

小麦ERF转录因子W17互作蛋白的筛选和解析

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Screening and Identification of Proteins Interacting with ERF Transcription Factor W17 in Wheat

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摘要

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摘要 来自小麦的ERF转录因子W17基因参与胁迫应答, 过表达W17可显著提高转基因拟南芥的抗旱性和抗病性。本研究构建了小麦cDNA文库, 通过酵母双杂技术筛选W17的互作蛋白, 以期进一步解析ERF蛋白的作用机制。将pGBKT7-W17质粒、pGADT7和小麦文库混合转入酵母细胞AH109, 在SD/-Trp/-Leu/-His/-Ade营养缺陷型平板上培养, 挑选直径大于2 mm的克隆, 在SD/Raf/Gal/X-gal平板上划线培养, 筛选蓝色克隆。将筛出的克隆测序、BLAST分析, 得到4类与W17相互作用的候选蛋白, 分别是胁迫相关功能蛋白、翻译后修饰蛋白、1,5-二磷酸核酮糖羧化酶/加氧酶(Rubisco)大亚基/小亚基以及功能未知蛋白。互作验证表明, Hsp90和PPR蛋白与W17有相互作用关系。这些候选蛋白参与信号转导或免疫过程, 暗示W17在植物的逆境信号转导、下游基因转录调控, 甚至在翻译过程都有重要作用。

关键词: 酵母双杂交系统 ERF 蛋白互作 信号转导 小麦

Abstract: Ethylene responsive factors (ERFs) regulate a variety of biotic- and abiotic-stress responses. Transcription factor W17 is an ERF isolated from wheat (*Triticum aestivum* L.), which participates in stress responses. To provide data for exploring the functional mechanism of ERF proteins, we constructed a wheat cDNA library and screened proteins interacting with W17 by yeast two-hybrid system. The mixture of recombinant plasmid pGBKT7-W17, pGADT7, and wheat cDNA library was introduced into yeast cell AH109. Transformed cells were incubated on SD/-Trp/-Leu/-His/-Ade plate for 3-5 d at 30°C before selection of clones with diameter larger than 2 mm, and further incubated on SD/Raf/Gal/x-gal for screening blue clones. Four types of proteins that interacted with W17 were obtained, namely stress-related functional protein, post-translational modification protein, ribulose-1,5-bisphosphate carboxylase/oxygenase, and unknown protein. The protein-protein interaction was retested using the co-transformation yeast system of pGBKT7-W17 and candidates of interaction protein carried by pGADT7. The result showed that Hsp90 and PPR proteins interacted with W17 in vivo. Most of candidate proteins involved in signal transduction and immune process, such as Tir cytoskeleton coupling protein (TCCP), 26S proteasome subunit, RNA binding protein, WD40, PPR, HSP90, and cysteine proteinase inhibitor. This result suggests that W17 possibly plays significant roles in stress signal transduction, transcription regulation of downstream genes, and translation process in stress environments.

Keywords: Yeast two-hybrid system Ethylene responsive factor (ERF) Protein interaction Signal transduction Common wheat

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