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A novel *Gossypium barbadense* ERF transcription factor, GbERFb, regulation host response and resistance to *verticillium dahliae* in tobacco

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Ethylene-responsive factors (ERFs) are commonly considered to play important role in pathogen defense responses. However, only few of ERF members have been characterized in Sea island cotton (*Gossypium barbadense*). Here we reported a novel AP2/ERF transcription factors gene, named GbERFb which was cloned and identified from *Gossypium barbadense* by RACE. The expression of GbERFb was significantly up-regulated by ET, MeJA, SA, wounding, H₂O₂ and *Verticillium dahliae*. Bioinformatics analysis showed that GbERFb protein contain a conserved ERF DNA binding domain and a nuclear localization signal sequence. Further experiments demonstrated that GbERFb could be bind to GCC box cis-acting element and interact with the GbMAPKb (MAP kinase) directly in yeast. Over-expression of GbERFb in tobacco could increase the disease resistance to *V.dahliae*. These results suggest that the GbERFb, encode an AP2/ERF transcription factor, could be utilized for enhancing *V.dahliae* tolerance.



Figure 1: The full length cDNA sequence and deduced amino acid sequence of GbERFb. The ERF domain was single underlined, deduced nuclear localization signal was shown in the grey box, The predict phosphorylated sites were shown in the white boxes.

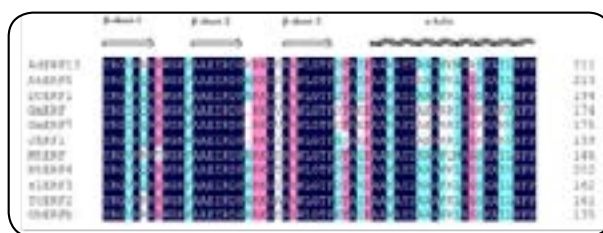


Figure 2: The conserved ERF domain of GbERFb with those of other closely related ERF proteins including AdERF13 (ADJ67442), AtERF5(NP_568679), DcERF1 (BAF75651), GmERF(AAQ10777), GmERF7(AEQ55266), JERF1(AAK95687),MIERF(XP_013467284), NtERF4(NP_001312428),siERF5(NP_001234512), cERF2(EOY22776). The three β -sheets and one α -helix of the ERF domain are indicated over the corresponding sequences.

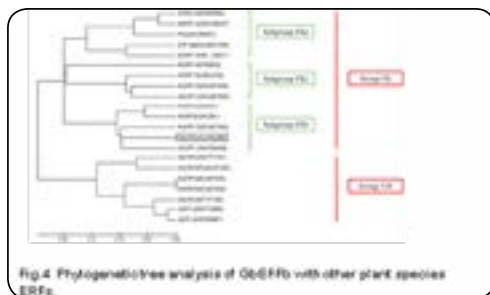


Fig.4 Phylogenetic tree analysis of GbERFb with other plant species ERFs.

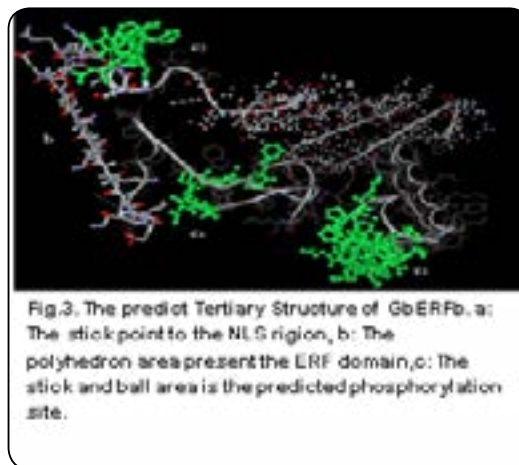


Fig.3. The predict Tertiary Structure of GbERFb. a: The stick point to the NLS region, b: The polyhedron area present the ERF domain,c: The stick and ball area is the predicted phosphorylation site.

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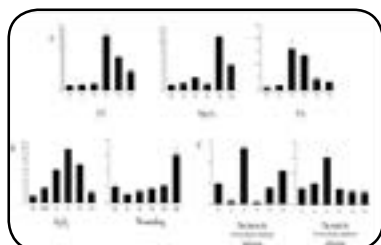


Fig.5. Expression patterns of GbERFb in Sea island cotton. A the effect of exogenous hormones treatments with ET, MeJA and SA in leaves, B the effect of exogenous H₂O₂ and wounding in leaves, C the effect of inoculating *Verticillium dahliae* in leaves and roots. Data are averages of three replicates by mean ±SD

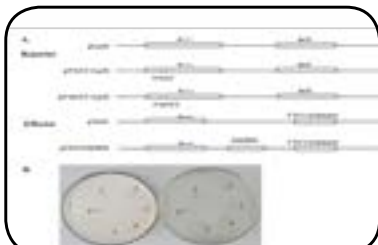


Fig.6. Analysis of the GbERFb transcriptional activation activity with the GCC sequence by yeast one hybrid. A: Diagram of the reporters, effectors construct used in the assays. PCYC1 the promoter of the minimal promoter of the yeast iso-1-cytochrome C gene, PGAL1 the promoter of galactose-inducible expression of gene, CYC1 terminator the terminator of the Cytochrome transcription gene. B: Comparison of the transactivation activity of GbERFb and other control by lifted filter in v yeast cells. 1 pLacZi /pYES3, 2 pLacZi/pYES3-GbERFb, 3p3×mGCC-LacZi/pYES3, 4 p3×mGCC-LacZi/pYES3-GbERFb, 5 p3×GCC-LacZi/pYES3, 6 p3×GCC-LacZi /pYES3-GbERFb.

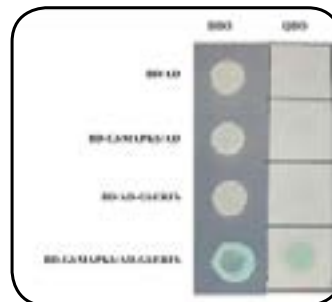


Fig.7. Interaction between the GbERFb and the candidate protein GbMAPKb by yeast two hybrid. The competent cell AH109 harbored the Gal4 DNA-binding domain (BD) and Gal4 activation domain (AD) fusion constructions. The pGbERFb-GADT7/pGbMAPKb-GBKT7 and other control were selected on SD medium lacking Leu and Trp (DDO) and interaction was selected on SD medium lacking Leu, Trp, His and Ade (QDO) for 5 days. All medium contained the X-a-gal.



Fig.8. Response of GbERFb transgenic tobacco plants to *Verticillium* wilt. The tobacco leaves were collected from non-transgenic tobacco line and three transgenic tobacco lines (L1-L3) of 4 weeks and inoculated with *Verticillium* wilt. The non-transgenic tobacco line acted as the CK.

Recent Publications

- Meng, X., Li, F., Liu, C., Zhang, C., Wu, Z. and Chen, Y. (2010) Isolation and Characterization of an ERF Transcription Factor Gene from Cotton (*Gossypium barbadense* L.). *Plant Mol Biol Rep*, 28, 176-183. doi:10.1007/s11105-009-0136-x
- Guo, W., Jin, L., Miao, Y., He, X., Hu, Q., Guo, K., Zhu, L. and Zhang, X. (2016) An ethylene response-related factor, GbERF1-like, from *Gossypium barbadense* improves resistance to *Verticillium dahliae* via activating lignin synthesis. *Plant Mol Biol*, 91, 305-318. doi:10.1007/s11103-016-0467-6
- Li, C., He, X., Luo, X., Xu, L., Liu, L., Min, L., Jin, L., Zhu, L. and Zhang, X. (2014) Cotton WRKY1 Mediates the Plant Defense-to-Development Transition during Infection of Cotton by *Verticillium dahliae* by Activating JASMONATE ZIM-DOMAIN1 Expression. *Plant Physiol*, 166, 2179-2194. doi:10.1104/pp.114.246694
- Cai, Y., Xiaohong, H., Mo, J., Sun, Q., Yang, J. and Liu, J. (2012) Molecular research and genetic engineering of resistance to *Verticillium* wilt in cotton: A review. *African Journal of Biotechnology*, 8, 7363-7372.
- Zhang, Z., Zhao, J., Ding, L., Zou, L., Li, Y., Chen, G. and Zhang, T. (2016) Constitutive expression of a novel antimicrobial protein, Hcm1, confers resistance to both *Verticillium* and *Fusarium* wilts in cotton. *Sci Rep-Uk*, 6, 20773. doi:10.1038/srep20773.

Biography

Hanshuang Zhang, professor, female, born in Hebei province. China. She has engaged in cotton breeding for over 20 years, and won the Technological Progress First Prize Award of Hebei province. Her team has successively presided and finished national or provincial cotton research projects more than 30 items, and breeding nearly 10 high-yielding, disease-resistant and high-quality cotton varieties, such as Jimian169, JiH 170, Ji178 etc.

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