

**EVALUATION OF DISEASE RESISTANCE IN COTTON PLANTS WITH REDUCED LEVELS OF METHYLATED PHYTOALEXINS****T. A. Wagner****Department of Plant Pathology/Texas A&M****College Station, TX****L. S. Puckhaber****A. A. Bell****USDA/ARS/ICCDRU****College Station, TX****C. Magill****Department of Plant Pathology/Texas A&M****College Station, TX****J. Liu****USDA/ARS/ICCDRU****College Station, TX****Abstract**

The production of sesquiterpenoids in cotton tissues contribute to the plant's constitutive and inducible defense against pathogens. In roots, there is constitutive expression of gossypol, and there is the inducible expression of desoxyhemigossypol (dHG), desoxyhemigossypol-6-methyl ether (dMHG), hemigossypol (HG), and hemigossypol-6-methyl ether (MHG). Of these compounds, dHG is the most toxic, followed by dMHG, then HG, and lastly MHG, with the non-methylated compounds being more toxic to fungal spores and hyphae than their methylated counterparts. These inducible compounds are also considered to be pathway intermediates leading to gossypol, so they can be found in roots at low levels without induction. We previously identified, cloned and sequenced the desoxyhemigossypol-6-*O*-methyltransferase (dHG-6-OMT) gene from *Gossypium barbadense* that is responsible for the conversion of dHG to dMHG. We hypothesized that knocking down the expression of dHG-6-OMT in *G. hirsutum* via RNAi would lead to an increase of dHG and HG (most toxic phytoalexins) in roots and that these plants would show increased resistance to soil borne pathogens. We generated eight independently transformed RNAi lines. All the lines suppressed the methylation of sesquiterpenoids in roots resulting in methylation between 0 and 58% of WT levels. Two RNAi lines were fertile, and homozygous WT and RNAi seed stocks were generated for further characterization. Data for one line is presented here. In unchallenged plant roots, the amount of methylated compounds in RNAi-containing plants is reduced to 3% of WT levels. Although HG, dHG, and gossypol are increased 1.6, 1.8, and 1.3X respectively in the RNAi-containing plants compared to WT, the total amount of phytoalexins present in roots is reduced almost by half in the RNAi-containing plants. Thus, feed-back regulation may prevent the continual synthesis and build-up of HG and dHG. HGAL (thought to be a breakdown product of dHG) is increased 28X. Next, we tested RNAi plants for increased resistance to Fov race 4. Homozygous WT and RNAi cotton plants were either mock inoculated or inoculated with three different Fov race 4 strains (Ca9 - highly virulent, China 869 - less virulent, and China 970 - moderately virulent) using a soil drench method. Plant measurements were taken at 6 weeks post inoculation. We did not observe any significant differences between the RNAi-containing plants and the WT sibs. Thus, the amount and/or composition of gossypol-related compounds in roots did not influence plant resistance to Fov race 4. Currently, we are testing if dHG can be induced to higher levels in the RNAi plants compared to the WT siblings by looking at induction of these compounds in the vascular tissue of stems; a tissue with no constitutive expression of this compound. We also plan to use bioassays with vascular competent Fov strains that utilize nematodes for pathogenesis. Thus, we will gain insight into the role of methylation in the synthesis of gossypol-related compounds and the involvement of these compounds in disease resistance *in planta*.