Hemigossypol, A Constituent in Developing Glanded Cottonseed (Gossypium hirsutum)

Tanya A. Wagner, Jinggao Liu*, Robert D. Stipanovic, Lorraine S. Puckhaber and Alois A. Bell

Southern Plains Agricultural Research Center, Agricultural Research Service

U. S. Department of Agriculture, 2765 F and B Road, College Station, TX 77845, USA

Supporting Information

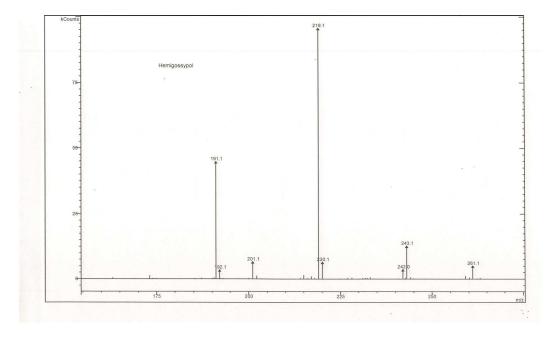


Figure S1. Hemigossypol mass spectum (MS/MS) from the LCMS analysis of an extract from 35 dpa Coker 312 embryos. Instrument Varian 500-MS Ion Trap Mass Spectrometer. LCMS details are given in the **MATERIAL AND METHODS** section.

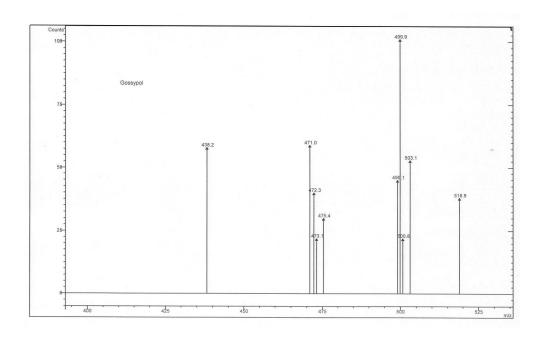


Figure S2. Gossypol mass spectum (MS/MS) from the LCMS analysis of an extract from 35 dpa Coker 312 embryos. Instrument Varian 500-MS Ion Trap Mass Spectrometer; Electrospray ionization; Chamber 50° C; Nebulizing gas presue 25 psi; Drying gas pressure 15 psi; Needle 5000 V, 2.7 μ Amp; Shield spary 600 V; Drying gas $350 ^{\circ}$ C; Capillary 80 V; rf 70%; CID 1.5 V.

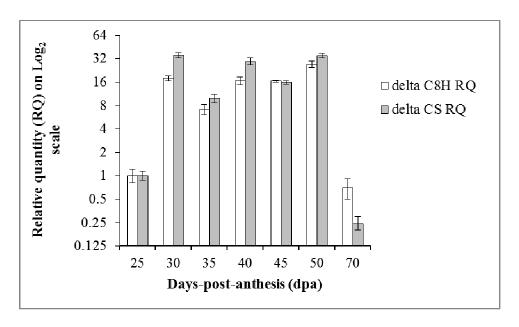


Figure S3. Time course during cotton boll development showing the relative quantities of (+)- δ -cadinene synthase (delta CS; grey bars) and (+)- δ -cadinene-8-hydroxylase (delta C8H; white bars) mRNA in developing embryos. For measurements of (+)- δ -cadinene synthase and (+)- δ -cadinene-8-hydroxylase mRNA accumulation, total RNA was extracted from Coker 312 embryos harvested at 25, 30, 35, 40, 45, 50, and 70 dpa during the winter. These embryos were from different bolls than those used for the qPCR experiment shown in Figure 3. The mRNA levels of (+)- δ -cadinene synthase and (+)- δ -cadinene-8-hydroxylase were determined by qPCR. This qPCR was done separately from the qPCR results shown in Figure 3. GhMZA (clathrin adaptor medium subunit) and GhPTB (polypyrimidine tract-binding protein) served as internal reference genes. The data are presented as relative quantities (RQ) with the levels at 25 dpa set to 1. The RQ minimum and maximum represent 1 standard deviation of the three technical replicates.

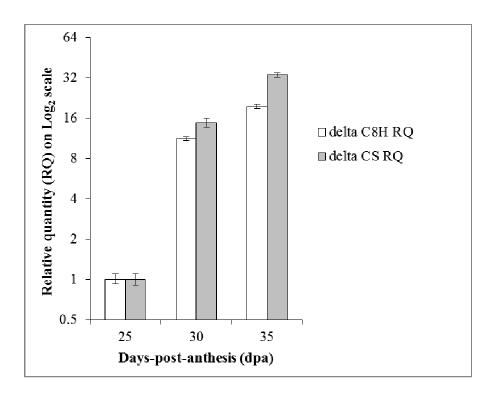


Figure S4. Time course during cotton boll development showing the relative quantities of (+)-δ-cadinene synthase (delta CS; grey bars) and (+)-δ-cadinene-8-hydroxylase (delta C8H; white bars) mRNA in developing embryos. For measurements of (+)-δ-cadinene synthase and (+)-δ-cadinene-8-hydroxylase mRNA accumulation, total RNA was extracted from Coker 312 embryos harvested at 30 and 35 dpa during the winter. These embryos were from different bolls than those used for the qPCR experiment shown in Figure 3 and Figure S3, but RNA extraction, reverse transcription, and qPCR was completed in the same experiment that was used to generate Figure 3 data. The mRNA levels of (+)-δ-cadinene synthase and (+)-δ-cadinene-8-hydroxylase were determined by qPCR. GhMZA (clathrin adaptor medium subunit) and GhPTB (polypyrimidine tract-binding protein) served as internal reference genes. The data are presented as relative quantities (RQ) with the levels at 25 dpa (the same data points were used as in Figure 3) set to 1. The RQ minimum and maximum represent 1 standard deviation of the three technical replicates.