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论文

鹰嘴豆锌指蛋白基因*ZF1*的克隆及表达分析

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摘要：

利用一段从PEG胁迫的鹰嘴豆幼苗叶片所构建的cDNA文库中得到的EST序列，通过3¢RACE方法克隆到一个鹰嘴豆C_2H_2型锌指蛋白基因*ZF1*，该基因不含内含子，编码一条244个氨基酸残基的多肽，含有两个典型的Cys2/His2锌指结构。其氨基酸序列含有一个可能的核定位型号，农杆菌介导的洋葱表皮细胞GFP瞬时表达实验表明，*ZF1*蛋白位于细胞核内。半定量RT-PCR分析表明，*ZF1*在鹰嘴豆的根、茎、叶、花、幼荚和幼胚中均有表达，在茎和叶中表达较弱，为组成型转录因子。半定量RT-PCR和实时荧光定量PCR检测结果显示，*ZF1*不但受高温及干旱诱导，而且还受6-苄基腺嘌呤(6-BA)、脱落酸(ABA)、乙烯利(Et)、赤霉素(GA_3)、吲哚-3-乙酸(IAA)、茉莉酸甲酯(MeJA)、水杨酸(SA)和氧胁迫诱导。这些结果表明，*ZF1*基因可能作为一个核调控因子参与植物的生长代谢以及多种生物与非生物胁迫的应答。

关键词： 鹰嘴豆 锌指蛋白 基因克隆 胁迫 表达分析

Cloning and Expression of Zinc Finger Protein Genetic ZF1 in Chickpea（*Cicerone arietinum* L.）

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Abstract:

Regulation of gene expression at the level of transcription controls many crucial biological processes including growth and development, stress response, signal transduction and disease resistance. A number of factors, such as C_2H_2 zinc finger protein, are required and factors play an important role in the transcription. Chickpea (*Cicer arietinum* L.) is the third important legume crop gown mainly in the arid and semi-arid regions in the world. Due to its taxonomic proximity with the model legume genome of *Medicago truncatula* and its ability to grow in soil with relatively low water content, chickpea is being investigated as a model legume crop for drought tolerance studies. In our laboratory, two cDNA libraries from the PEG-treated and non-treated seedling leaves of chickpea XJ209 were constructed and many genes were found to express differentially and involved in diverse biological processes, such as metabolism, transcription, signal transduction, protein synthesis and others. According to an EST in the cDNA libraries, a zinc finger protein gene *ZF1* was cloned by RT-PCR and rapid amplification of cDNA ends (RACE). *ZF1* did not include any intron, encoding a 26.33 kD protein with 244 amino acids, containing two typical C_2H_2 zinc finger domains. The deduced protein sequence had a potential nuclear localization signal (NLS). Meanwhile, transient expression of the ZF1-GFP protein in onion epidermal cells showed that ZF1 protein was localized in cell nuclei. Semi-quantitative RT-PCR analysis showed that *ZF1* expressed in root, stem, leaf, flower, immature pod, and embryo of chickpea with different expression patterns. The expression of *ZF1* investigated by semi-quantitative PCR had no obvious changes under stresses of cold, salt and wounding, while was increased under the treatments of heat and drought, as well as N-6-benzyl-adenine (6-BA), abscisic acid (ABA), ethephon (Et), gibberellin (GA_3), indole-3-acetic acid (IAA), methyl jasmonate (MeJA), salicylic acid (SA), and H_2O_2. These results from semi-quantitative PCR in several treatments were further confirmed to be mainly in accord with those from real-time quantification PCR. Our results suggest that *ZF1* may play multiple roles in abiotic and biotic resistance pathways, as well as in plant growth.

Keywords: Chickpea Zinc finger protein Gene cloning Stress Expression analysis

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