

RNAI CONSTRUCT OF A P450 GENE BLOCKS AN EARLY STEP IN HEMIGOSSYPOLONE AND GOSSYPOL SYNTHESIS IN TRANSGENIC COTTON PLANTS

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Abstract

Naturally occurring terpenoid aldehydes from cotton such as gossypol, hemigossypolone, and heliocides, are important components of disease and herbivory resistance in cotton. These terpenoids are predominately found in the glands. Differential screening identified a P450 cDNA clone (GHC28) that only hybridized to mRNA from glanded cotton and not from glandless cotton. The protein encoded by GHC28 is predicted to be a P450 based on sequence analysis. The predicted protein shares 48% amino acid identity with cytochrome P450 82A3 (P450 CP6, the closest match, GenBank accession number O49858), and contains a Pfam P450 domain, including common motifs and the heme-binding site found in eukaryotic P450s. We transformed *G. hirsutum* with an RNAi construct specific to this gene and generated 6 transgenic plants stemming from at least four independent transformation events. Using HPLC, we found that plants containing the RNAi construct have less than 10% of wild-type (WT) levels of hemigossypolone and heliocides and a 70% reduction in WT gossypol levels in the terminal leaves. The Lacinilene-related compounds were identified in the leaves and roots of RNAi plants. Volatile terpenes extracted from WT and RNAi leaves were analyzed by GC-MS and a novel terpene (MW: 218) was identified in RNAi leaf extracts. ¹H and ¹³C NMR analysis identified this compound as δ -cadinen-2-one. Simple double bonds rearrangement can transform this compound into 7-hydroxycalamenene, a lacinilene pathway intermediate. δ -cadinen-2-one can be derived from δ -cadinene via yet an unidentified intermediate, 2-hydroxy- δ -cadinene. Our results indicate that RNAi construct of GHC28 is blocking the synthesis of desoxyhemigossypol, and there is likely cross-talk between the lacinilene C and gossypol pathways.