

研究报告 Original Papers

组成型表达的 *ClpB* 基因提高番茄植株的耐冷性秦佳¹, 杨金莹², 伊淑莹¹, 赵春梅¹, 李明辉¹, 刘箭^{1,*}¹ 山东师范大学生命科学学院, 济南 250014; ² 中国石油大学(华东)生物工程与技术中心, 山东青岛 266555

摘要: 用农杆菌介导法将 CaMV35S 启动子驱动的 *ClpB* cDNA 导入番茄, 并比较了转基因和未转基因番茄的抗冷能力。当受冷胁迫后, 转基因番茄比未转基因番茄表现出较轻的冷胁迫症状, 并维持较高的 PSII 水平。

关键词: *ClpB*; 耐冷性; 转基因番茄; PSII

Improvement of Chilling Tolerance in Tomato (*Lycopersicon esculentum* Mill.) Plant by Constitutive Expression of *ClpB* Gene

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Abstract: *ClpB* cDNA under the control of CaMV35S promoter was introduced into tomato (*Lycopersicon esculentum*) plants by *Agrobacterium tumefactions*-mediated. The chilling tolerances of transgenic and non-transformed tomato lines were evaluated. After exposure to chilling stress, transgenic plants exhibited lighter symptoms of chilling injury and kept higher values of F_v/F_m in PSII than those of non-transformed plants. All results indicated that the chilling tolerance in transgenic tomato plants was improved.

Key words: *ClpB*; chilling tolerance; transgenic tomato (*Lycopersicon esculentum*); PSII

热激蛋白(heat shock protein, HSP)不但受高温诱导, 而且也受低温胁迫诱导。受低温诱导的热激蛋白包括大豆(*Glycine max*)、菠菜(*Spinacia oleracea*)和拟南芥(*Arabidopsis thaliana*) (Sung等2001)中的 Hsp70, 水稻(*Oryza sativa*)、芸苔(*Brassica campestris*)和洋葱(*Allium cepa*) (Giménez-Abián等2004)中的 Hsp90, 以及马铃薯(*Solanum tuberosum*)、桑树(*Morus Alba*)和番茄(*Lycopersicon esculentum*) (Liu和Shono 2001)中的小分子热激蛋白。根据热激蛋白受低温诱导的现象, 有人推测, 低温诱导的热激蛋白可能与植物抗冷性有一定的关系(Sung等2001)。

李筱媛等(2003)用基因工程方法, 在番茄中组成型地表达拟南芥热激蛋白转录因子(AtHsf1b), 导致高温诱导的热激蛋白可在常温和低温条件下表达, 转基因番茄的耐冷性也得到相应的提高。Wang等(2005)和Neta-Sharir等(2005)也分别用基因工程方法证明, 组成型表达的叶绿体小分子热激蛋白可保护叶绿体 PSII, 从而提高

番茄的耐冷性。这些结果说明, 热激蛋白确实可以提高植物的抗冷性。

在庞大的植物热激蛋白家族中, *ClpB* 是分子量最大的热激蛋白(分子量约100 kDa), 是微生物和植物获得耐热性的重要因子。蓝藻 *ClpB* 是高等植物 *ClpB* 的近源基因, 此基因的突变会增加其对高温和低温的敏感性(Porankiewicz 和 Clarke 1997), 但目前还不清楚的是高等植物叶绿体 *ClpB* 是否也对植物的抗冷性有影响。我们实验室从番茄的 cDNA 文库中克隆到一个属于 *ClpB* 家族、由核基因编码、表达蛋白定位于叶绿体基质的热诱导型 *ClpB* 基因(Yang等2006)。采用反义 RNA 技术, Yang等(2006)证明此叶绿体型 *ClpB* 是植物获得耐热性的必须基因。本文用基因工程方法, 将叶绿体型 *ClpB* 导入番茄并在叶绿体中呈组成型表

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