的克隆与功能鉴定

丛 郁，杨顺瑛，金 曼，郝东利，苏彦华

中国科学院南京土壤研究所土壤与农业可持续发展国家重点实验室，南京 210008

Molecular Cloning and Function Analyses of Two Ammonium Transporter Protein Genes from Pyrus calleryana

CONG Yu, YANG Shun-ying, JIN Man, HAO Dong-li, and SU Yan-hua

State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China

摘要 为探明豆梨（Pyrus calleryana Dcne.）铵转运蛋白基因家族成员的序列特征、生理功能和表达特点，以豆梨幼苗为材料，运用电子克隆与3′-RACE 技术获得2 个铵转运蛋白基因PcAMT1-1 和PcAMT1-2，采用酵母互补和半定量RT-PCR 方法研究它们的生理功能与表达特点。结果表明：PcAMT1-1和PcAMT1-2 开放阅读框为1 518 bp 和1 515 bp，编码的蛋白分别含505 和504 个氨基酸残基。PcAMT1-1和PcAMT1-2 分别与甜橙× 枳后代CpAMT（ABI52423）和茶CsAMT1;2（AB114913）的同源性最高（81.96%和78.31%）。PcAMT1-1 和PcAMT1-2 转入酵母均能使铵转运体缺失突变菌株31019b 恢复NH_4^+吸收能力，在酸性条件下（pH 4.8 或5.8）PcAMT1-1 和PcAMT1-2 对31019b 的互补效果均优于中性条件（pH 6.8）。NH_4^+有毒类似物MeA+可以显著抑制转PcAMT1-1 酵母31019b 的生长，该物质对转PcAMT1-2酵母31019b 无抑制效果；谷氨酰胺合成酶抑制剂MSX 可有效抑制PcAMT1-2 对酵母31019b 的互补效果，对PcAMT1-1 的互补效果无显著影响。正常供铵，PcAMT1-1 主要在叶中表达，PcAMT1-2 主要在根中表达；无氮处理后，PcAMT1-1 和PcAMT1-2 在根中的表达先上调后下降；重新供铵后，它们的表达量恢复；地上部的表达对上述处理无显著响应。综上所述，PcAMT1-1 和PcAMT1-2 在豆梨中具有吸收或转运NH_4^+的功能，并可能具备不同的调控机制。

关键词： 豆梨 铵转运蛋白基因 克隆 表达特点 功能互补

Abstract： The objective of this study was to illuminate sequence feature，physiological function and expression characteristic of PcAMT1-1 and PcAMT1-2 from ammonium transport protein gene family in bean pear（Pyrus calleryana Dcne.）. The cDNA sequences of PcAMT1-1 and PcAMT1-2 were cloned from bean pear seedlings by EST splicing and rapid amplification of cDNA ends（RACE）methods. The yeast complementation was adapted to preliminary study PcAMT1-1 and PcAMT1-2 physiological functions.Furthermore，their expression patterns were analyzed by semi-quantitative RT-PCR under different ammonium concentrations. The results showed that PcAMT1-1 cDNA had an open reading frame of 1 518 nucleotides encoding a polypeptide with 505 residues，while PcAMT1-2 cDNA had an opening reading frame of 1 515 nucleotides encoding a polypeptide with 504 residues. The deduced proteins of PcAMT1-1 and PcAMT1-2 had higher homologies（81.96% and 78.31%）with Citrus sinensis × Citrus trifoliata CpAMT（ABI52423）and Camellia sinensis CsAMT1;2（AB114913），respectively. Both PcAMT1-1 and PcAMT1-2 were able to complement the growth defect of yeast（Saccharomyces cerevisiae）ammonium transport mutant strain 31019b. Furthermore，the complementary effect under acid condition（pH 4.8 or 5.8）was slightly better than neutral condition（pH 6.8）. The presence of toxic NH_4^+ analog methylamine（MeA+）markedly inhibited the growth of yeast with PcAMT1-1，whereas yeast cells transformed with PcAMT1-2 can grow normally. L-methionine sulfoximine（MSX），the glutamine synthetase inhibitors，could effectively inhibit PcAMT1-2 complementary. However，it had no significant effect on PcAMT1-1.Under the normal NH_4^+ concentration condition，transcription of PcAMT1-1 was mainly in the leaves andPcAMT1-2 expressed mainly in the root. When NH_4^+ was depleted，PcAMT1-1 and PcAMT1-2 expression levels in roots increased firstly and then decreased. Moreover，their expression levels were recovered upon NH_4^+ resupply. However，their expression levels in shoots were not observably changed when the NH_4^+ concentration was changed. Taken together，both PcAMT1-1 and PcAMT1-2 play the roles in absorption or translocate NH_4^+ in bean pear，which maybe have different regulatory mechanisms.

Keywords： *Pyrus calleryana*， ammonium transport protein gene， cloning， expression feature， functional complementation

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