



青花菜 C3H 型锌指蛋白基因 *BoCCCH2* 的克隆与表达

蒋明^{1*}, 刘青娥², 章燕如¹, 祝琦¹, 龚秀¹, 俞可可¹, 周秀倩¹

(1. 台州学院生命科学学院生态学省重点学科, 浙江 椒江 318000; 2. 丽水学院生态学院, 浙江 丽水 323000)

摘要: 以青花菜为材料, 在克隆 C3H 型锌指蛋白基因 *CS###K?* 的基础上, 研究该基因在不同器官及霜霉菌和灰葡萄孢菌侵染叶片中的表达模式。测序结果表明, *CS###K?* 没有内含子, 编码区全长为 11740

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* 通信作者 (Corresponding author): 蒋明 (<http://orcid.org/0000-0001-9556-2249>), E-mail: jiangming1973@139.com

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various biological activities, such as replication, transcription, translation, repair, metabolism and signaling. According to the number and order of cysteine and histidine residues, zinc finger proteins were classified into several different types, such as C₂H₂, C₂C₂, C₂C₂C₂, C₂HQ and C₃H. For example, C₃H-type ones contain one to six typical motifs with three cysteine residues and one histidine residue. However, their functions are little known, and no gene has been reported in broccoli.

In this study, a C₃H-type zinc finger protein gene *CsZFP1* was isolated from broccoli, and later the expression patterns in different organs as well as leaves infected by *K. M15/1* and *C. /48* were studied.

Results indicated that *CsZFP1* contained no intron, and the full length of coding sequence was 1740 bp encoding 579 amino acids. The deduced protein sequence contained two ANK domains and two CCCH zinc finger structures, respectively, and the CCCH zinc finger types were C-X₈-C-X₅-C-X₃-H and C-X₅-C-X₄-C-X₃-H. Reverse transcription-polymerase chain reaction results showed that the *CsZFP1* was expressed in roots, leaves, stalks, young siliques, flower buds and flowers, with highest level in roots. Expression levels increased when challenged by both *K. M15/1* and *C. /48*. When infected by *K. M15/1*, expression levels increased after 24 h, and decreased after 72 h, while infected by *C. /48*, the highest level was detected after 6 h, and slowed down in 12 h. Homologous sequences were downloaded from NCBI (National Center for Biotechnology Information) website, including *At1G54855*, *ES56MG21** and *D1*.

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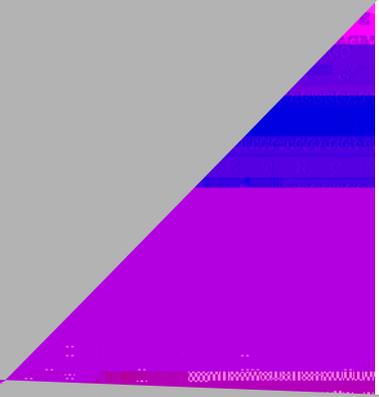
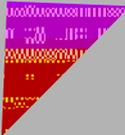
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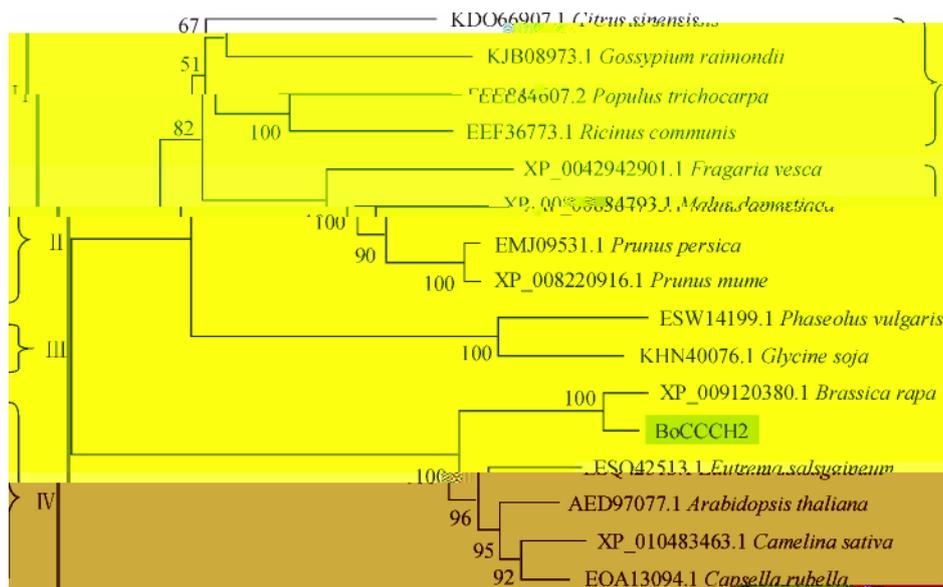


图 5| 用邻接法构建的 BoCCCH2 及其同源序列的系统进化树

Fig. 5| Phylogenetic tree of BoCCCH2 and its homologous sequences constructed using neighbor-joining method

氨酸之间的氨基酸残基数, C3H 型锌指结构共有序列的最初定义是 $C-X_{6-14}-C-X_{4-5}-C-X_3-H$ ^[22], 在对拟南芥和水稻的 C3H 进行全基因组鉴定时发现了一些新类型, 重新定义为 $C-X_{4-15}-C-X_{4-6}-C-X_3-H$ ^[23]。锌指蛋白通常含 1~6 个锌指结构, 但也有例外, 如玉米的 ZmC3H3 含 7 个 C3H, 类型为 $C-X_{17}-C-X_6-C-X_3-H$, 除此之外, 还有 $C-X_8-C-X_5-C-X_3-H$ 、 $C-X_7-C-X_5-C-X_3-H$ 、 $C-X_7-C-X_4-C-X_3-H$ 、 $C-X_5-C-X_4-C-X_3-H$ 、 $C-X_7-C-X_6-C-X_3-H$ 和 $C-X_8-C-X_4-C-X_3-H$ ^[24]。在本研究中 BoCCCH2 具 2 个锌指结构, 分别为 $C-X_8-C-X_5-C-X_3-H$ 和 $C-X_5-C-X_4-C-X_3-H$, 它们均为常见类型, 拟南芥 C3H14 和 C3H15 各具 2 个锌指结构, 都是 $C-X_8-C-X_5-C-X_3-H$ 类型^[23]; 在毛果杨的 211 个 C3H 锌指结构中, $C-X_8-C-X_5-C-X_3-H$ 有 96 个, $C-X_7-C-X_5-C-X_3-H$ 有 76 个^[25], 而在拟南芥的 148 个 C3H 锌指结构中, $C-X_8-C-X_5-C-X_3-H$ 和 $C-X_7-C-X_5-C-X_3-H$ 类型的 C3H 分别为 78 和 43 个。

锚蛋白重复序列 (ankyrin repeats, ANK) 结构域在蛋白质-蛋白质互作中起着重要作用, 它们参与转录起始、细胞周期调控、细胞骨架、

一步我们将开展载体构建和转基因研究,以明确 *ZNF116* 在霜霉病和灰霉病抗性反应中的功能。

参考文献 (References):

[1] BUCKLE P A. Origin and