园艺学报 » 2012, Vol. 39 » Issue (5) :869-878 DOI:

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黄瓜Rubisco 活化酶基因CsRCA 表达载体构建与遗传转化

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Expression Vector Construction of Rubisco Activase Gene *CsRCA* and Genetic Transformation to Cucumber

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摘要 核酮糖－1,5－二磷酸羧化/加氧酶（Rubisco）是光合碳循环中的关键酶，为了探明其在植物体内的活化机制，用农杆菌介导法将Rubisco活化酶（RCA）基因*CsRCA*导入黄瓜，分别用PCR、real-time PCR和Western杂交法进行分子检测，分析转基因植株的表达量。酶切鉴定结果显示，*CsRCA*正向插入植物表达载体pCAMBIA1301的CaMV 35S启动子和NOS终止子之间，成功构建*CsRCA*的正义表达载体。将pCAMBIA1301-*CsRCA*导入黄瓜自交系‘08-1’，获得7株转基因植株，拷贝数均为2（非转基因植株的拷贝数为1），转化率约为3.5%。表达分析结果表明，7株T0代转基因植株叶片的*CsRCA* mRNA表达量为野生型（WT）的1 ~ 1.98倍，在蛋白水平的表达信号显著强于WT。T1代转基因植株的叶片叶绿素和类胡萝卜素含量、光合速率（P_n）及可溶性糖和淀粉含量均显著高于WT。研究结果表明，利用农杆菌介导法获得了稳定遗传的黄瓜*CsRCA*转基因植株，*CsRCA*过量表达能显著提高黄瓜叶片的P_n，增加干物质积累。

关键词： 黄瓜 核酮糖&ndash 1 5&ndash 二磷酸羧化/加氧酶（Rubisco） Rubisco 活化酶（RCA） 遗传转化 过量表达

Abstract: Ribulose-1,5-bisphosphate carboxylase/xygenase（Rubisco）is a key enzyme in the photosynthetic carbon cycle. In order to elucidate the activating mechanism of Rubisco in plants, the Rubisco activase gene *CsRCA* was introduced into inbred line of cucumber with the *Agrobacterium*-mediated method. The transgenic plants were screened by PCR, and their expression in mRNA and protein level were analysed by real-time PCR and western blot respectively. The restriction enzyme result showed that the *CsRCA* was inserted into the binary vector pCAMBIA1301, a sense expression vector containing *CsRCA* gene was constructed. The resulting plasmid was introduced into cucumber inbred lines '08-1', and seven transgenic plants which contain two *CsRCA* copies were obtained（The wild type plants contain one *CsRCA* copy）. The transformation rate was about 3.5%. The seven T0 transgenic cucumber plants showed 1－1.98 folds in *CsRCA* mRNA abundance and a significant increase in expression of protein level compared with the wild type（WT）plants. *CsRCA* over expression led to significant increase in the pigment content, photosynthetic rate（P_n）, soluble sugar and starch contents in T1 transgenic plant leaves. These data indicated that stable genetic *CsRCA* transgenic cucumber plants were obtained with *Agrobacterium*-mediated method. *CsRCA* over expression increased the P_n and carbohydrate significantly in cucumber leaves.

Keywords: cucumber, Ribulose-1,5-bisphosphate carboxylase/oxygenase（Rubisco）, Rubisco activase（RCA）, genetic transformation, over expression

引用本文:

刘培培, 姜振升, 王美玲等 .黄瓜Rubisco 活化酶基因CsRCA 表达载体构建与遗传转化[J] 园艺学报, 2012,V39(5): 869-878

LIU Pei-Pei, JIANG Zhen-Sheng, WANG Mei-Ling etc .Expression Vector Construction of Rubisco Activase Gene *CsRCA* and Genetic Transformation to Cucumber[J] ACTA HORTICULTURAE SINICA, 2012,V39(5): 869-878

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