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**HISTOCHEMICAL LOCALIZATION OF POLYPHENOLIC ALDEHYDES IN
GOSSYPIUM BARBADENSE**

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ABSTRACT

Histochemical study was carried out to localize polyphenolic terpenoid aldehydes and fixed oil in healthy seeds, stems, leaves and roots of *Gossypium Barbadense L. var. Giza 86*. In all examined organs, polyphenolic terpenoid aldehydes and fixed oil were mainly detected inside lysigenous glands. In young Leaves and roots, polyphenolic aldehydes were also observed as fine particles inside the cytoplasm of some parenchymatous cells around glands. Lysigenous terpenoid-containing glands were noticed in all tap root regions except the apical 3 cm. The number of glands increased with increasing distance from the root tip. This may explain why the antimutagenic activity of gossypol does not affect the growing tip of the plant.

KEYWORDS

Gossypium Barbadense, Histochemistry, Cotton, Polyphenol and Gossypol.

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INTRODUCTION

Gossypium species are characterized by their metabolic ability to synthesize a unique array of polyphenolic aldehydes, the most famous of them is gossypol, these compounds include sesquiterpenes (hemi gossypol, 6-Methoxyhemigossypol), sesquiterpenequinones (hemigossypolone, hemigossypolone-7-methyl ether), dimeric sesquiterpene (gossypol, 6-methoxygossypol, 6, 6-dimethoxygossypol) and sesterterpenes (heliocides H 14 and Ba4). It was reported that these compounds are responsible for plant resistance to insects, fungi, bacteria and nematodes. However, deposition of these aldehydes

in cultivated cottonseed renders cottonseed meal and oil toxic to non-ruminant animals and humans (Brubaker *et al.*, 1996)¹. Triplett *et al.*, 2008² reported that induction of hairy root cultures from *Gossypium hirsutum* and *Gossypium barbadense* to produce gossypol and related compounds. Analysis of [*Gossypium capitis-viridis* × (*G.hirsutum* × *G.australe*)²].

Trispecific Hybrid and Selected Characteristics

Recently, it is proved that gossypol effectively depressed the mitotic process in both mammalian (Ligueros *et al.*, 1997) and plant cells (Houssen *et al.*, 1999). However, a question was raised why gossypol does not interfere with the mitotic process in cotton plant. So, the localization and distribution of gossypol and other polyphenolic terpenoid aldehydes in cotton plant may help in understanding such phenomenon. Mace *et al.*, (1974) used histochemical techniques to localize such compounds in roots of *Gossypiumhirsutum L. var. Acala 4-42*. This paper reports the histochemical localization of such terpenoids on seeds, stems, leaves and roots of *Gossypium Barbadosense L. var. Giza 86*.

MATERIAL AND METHODS

Plant materials

Seeds, stems, leaves and roots of *Gossypium Barbadosense L. var. Giza 86* were used for the preparation of free-hand sections for histochemical studies.

Histochemical reagents

The following reagents may be used to localize polyphenolic terpenoid aldehydes in fresh plant sections. Unless otherwise specified, fresh sections were incubated in the reagent for 10 minutes and rinsed in distilled H₂O before observations were recorded.

1. A saturated solution of SbCl₃ in 60% HClO₄ (Mace, 1974).
2. A saturated solution of 2, 4-dinitrophenylhydrazine (DNP) in 2N HCl (Mace, 1974).
3. A 10% FeCl₃ solution in distilled H₂O.
4. A 0.1 M NaOH solution.

5. A 66% H₂SO₄ in distilled H₂O.
6. A solution of 1% phloroglucinol in a mixture of 95% ethanol: conc. HCl (1:1 v/v).
7. A 1% solution of sodium borohydride (NaBH₄) in 1% aqueous NaH₂PO₄ was prepared to make in situ reduction of aldehyde group of gossypol-like compounds.

Fixed oil and protein were detected using the following reagents

1. Sudan III reagent was prepared by dissolving 0.01 g in 5 ml of 90% ethanol followed by 5 ml glycerin (British pharmacopeia, 1998).
2. Ninhydrin-stannous chloride reagent was prepared by dissolving 1.5 g ninhydrin and 0.05 g stannous chloride in 50 ml ethanol and completing the volume to 100 ml with acetate buffer pH 5 (British pharmacopeia, 1998).

RESULTS AND DISCUSSION

Although, cottonseed is a rich source of edible oil (ca. 20%) and protein (20-25%), its utilization as food is restricted because of toxic polyphenolic terpenoids in pigment glands of the seed embryo. On 1950, cotton with glandless seeds were produced. Many reports, however, showed that glandless cottons are more susceptible to insects (Bell *et al.*, 1977)³. It was stated that gossypol and related aldehydes are responsible for plant resistance to insects (stipanovic *et al.*, 1977). Histochemical localization of these natural insecticides in different plant tissues is highly important from the phytopathological point of view. It may give a guide for tracing and removing such compounds without affecting the high oil and protein contents.

Recently, it was stated that gossypol can markedly decrease the mitotic rate of plant cells (Houssen *et al.*, 1999). Oddly enough, cotton plant contains a high concentration of gossypol without being affected by its mitodepressant effect. However, scant attention was given to the localization of gossypol and other related aldehydes in cotton plant. Mace *et al.* (1974) reported histochemical

techniques to localize such compounds in roots of *Gossypiumhirsutum* L. var. Acala 4-42. They reported that terpenoids were localized in epidermis and in scattered cortical parenchyma cells of the healthy tap root of *Gossypiumhirsutum* L. var. Acala 4-42. Also, Mace and his group (Mace *et al.*, 1974) did not detect these compound in the first 3 cm back of the root tip. They suggested that the absence of these terpenoids in the root tip zone might be related to the susceptibility of this zone to nematode penetration.

Therefore, this study was conducted to provide more details about the histochemical localization of such compounds in seeds. Stems, leaves and roots of *Gossypiumbarbadense* L. var. Giza 86.

Gossypol and related compounds are located in pigment glands that present in stems, leaves, roots and seed embryos. This finding was manifested by the action of histochemical reagents on the color of pigment glands. A yellowish brown color of the pigment glands is seed embryo mounted with distilled water was observed Figure No.1. The yellowish brown color was turned black upon treatment with 10% FeCl₃ (Figure No.2), orange-red with either DNP or Phloroglucinol reagent (Figure No.3 and 4), reddish brown with either 0.1 M NaOH or 66% H₂SO₄ (Figure No.5 and 6) and red with SbCl₃-HClO₄ reagent (Figure No.7). Chelation of SbCl₃ between the aldehyde group and the 7-hydroxyl group of gossypol and related terpenoids probably is the reaction mechanism for formation of the red-colored complex (Mace *et al.*, 1974). Polyphenolic terpenoid aldehyde formed a yellow-orange color with 60% HClO₄.

Sodium borohydride (NaBH₄) was used to reduce the aldehyde group of gossypol-like compounds in root sections and thereby verify the *in vivo* occurrence of the terpenoids in the aldehyde form (Mace *et al.*, 1974). DNP did not stain the pigment gland in sections pretreated with NaBH₄ and this confirms that the original compounds are present as aldehyde (Figure No.8). Color response with DNP

(Figure No.3) is due to aldehyde group-containing pigments.

Fixed oil was localized inside the cavity of the pigment gland in all examined organs and this was confirmed by the action of Sudan III (Figure No.9). Protein was observed in seed embryo outside the pigment gland. It gives a blue color with ninhydrin-stannous chloride reagent (Figure No.10). This color had not been noticed in control sections treated with stannous chloride only (Figure No.11). The red coloration of the pigment gland was observed in stem upon treatment with phloroglucinol-HCl reagent (Figure No.12). Pigment glands are located at least two-cell-layers deep and contain phenolic terpenes. No change in the color of the epidermis was observed upon treatment with DNP reagent.

In leaf and root (1 month old), it was noticed that there is fine particles of phenolic terpenes present in the parenchymatous cells around the pigment glands. These particles turned red upon treatment with DNP reagent (Figure No.13). In young roots, 2-weeks old, it was observed that the epidermis turned black upon treatment with FeCl₃ which indicates that it contains phenolic materials (Figure No.15 and 16). No change was observed in the color of the epidermis upon treatment with DNP or phloroglucinol reagents (Figure No.17 and 18). Thus, it is concluded that root epidermis of *Gossypium barbadense* L. var. Giza 86 contains a phenolic constituents other than gossypol and its related aldehydes. No pigment glands were observed in the first apical 3cm of the tap root. Generally, in young roots, it was observed that the number of pigment glands was decreasing towards the apex leaving the last few cm free from any glands. These results may offer a plausible answer for the question which we had addressed earlier about why gossypol does not interfere with the mitotic process in cotton plant.

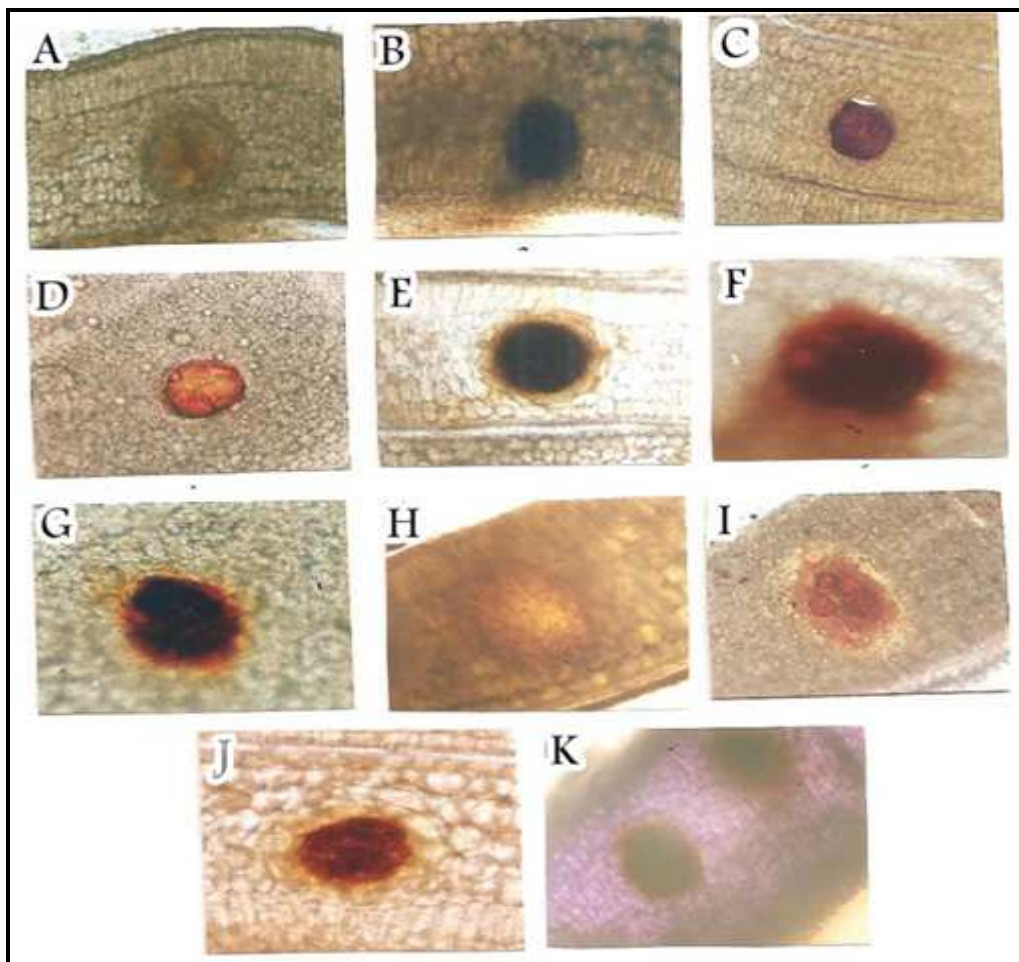


Figure No.1: T.S in mature cottonseed embryo treated with (A) Dis. H₂O. (B) 10% FeCl₃. (C) DNP reagent. (D)Phloroglucinol/HCl reagent. (E) 0.1 M NaOH. (F) 66% H₂SO₄. (G) SbCl₃/HClO₄ reagent. (H) NaBH₄ followed by DNP reagent. (I) Sudan III. (J) Stannous chloride in acetate buffer. (K) Stannous chloride/ninhydrine reagent

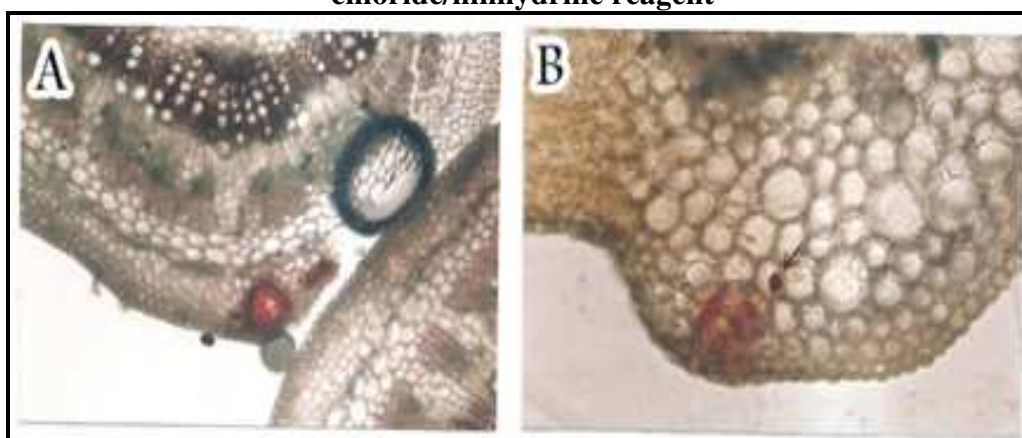


Figure No.2: T.S in stem treated with (A) Phloroglucinol/HCl reagent. (B) T.S in leaf (1 month old) treated with by DNP reagent. Arrows refers to a fine particles of terpenoids in cytoplasm of parenchyma around the gland

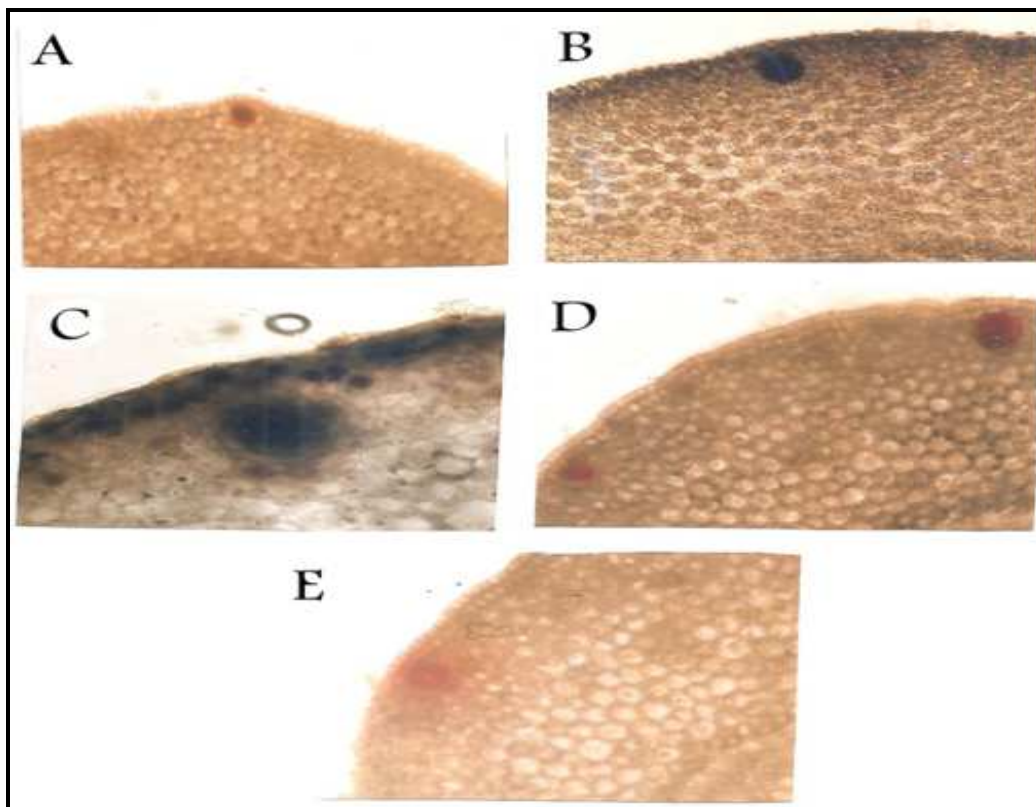


Figure No.3: Showed T. S one-week-old root (5 cm far from the root apex) treated with (A) Dis. H₂O. (B) 10% FeCl₃ (X10) (C) 10% FeCl₃ (X20). (D) DNP reagent. (E) Phloroglucinol/HCl reagent

CONCLUSION

Generally, in young roots, it was observed that the number of pigment glands was decreasing towards the apex leaving the last few cm free from any glands. These results may offer a plausible answer for the question which we had addressed earlier about why gossypol does not interfere with the mitotic process in cotton plant.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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