

植物诱变育种·农业生物技术

棉花*Bax inhibitor-1*基因启动子的克隆及初步验证

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摘要: 为研究克隆自陆地棉的*GhBI-1A*和*GhBI-1B*基因的表达差异及其响应不同物生和非生物胁迫的分子机制, 利用BD GenomeWalkerTM Universal Kit的染色体步移技术得到了2个棉花*GhBI-1A*和*GhBI-1B*基因5'端上游的启动子序列, 长度分别为1650bp和2001bp。生物信息学分析表明, *GhBI-1A*和*GhBI-1B*的启动子序列均存在TATA-box和CAAT-box及多个与植物非生物胁迫相关的响应元件, *GhBI-1B*启动子中还含有真菌诱导应答元件。以表达载体 pBI101为基础, 用所克隆的2个棉花*GhBI-1*基因启动子序列与GUS报告基因融合, 构建新的植物表达载体并转入农杆菌。用叶盘法侵染烟草进行瞬时表达, 结果表明2个启动子均能够驱动报告基因的表达。

关键词: 棉花 *GhBI-1*基因启动子 克隆 瞬时表达

Cloning and Test of *Bax Inhibitor-1* Gene Promoters from Upland Cotton (*Gossypium hirsutum* L.)

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Abstract: To investigate the expression scenario of *Bax inhibitor-1* (*GhBI-1A* and *GhBI-1B*) genes cloned from Upland Cotton (*Gossypium hirsutum* L.) and the molecular mechanism of their diverse response to biotic and abiotic stress, the promoter fragments of 1650bp and 2001bp upstream the 5' end of *GhBI-1A* and *GhBI-1B* were isolated from the genomic DNA of Upland Cotton by BD GenomeWalkerTM Universal Kit technique. Bioinformatics analysis revealed that the two promoter sequences contained basic cis-elements, such as TATA-box and CAAT-box and many elements involved in the plant abiotic stress response. *GhBI-1B* also included a Box-W1, which is involved in fungal response. Plant expression vectors were constructed by inserting the two *GhBI-1* promoter sequences into the upstream of the GUS gene of the binary vector pBI101, and, transferred into *Agrobacterium tumefaciens*. The result of transient expression indicated that both sequences had the function to drive reporter gene *GUS* in tobacco.

Keywords: *Gossypium hirsutum* *GhBI-1* promoters Clone Transient Expression

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参考文献：

- [1] 万小荣,莫爱琼,刘 帅,梁建华,李 玲,余土元,郑奕雄. 粤油7号花生*AhNCED1*基因启动子克隆及其活性分析[J].核农学报,2011,25(4):692-699
- [2] Behnam B, Kikuchi A, Celebi-Toprak F, Kasuga M, Yamaguchi-Shinozaki K, Watanabe K, Arabidopsis rd29A:: DREB1A enhances freezing tolerance in transgenic potato [J]. Plant Cell Reports, 2007, 26(8): 1275-1282
- [3] Behnam B, Kikuchi A, Celebi-Toprak F, Yamanaka S, Kasuga M, Yamaguchi-Shinozaki K, Watanabe KN. The Arabidopsis DREB1A gene driven by the stress-inducible rd29A promoter increases salt-stress tolerance in proportion to its copy number in tetrasomic tetraploid potato (*Solanum tuberosum*) [J]. Plant Biotechnology, 2006, 23(2): 169-177
- [4] Bihmidine S, Lin J, Stone J, Awada T, Specht J, Clemente T. Activity of the Arabidopsis RD29A and RD29B promoter elements in soybean under water stress [J]. Planta, 2013, 237(1):55-64
- [5] Devi M J, Bhatnagar-Mathur P, Sharma K K, Serraj R, Anwar SY, Vadez V. Relationships between transpiration efficiency and its surrogate traits in the rd29A: DREB1A transgenic lines of groundnut [J]. Journal of Agronomy and Crop Science, 2011, 197(4): 272-283
- [6] Kasuga M, Miura S, Shinozaki K, Yamaguchi-Shinozaki K. A combination of the Arabidopsis DREB1A gene and stress-inducible rd29A promoter improved drought-and low-temperature stress tolerance in tobacco by gene transfer [J]. Plant and Cell Physiology, 2004, 45(3): 346-350
- [7] Qiu W, Liu M, Qiao G, Jiang J, Xie L, Zhuo R. An isopentyl transferase gene driven by the stress-inducible rd29a promoter improves salinity stress tolerance in transgenic tobacco [J]. Plant Molecular Biology Reporter, 2012, 30(3): 519-528
- [8] Polizel A M, Medri M E, Nakashima K, Yamanaka N, Farias J R B, Oliveira M C N, Marin S R R, Abdelnoor R V, Marcelino-Guimarães F C, Fuganti R. Molecular, anatomical and physiological properties of a genetically modified soybean line transformed with rd29A: AtDREB1A for the improvement of drought tolerance [J]. Genetics and Molecular Research, 2011, 10(4): 3641-3656
- [9] Pellegrineschi A, Reynolds M, Pacheco M, Brito RM, Almeraya R, Yamaguchi-Shinozaki K, Hoisington D. Stress-induced expression in wheat of the Arabidopsis thaliana DREB1A gene delays water stress symptoms under greenhouse conditions [J]. Genome, 2004, 47(3): 493-500
- [10] Sa Q, Wang Y, Li W, Zhang L, Sun Y. The promoter of an antifungal protein gene from *Gastrodia elata* confers tissue-specific and fungus-inducible expression patterns and responds to both salicylic acid and jasmonic acid [J]. Plant Cell Reports, 2003, 22(1): 79-84
- [11] 李晓荣,王冬梅,李建平,李 静,张帅,陈国华,黄乐平.小麦Wcor719基因的原核表达及转化烟草和棉花提高抗冷性研究[J].核农学报,2012,26(3):420-426
- [12] 李 蒙,刘珊珊,李 钰. *BI-1*基因的功能研究进展[J]. 国外医学遗传学分册,2005, 28(6):329-333
- [13] Walter L, Dirks B, Rothermel E, Heyens M, Szpirer C, Levan G, Günther E. A novel, conserved gene of the rat that is developmentally regulated in the testis [J]. Mammalian Genome, 1994, 5(4): 216-221
- [14] Walter L, Marynen P, Szpirer J, Levan G, Günther E. Identification of a novel conserved human gene, TEGT [J]. Genomics, 1995, 28(2): 301-304
- [15] 张美红,周克元. 进化中保守的凋亡抑制因子*Bax inhibitor-1*[J]. 肿瘤防治研究, 2007, 34(7):534-537
- [16] Isbat M,Zeba N,Kim S R,Hong C B. A *Bax inhibitor-1* gene in *Capsicum annuum* is induced under various abiotic stresses and endows multi-tolerance in transgenic tobacco [J]. Plant Physiology,2009,166: 1685-1693
- [17] 郑 玲,张玉芹,张传云,刘国栋,王芙蓉,张 军.棉花*GhBI-1*基因全cDNA的克隆与表达分析[J].核农学报,2011,25(1):20-25
- [18] 郑 玲.棉花BI基因的克隆与功能分析[D].济南:山东师范大学,2011
- [19] Paterson A H, Brubaker C L, Wendel J F. A rapid method for extraction of cotton (*Gossypium* spp.) genomic DNA suitable for RFLP or PCR analysis[J]. Plant Molecular Biology Reporter, 1993, 11 (2):122-127
- [20] 朱海生,潘东明,林义章,张志忠,温庆放. 根癌农杆菌介导草莓遗传转化研究[J]. 核农学报,2008,22(1):36-40
- [21] Jefferson R A, Kavanagh T A, Bevan M W. GUS fusions: β -glucuronidase as a sensitive and versatile gene fusion marker in higher plants[J]. EMBO Journal, 1987, 6: 3901-3907
- [22] Lescot M, Déhais P, Thijs G, Marchal K, Moreau Y, Van de Peer Y, Rouzé P, Rombauts S. PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in

- silico analysis of promoter sequences[J]. Nucleic Acids Research, 2002, 30: 325-327
- [23] Pastuglia M, Roby D, Dumas C, Cock J M. Rapid induction by wounding and bacterial infection of an S gene family receptor-like kinase in Brassica oleracea[J]. Plant Cell, 1997, 9:1-13
- [24] Baker S S, Wilhelm K S, Thomashow M F. The 5'-region of Arabidopsis thaliana cor15a has cis-acting elements that confer cold-, drought-and ABA-regulated gene expression[J]. Plant Molecular Biology, 1994, 24: 701-713
- [25] Rushton P J, Torres J T, Parniske M, Wernert P, Hahlbrock K, Somssich IE. Interaction of elicitor-induced DNA-binding proteins with elicitor response elements in the promoters of parsley PR1 genes [J]. EMBO Journal, 1996, 15: 5690-5700
- [26] Liu Z B, Ulmasov T, Shi X, Hagen G, Guilfoyle T J. Soybean GH3 promoter contains multiple auxin-inducible elements[J]. Plant Cell, 6: 645-657
- [27] Pastuglia M, Roby D, Dumas C, Cock J M. Rapid induction by wounding and bacterial infection of an S gene family receptor-like kinase gene in Brassica oleracea[J]. Plant Cell, 1994, 9: 49-60
- [28] Dunn MA, White AJ, Vural S, Hughes MA. Identification of promoter elements in a low-temperature -responsive gene (blt4.9) from barley (*Hordeum vulgare* L.)[J]. Plant Molecular Biology, 1998, 38: 551-564
- [29] Yamaguchi-Shinozaki K, Shinozaki K. Arabidopsis DNA encoding two esiccation-responsive rd29 genes [J]. Plant Physiology, 1993, 101(3): 1119-1120
- [30] Rushmore TH, Morton MR, Pickett CB. The antioxidant responsive element. Activation by oxidative stress and identification of the DNA consensus sequence required for functional activity [J]. Journal of Biological Chemistry, 1991, 266: 11632-11639
- [31] Rouster J, Leah R, Mundy J, Cameron-Mills V. Identification of a methyl jasmonate-responsive region in the promoter of a lipoxygenase 1 gene expressed in barley grain[J]. Plant Journal, 1997, 11: 513-523
- [32] Pastuglia M, Roby D, Dumas C, Cock JM. Rapid induction by wounding and bacterial infection of an S gene family receptor-like kinase in Brassica oleracea[J]. Plant Cell Physiology, 1997, 9: 1-13
- [33] Diaz-De-Leon F, Klotz K L, Lagrimini M. Nucleotide sequence of the tobacco (*Nicotiana tabacum*) anionic peroxidase gene [J]. Plant Physiology, 1993, 101: 1117-1118
- [34] Henning J, Dewey R E, Cutt J R, Klessig D F. Pathogen, salicylic acid and development dependent expression of a beta-1,3 glucanase/GUS gene fusion in transgenic tobacco plant [J]. Plant Journal, 1993, 4: 481-493
- [35] Xu P, Blancaflor EB, Boosinck MJ. In spite of induced multiple defense responses, tomato plants infected with Cucumber mosaic virus and D satellite RNA succumb to systemic necrosis [J]. Molecular Plant Microbe Interaction, 2003, 16(6): 467-476
- [36] Kawai-Yamada M, Jin L, Yoshinaga K, Hirata A, Uchimiya H. Mammalian Bax-induced plant cell death can be down-regulated by overexpression of Arabidopsis Bax Inhibitor-1 (AtBI-1) [J]. Proceedings of the National Academy of Sciences, U S A, 2001, 98(21): 12295-12300
- [37] Watanabe N, Lam E. Arabidopsis Bax inhibitor-1 functions as an attenuator of biotic and abiotic types of cell death [J]. Plant Journal, 2006, 45(6): 884-894
- [38] Isbat M, Zeba N, Kim SR, Hong CB. A BAX inhibitor-1 gene in Capsicum annuum is induced under various abiotic stresses and endows multi-tolerance in transgenic tobacco [J]. Plant Physiology, 2009, 166(15): 1685-1693
- [39] 路 静,赵华燕,何亦昆,宋艳茹.高等植物启动子及其应用研究进展[J].自然科学进展,2004,14