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芥菜开花调控蛋白SVP 与FLC 酵母表达载体的构建及其相互作用研究

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Determination of Interactions Between SVP and FLC in *Brassica juncea* Coss. by Yeast Two-Hybrid System

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摘要 为深入研究芥菜开花信号整合子的两个核心调节因子SHORT VEGETATIVE PHASE（SVP）与FLOWERING LOCUS C（FLC）相互作用的分子机理，通过PCR扩增，从芥菜材料‘QJ’中分别克隆含*Eco*RⅠ/*Bam*HⅠ双酶切位点的*SVP*和*FLC*编码区全长，并利用酵母双杂交体系，将FLC与GAL4报告基因DNA 激活域融合（pGADT7FLC），SVP与GAL4报告基因DNA 结合域融合（pGBKT7SVP）。两种重组质粒分别转化酵母Y187和Y2HGold后未出现自激活和毒性现象。融合的二倍体酵母（pGADT7FLC × pGBKT7SVP）能在选择性固体培养基QDO/X/A（SD/-Ade/-His/-Leu/-Trp/X-α-Gal/AbA）上生长，并且菌落呈蓝色。将诱饵质粒（pGBKT7SVP）与猎物质粒（pGADT7FLC）载体互换（pGADT7SVP、pGBKT7FLC），再次转化酵母后仍能融合成二倍体酵母（pGADT7SVP × pGBKT7FLC），并同时激活报告基因*AUR1-C*、*HIS3*、*ADE2*、*MEL1*，由此表明SVP与FLC蛋白能够相互结合。

关键词： 芥菜 SVP FLC 酵母双杂交

Abstract： The fate of the flowering signal integrators is determined by SHORT VEGETATIVE PHASE（SVP）and FLOWERING LOCUS C（FLC）. For further study on the mechanism of the mutual recognition between SVP and FLC in *Brassica juncea* Coss.（Mustard） variety ‘QJ’，the coding sequences of SVP and FLC with digestion sites of *Eco*RⅠ/*Bam*HⅠwere respectively amplified via PCR，and the interactions between SVP and FLC were detected by the yeast two-hybrid system. The full-length FLC was fused to the GAL4 DNA activation domain，which was designated as pGADT7FLC and then transformed into Y187 yeast stain. While SVP was fused to the GAL4 DNA binding domain，which was designated as pGBKT7SVP and then transformed into Y2HGold yeast stain. The two transformed yeast stains did not exhibit autoactivation and toxicity. The yeast stains of pGADT7FLC and pGBKT7SVPcouldmate into yeast diploids. The zygote diploids grew on selective agar plates QDO/X/A （SD/-Ade/-His/-Leu/-Trp/X-α-Gal/AbA）with blue stains. The results strongly indicated that SVP and FLC could combine with each other. Furthermore，the expression vectors of bait plasmid（pGBKT7SVP）and prey plasmid（pGADT7FLC）were exchanged with each other. Then the recombinated yeast plasmids of pGADT7SVP and pGBKT7FLC were reconstructed and respectively transformed into Y187 and Y2HGold yeast stains. The yeast zygote diploids（pGADT7SVP × pGBKT7FLC）exhibit on selective agar plates QDO/X/A，and the DNA-BD and AD were brought into proximitry to activate transcription of four independent reporter genes（*AUR1-C*，*HIS3*，*ADE2*，*MEL1*）. The results showed that SVP and FLC could act with each other to combine and form a complex.

Keywords： *Brassica juncea*, SVP, FLC, yeast two-hybrid system

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