

甘蓝花粉管钙感应蛋白CaM与SRK相互作用研究

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Studies on the Interactions Between the Pollen Tube Calmodulin (CaM) and SRK from *Brassica oleracea* var. *capitata*

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摘要 为研究甘蓝花粉管钙感应蛋白CaM与SRK相互作用的分子机理及其可能相互作用的区域, 从自交不亲和甘蓝材料‘E1’中分别克隆得到CaM12基因450 bp及S位点受体激酶(SRK7)基因全长序列2 118 bp, 并亚克隆得到SRK胞外域(eSRK)和胞内激酶域(iSRK), 构建原核表达载体pGEX-CaM12、pCold-eSRK和pCold-iSRK, 转化E. coli BL21(DE3)进行原核表达, 表达产物纯化后进行体外相互作用, 结果表明CaM12能够与SRK7进行相互作用, 但作用区域是iSRK7而不是eSRK7。为进一步验证其相互作用, 本研究利用酵母双杂交系统, 构建pGBKT7-CaM12、pGADT7-eSRK7、pGADT7-iSRK7和pGADT7-SRK7酵母表达载体, 转化相应酵母Y2HGOLD和Y187感受态细胞后未出现自激活和毒性现象, 相互作用结果与原核表达检测一致。同时将CaM12的3个EF-hands结构域突变体CaM12-2-、CaM12-23-和CaM12-234-与iSRK7分别构建酵母表达载体pGADT7-CAM12-2-、pGADT7-CAM12-23-、pGADT7-CAM12-234-, 检测其相互作用。结果表明CaM12 EF-hands突变体CaM12-2-、CaM12-23-和CaM12-234-

在酵母双杂交系统中均不能与iSRK7片段发生相互作用, 说明CaM12的EF-hands结构域突变后失去结合Ca²⁺能力而不能与iSRK7相互作用。该研究可为自交不亲和机理提供新的参考依据。

关键词

关键词: [结球甘蓝](#) [CaM12](#) [S位点受体激酶\(SRK7\)基因](#) [原核表达](#) [酵母双杂交](#)

Abstract: In order to study the molecular mechanism and possible interaction domains between pollen tube calmodulin (CaM) protein and S locus receptor kinase (SRK) from *Brassica oleracea* L. var. *capitata* L. We got the full length sequence of CaM12 with 450 bp and SRK7 gene with 2 118 bp from self-incompatibility of *Brassica oleracea* var. *capitata* E1, respectively, and got extracellular domain of SRK (eSRK7) and intracellular kinase domain (iSRK7), then constructed prokaryotic expression vectors of pGEX-CaM12, pCold-eSRK7 and pCold-iSRK7, transformed into E. coli BL21 (DE3) and checked the interactions with purified expression products in vitro. The results showed that CaM12 protein and SRK7 could do interaction, and interactive domain is iSRK7 rather than eSRK7. In order to verify their interaction furtherly, yeast two-hybrid system was used in this study, we constructed yeast expression vectors pGBKT7-CaM12, pGADT7-eSRK7, pGADT7-iSRK7 and pGADT7-SRK7, transformed into corresponding Y2HGOLD and Y187 yeast cells, and made sure that they did not appear the self-activation and toxicity. The results was consistent with prokaryotic expression. Simultaneously, we constructed yeast expression vectors pGADT7-CAM12-2-, pGADT7-CAM12-23-, pGADT7-CAM12-234- and pGBKT7-iSRK7 with CaM12-2-, CaM12-23-, CaM12-234- mutants from three EF-hands of CaM12 and iSRK7, and tested their interactions. The results showed that all CaM EF-hands mutants CaM12-2-, CaM12-23- and CaM12-234- cannot interact with iSRK7 in yeast two-hybrid system. CaM12 protein lost the ability to combine Ca²⁺ and cannot interact with iSRK7 after EF-hands structure domains were mutated. This study could provide a new reference for the mechanism of self-incompatibility in *Brassica oleracea*.

Keywords: [Brassica oleracea](#), [CaM12](#), [SRK7](#), [prokaryotic expression](#), [yeast two-hybrid](#)

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引用本文:

许俊强, 孙梓健, 宋 明等 . 甘蓝花粉管钙感应蛋白CaM 与SRK 相互作用研究[J] 园艺学报, 2013,V40(12): 2429-2440

XU Jun-Qiang, SUN Zi-Jian, SONG Ming etc . Studies on the Interactions Between the Pollen Tube Calmodulin (CaM) and SRK from *Brassica oleracea* var. *capitata*[J] ACTA HORTICULTURAE SINICA, 2013,V40(12): 2429-2440

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