EXPLORING THE CLCrV-VIGS FOR MODULATING ACTIN AND CHITINASE GENE EXPRESSION IN COTTON

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Abstract

A vector for virus induced gene silencing (VIGS) based on the *Cotton leaf crumple virus* (CLCrV) DNA-A, was previously constructed and shown to silence host genes when biolistically delivered to cotton seedlings (Idris and Brown, 2004; Idris et al., 2010). The CLCrV VIGS vector is approximately 1.9 Kbp in size, and comprises the CLCrV DNA-A component that lacks most of the coat protein (CP) ORF, and the DNA-B component, which has not been modified (wild type). In this study, we used the CLCrV VIGS vector to transiently silence cotton genes involved in fatty acid synthesis and adaptation to cold stress, fiber initiation, fiber elongation, and cellulose synthesis in primary and secondary cell walls in upland cotton (*Gossypium hirsutum*): 1) *fatty acid desaturase* (FAD2-4); 2) *actin* (GhACT1); 3) *two chitinase like genes* (GhCTL1 and GhCTL2). ClustalV alignment of the cotton mRNAs was carried out using available sequences of each target gene. Aligned sequences were used to design specific primers for each gene. Total RNA was isolated from cotton (leaves, roots, cotton boll) and used as template to amplify (by RT-PCR) fragments of the different target genes. These fragments were cloned into the VIGS CLCrV vector. VIGS CLCrV carrying the FAD-2-4, ACT1, CTL1 and CTL2 gene fragments were constructed and sequenced to confirm the integrity of the cloned gene fragment in relation to published sequences available in GenBank used to guide primer design. Cotton seedlings (*G. hirsutum* cv Deltapine 5415) were bombarded with 1.1-µm diameter tungsten microprojectiles (BioRad) coated with a mixture of 1 µg each of the following CLCrV DNA-A plasmid and 1 µg of the wild type CLCrV DNA-B component: (1) VIGS CLCrV –FAD2-4, (2) VIGS CLCrV –ACT1 , (3) VIGS CLCrV –CTL1 (4) CLCrV wild type virus, and (4) VIGS 'empty' (no insert control) plasmid. Inoculated plants were maitained in the growth room at 28°C with a 16/8h photoperiod and ~ 80% RH. Mock-inoculated (water without of DNA template), virus-free cotton plants were maintained as controls. Results showed that cotton plants inoculated with the VIGS vector carrying the FAD2-4, ACT1, CTL1 and CTL2, each co-inoculated with CLCrV DNA B dimer developed a range of mild symptoms four weeks post inoculation (PI). In comparison, wild type CLCrV-inoculated plants developed typical, severe CLCrV disease symptoms about 12 days PI. Analysis of gene expression and assessment of gene silencing is underway.