Asian Journal of Basic Science & Research
Volume 8, Issue 3, Pages 89-94, July-September 2026

Evaluation of Seed and Static Culture Systems for Gossypol Production in *Gossypium* Cultivars

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DOI: https://doi.org/10.38177/ajbsr.2026.8306

Article Received: 27 May 2026 *Article Accepted: 28 July 2026* *Article Published: 16 August 2026*

ABSTRACT

In the present study, seeds and static cultures of three *Gossypium* cultivars (GA, RG-8 and Pusa 8-6) were analyzed for gossypol. Seeds and callus tissues of all three *Gossypium* cultivars were dried. Each sample was extracted twice with methanol saturated with sodium bisulfite. The extracts so obtained were analyzed by thin-layer chromatography (TLC). Results demonstrated that seeds and 18-month-old static cultures of *Gossypium* contain secondary metabolite 'gossypol'. These findings indicate that seeds and 18-month-old static cultures of *Gossypium* cultivars retain the biosynthetic potential to produce gossypol.

Keywords: Gossypol; G. Arboreum (RG-8); G. Hirsutum (GA, Pusa 8-6); Static Cultures; Seeds; Thin-Layer Chromatography; Callus; Extracts.

1.0. Introduction

Cotton is a major cash crop cultivated for its fibre, which serves as a primary raw material for the textile industry. The plant, a shrub belonging to the family Malvaceae, naturally grows as a perennial [1]. To improve fibre quality, numerous varieties of Gossypium have been developed through conventional breeding as well as genetic engineering [2]. Gossypium is also an important oil-yielding plant. However, during industrial-scale extraction of cooking oil from the seeds, gossypol is released in its free form, rendering the oil toxic [3].

Gossypol is a prominent polyphenolic triterpenoid yellow pigment [4]. It has been reported as an important secondary metabolite that provides resistance to cotton plants by attracting, repelling, or poisoning insects such as Spodoptera and Heliothis [5]. In addition, it exhibits antifungal and antibacterial activity against pathogens including *Verticillium dahliae, Rhizoctonia solani,* and *Xanthomonas campestris* [6]. Higher gossypol content is associated with greater resistance in plants [7]. Gossypol is also regarded as an effective oral contraceptive in humans [8], monkeys, boars, dogs, bulls, and hamsters [9-11], as well as in guinea pigs and rats [12-14]. It reduces sperm counts and causes sperm malformation, immobility, and inviability. Furthermore, gossypol has been investigated as a novel target for lymphoma treatment [15] and for its antiviral properties [16,17]. Its effects on in vitro pollen germination, pollen tube growth, and male gametogenesis in Impatiens balsamina have also been reported [18]. Both total and free gossypol have been detected in seeds of many Bt and non-Bt cotton varieties [19]. Plant tissue culture represents a potent alternative to whole plants as a source of important secondary metabolites [20].

1.1. Study Objectives

Considering the significance of gossypol, current study was planned with following objectives:

1. To screen seeds and static cultures of G. arboreum (RG-8) and G. hirsutum (GA and Pusa 8-6) for gossypol production.

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2. To detect gossypol in static cultures.
3. To analyze gossypol using TLC.

2.0. Methodology

2.1. Tissue Culture

Seeds of Gossypium arboreum var. RG-8, G. hirsutum var. GA, and Pusa 8-6 were delinted with concentrated H_2SO_4, thoroughly washed with water, sterilized for 2–3 min in 0.2% mercuric chloride solution, and rinsed with sterile water before germination in sterilized Petri dishes. Two to a few days after germination, seedlings were cut into 0.5–1 cm segment of roots, cotyledons, and hypocotyls, which were cultured on MS medium [21] supplemented with different concentrations and combinations of indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and kinetin. The pH of the medium was adjusted to 5.6–5.8 and solidified with 8% agar. Approximately 30–35 ml of medium was dispensed into 100 ml Corning flasks and autoclaved at 121 °C and 18 lbs pressure for 20 min. Inoculations were performed under aseptic conditions in a laminar flow cabinet. Cultures were maintained in the dark at 27 ± 1 °C. Cultures initially raised on MS medium were later transferred to LS medium [22], and stock cultures were maintained on this medium by frequent subculturing. Six-week-old cultures were analyzed for gossypol estimation.

2.2. Isolation of Gossypol

Seeds and callus tissues of all three Gossypium cultivars were dried at 60 °C for 72 hours to determine dry weight. Each sample was extracted twice with methanol saturated with sodium bisulfite. The extracts were evaporated to dryness under vacuum at temperatures below 50 °C in the dark. The residue of each sample was dissolved in a small amount of ethyl acetate, and aliquots were analyzed by thin-layer chromatography (TLC). Several solvent systems were tested for the separation of gossypol, including hexane–ether–formic acid (65:35:1), benzene–chloroform–methanol–acetic acid (130:60:10:2), petroleum ether–ethanol (1:1), benzene–chloroform–ethyl acetate (40:10:3), petroleum ether–chloroform–ethyl acetate (30:15:5), and cyclohexane–chloroform–ethyl acetate (30:15:5). Among these, the benzene–chloroform–methanol–acetic acid system (130:60:10:2, v/v) was found to be the most effective [23]. Gossypol was visualized on TLC plates by spraying with a solution consisting of equal parts 5% phloroglucinol in 95% ethanol and concentrated HCl.

2.3. Preparative TLC

One spot (Rf. 0.61) coinciding with that of gossypol was separately eluted with preparative TLC (Silica gel 'G' dry thickness 4-.5 mm, solvent system benzene-chloroform-methanol-acetic acid, 130:60:10:2) from unsprayed plates along with silica gel. Each of the mixture was eluted with 95% ethanol dried in vaccum and redissolved in 95% ethanol. Intensity of the colour of each extract was measured at 550 nm.

3.0. Results And Discussion

The presence of gossypol in three cultivars of Gossypium arboreum var. RG-8 and G. hirsutum var. GA and Pusa 8-6 was confirmed by thin layer chromatography (Fig.1). These findings indicate that seeds and 18-month-old static cultures of Gossypium cultivars retain the biosynthetic potential to produce gossypol. Gossypol has also been detected in leaves and seeds from some wild malvaceae species [24] commercial cattle feed [25] and reported as a toxic compound in cottonseed products [26]. Meyer et al. [27] reported gossypol in seeds of G. hirsutum cultivars Acala 1517-70 and OR 19, while Baksha et al. [28] reported gossypol content in the seeds of five cotton cultivars. Nag et al. [29] detected gossypol in seeds and calli of six cotton cultivars, namely AKA 8401, CINA 316, CINA 343, CNH 120 MB, CNH 36, and LRK 516. Sharma et al., [30] reviewed various bioactivities viz. anticancer, antimicrobial, and antioxidant effects of gossypol. Studies also showed that gossypol toxicity could be reduced the synthesis of derivatives [30].

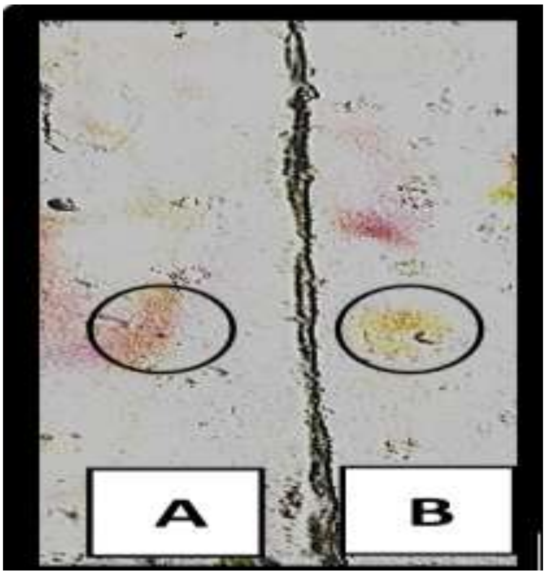

Figure 1. Thin-layer chromatography (TLC) plate showing spots of standard gossypol (A) and crude extract (B).

4.0. Conclusions and Future Recommendations

These findings indicate that seeds and 18-month-old static cultures of Gossypium cultivars retain the biosynthetic potential to produce gossypol. Further studies will include:

- Effect of hormones or adjuvants on gossypol production.
- Altering culture conditions to increase gossypol yield.
- Use advanced methods to quantity gossypol.
- Develop commercial scale culture approaches for gossypol production

Declarations

Source of Funding

This study did not receive grant from any funding agencies in the public, commercial, or not-for-profit sectors.

Consent for Publication

The authors declare that they consented to the publication of this study.

Competing Interests Statement

The authors declare that they have no conflict of interest.

Authors' contributions

AK carried out major experimental work. Both AK and MK carried out analysis and wrote the manuscript.

Informed Consent

Not Applicable

Availability of data and material

Not Applicable

Institutional Review Board Statement

Not Applicable

Ethical Approval

Not Applicable

Declaration of Artificial Intelligence

Authors have used AI such as ChatGPT of minor editing or formatting of references.

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