

Characterization of an Organ Specific and Pathogen Responsive CC-NBS-LRR Gene from Cotton (*Gossypium hirsutum* L.)

ZHANG Bao-long, NI Wan-chao, YANG Yu-wen, SHEN Xin-lian

(*Institute of Agro-biotechnology, Jiangsu Academy of Agricultural Sciences, Zhongling Street 50, Nanjing 210014, China*)

Cotton diseases represent a major challenge to cotton growth. Cloning of a cotton pathogen response gene and promoter is of great importance to improve disease resistance. In this study, a full length CC-NBS-LRR gene (GHNBS) and its 5L flanking sequence have been cloned by race and tail PCR and further studied. The entire coding region is 2583 bp and encodes a polypeptide of 861 amino acids with 28% maximum homology to an R gene of *Arabidopsis* deposited in the GenBank. Semi-quantitative RT-PCR showed that GHNBS was expressed in floral buds, petals, phloem, roots, and leaves, and it has a greater expression pattern in roots and leaves. In order to study the function of this protein, prokaryotic expression was done to use the whole gene and partial gene fused with this tag protein. SDS-PAGE gel results showed that 99 kD and 66 kD proteins appeared after IPTG induction. Western blotting using His tag as an antibody proved that 99 kD and 66 kD proteins separately represent the fusion product of the two plasmid carriers. The 5' flanking sequence of GHNBS contained CAAT-box, TATA-box, several pathogen, SA, MeJA, and ethylene responsive elements identified by PLACE analysis. Different 5' promoter-deletion derivatives with the coding region of the *GUS* gene fusions were transformed into *Arabidopsis*. Histochemical localization showed strong staining in roots, stem phloem, and leaf veins. GHNBS promoter was up regulated after SA, ABA, MeJA, ethylene, and pathogen DC3000 treatments. This study established a good foundation for further research on the study of enzyme activity of this gene and interaction of different domains.