ANALYSIS OF GOSSYPOL AND RELATED TERPENOIDS IN ANTISENSE TRANSGENIC COTTON PLANTS

K. S. Rathore
Crop Biotechnology Center & Dept. of Soil & Crop Sciences
Texas A&M University
College Station, TX
R. D. Stipanovic
Southern Crops Research Laboratory, USDA, ARS
College Station, TX

Abstract

Elimination of gossypol from cottonseed is highly desirable because it will both enhance the feed value of the meal and reduce the processing cost of cottonseed oil. We are evaluating a transgenic approach to reduce gossypol in the seed without affecting its levels in other mature parts of the plant where it serves a beneficial function. To this end, we are making use of antisense technology to down-regulate the expression of the delta-cadinene synthase gene. delta-Cadinene synthase is a key enzyme involved in the biosynthesis of gossypol. We made antisense, binary vector constructs using the two cDNA clones for delta-cadinene synthase isolated from *G. arboreum* by Chen *et al*. (Arch. Biochem. biophys. 324:255, 1995). For the initial testing of the antisense strategy, we have used the CaMV 35S promoter to drive the antisense genes. *nptII* gene was used as a selectable marker. We have utilized *Agrobacterium tumefaciens*-mediated transformation using strains LBA4404 and EHA105. Cotyledon and hypocotyl of var. Coker 312 were used as tissue explants for cocultivation. We have regenerated over forty plants from kanamycin resistant calli via somatic embryogenesis. These have been successfully transferred to soil and most of them are fertile. PCR and Southern analysis on the DNA from these plants confirmed their transgenic status. Leaves from plants that were expressing the *nptII* gene are being analyzed for levels of gossypol and related compounds that are derived via the same biosynthetic pathway. Leaves from two plants when tested initially, showed reduced levels or undetectable levels of gossypol, hemigossypolone and heliocides (H1-H4). However, over a period of twelve months, these plants began to show an increase in the levels of these terpenoids. We are further investigating this interesting result to check whether some type of trangene or promoter silencing mechanism is responsible for the increase in terpenoid levels.

We have isolated a delta-cadinene synthase cDNA clone from a seed-specific library of *G. hirsutum*. We have constructed two antisense binary vectors utilizing this gene driven by two different seed-specific promoters (alpha-globulin promoter from *G. hirsutum* and phaseolin promoter from *Phaseolus vulgaris*). Several transformation experiments have been carried out using these new constructs. Transgenic plants recovered from these experiments will be grown to maturity and seeds will be analyzed for gossypol and related terpenoids.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:445-445 (1999)
National Cotton Council, Memphis TN