An optimized gossypol high-performance liquid chromatography assay and its application in evaluation of different gland genotypes of cotton

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A comparative study on gossypol content of various genetic types of pigment glands of cotton varieties was conducted through an optimized high-performance liquid chromatography (HPLC) on a C18 column (4.6 mm × 250 mm, 5 μm particle) with methanol–0.5% acetic acid aqueous solution, 90 : 10 (v/v), as mobile phase, at a flow rate of 0.8 ml/min and UV detection at 254 nm. The method was shown to be highly reproducible, with precision [as relative standard deviation (RSD)] and accuracy [as relative mean error (RME)] < 10%, both intra-day and inter-day. Absolute recoveries were > 94%. The results revealed major differences among the different gland varieties or species of cotton, including the special and ordinary glandless and glanded *Gossypium hirsutum*, *G. barbadense*, and displayed the precious resources of different glands of extraordinary cotton.

1. Introduction

Gossypol (figure 1), a polyphenolic binaphthyl dialdehyde stored in the pigment glands of cotton, is not only an important resistant substance for cotton but also an important phytochemical component of immense interest due to its several biological properties including anti-cancer, antimicrobial, anti-HIV, anti-oxidation and male contraceptive (Pharmacia Institute of China Medicine Academy 1995).

Gossypol content of cotton is mainly dependent on different genetic types of pigment glands. The glanded cotton normally contains gossypol in both seeds and plants that is toxic to human and non-ruminant animals. Ordinary glandless cotton contains no or low-gossypol in seeds, roots, stems as well as leaves, but its resistance to diseases, pests and even rats is reduced greatly (Zhang et al 1999, 2001). Therefore a cotton variety which is characterized by the presence of glanded roots, leaves and stems (to maintain the resistance trait) but glandless seeds (for safe utilization) is highly desired. Biologists and agronomists have long been studying the glanded characters and the glandless characters of cotton as well as their gossypol content (Wang et al 1985; Hron et al 1990, 1999; Xiang et al 1993; Yang et al 1995; Yuan et al 1999). There are many methods to determine gossypol, such as spectrophotometry, the non-aqueous titrimetric method, gas chromatography and high-performance liquid chromatography (HPLC) (Pharmacia Institute of China Medicine Academy 1995; Yang et al 1995). Each of these methods can reflect the relative levels of gossypol. However, the chemical methods are not very specific and gossypol analogs give positive values resulting into significant overestimation. In contrast, the HPLC method is more accurate, effective and specialized (Nomeir 1982; Wang et al 1985; Yang et al 1995). Recently, Chinese scientists have bred some special varieties and genotypes of cotton

Keywords. Cotton (*Gossypium*); gossypol; high-performance liquid chromatography (HPLC); pigment gland

Abbreviations used: HPLC, High-performance liquid chromatography; ICIS, Industrial Crop Institute of Sichuan Academy of Sciences; RME, relative mean error; RSD, relative standard error.
(Zhang et al 2001, 2002). There is no report yet about their gossypol content measured by HPLC. In this experiment the comparative studies on gossypol content of various genetic types of pigment glands of cotton, including the new special and ordinary glandless and glanded *Gossypium hirsutum* and *G. barbadense*, were conducted through HPLC. The purpose is to stimulate the extensive exploitation of these resources, to provide a basis for isolating specific genes and to help understand the molecular mechanisms involved.

2. Materials and methods

The cotton materials tested were divided according to their pigment gland and species, into 6 groups (numbered 1–16, figure 3): (a) three glanded varieties of *G. barbadense* – Mexico 8390 (No. 1), 5593Φ (No. 2) and 72-69 (No. 3); (b) four glandless varieties of *G. hirsutum* – Wufen383 (No. 4), Jijiaowufen (No. 5), SP21 (No. 6) and Xiangwu 93 (No. 7); (c) one special new cv. Xiangmian 18 (No. 8) with glandless seed but glanded root, leaf and stem, obtained from National Center of Hybrid Cotton Development and Extension, Hunan (Zhang et al 2001); (d) two special glanded germplasms of *G. hirsutum* resistant to many races of Verticillium wilt dahlieae, Chuan 737 (No. 9) and Chuan 2802 (No. 10) bred by Industrial Crop Institute of Sichuan Academy of Agricultural Sciences (ICIS) (Li et al 1996; Zhang and Cai 2002); (e) three glanded new resistant varieties of *G. hirsutum* Chuanmian 239 (No. 11), Chuanmian 243 (No. 12), Chuanmian 65 (No. 13) and 1 susceptible Chuanmian 45 (No. 14) to Verticillium wilt (Zhang and Cai 2002); and (f) two Bt transgenic lines or varieties, Zhongmiansuo 30 (No. 15), RP4-4 (No. 16) obtained from Cotton Institute of Academy of Agricultural Sciences of China. Materials in groups (a–e) were obtained from ICIS.

2.1 Chemicals

Acetonitrile and methanol (HPLC-grade) were purchased from Tedia (Fairfield, OH, USA). All other reagents (analytical grade) were purchased from Beijing Chemical Company (Beijing, People’s Republic of China). Calibration gossypol was purchased from Sigma (USA).

![Figure 1. Structure of gossypol.](image)

2.2 Sample preparation

Samples were prepared according to Wang et al (1985). The dried and powdered samples (about 0.1 g) were macerated with acetone for 16 h, then filtered through 0.45 µm micro-filter membrane and the residue washed. The extract was evaporated to dryness under vacuum. The residue was resuspended in 1% HOAc-CHCl₃ solution to 25 ml.

2.3 High-performance liquid chromatography

HPLC was performed on a Waters system (Milford, MA, USA) 2487 dual-wavelength absorbance detector, a Waters 515 pump, and a Waters Rheodyne 7725i consisting of manual injector (Wang et al 1985). Compounds were separated on a Hewlett-Packard (Palo Alto, CA,USA) Zorbax Eclipse XDB-C18 column (4.6 mm × 250 mm, 5 µm particle) by a Supelco (Bellefonte, PA, USA) C₁₈ pre-column (4.6 mm × 20 mm, 5 µm) (Wang et al 1985). The mobile phase was 90 : 10 (v/v) methanol-0.5% acetic acid aqueous solution at a flow rate of 0.8 ml/min. The wavelength for UV detection was 254 nm. A 5 µl sample was injected. The assays were performed at room temperature.

2.4 Calibration procedure

Calibration curves were produced by analysis of solutions containing 3, 6, 15, 30, 45, 60 µg/ml standard gossypol (purity, > 99%) in chloroform containing 1% acetic acid.

2.5 Precision, accuracy, and limit of detection

The studies were performed with solutions containing gossypol at concentrations of 3 µg/ml (low), 15 µg/ml (medium), and 60 µg/ml (high). The solutions were stored in dark at room temperature, 4°C, and at –20°C. The interday study was performed over a period of 15 days on days 0, 3, 6, 9, 12 and 15. The equations used to calculate relative standard deviation of the mean (RSD) and relative mean error (RME) were: RSD (%) = [standard deviation/mean] × 100; RME (%) = [(measured value – true value)/true value] × 100. RSD and RME were used as measures of precision and accuracy, respectively. Limit of detection was calculated as the lowest concentration of standard for which both RSD and RME were less than 20% (Causon 1997).

2.6 Recovery

Recovery was studied by use of three sets of samples: (i) standard solutions containing 3 µg/ml, 15 µg/ml, and 60 µg/ml, (a); (ii) 0.2 g sample, (b); and (iii) standards added to...