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Flavonoid Contents From Seeds And Callus Culture Of *Gossypium* Varieties.

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ABSTRACT

Seeds and unorganized callus tissues of various cotton varieties (GA,RG-8,PUSA 8-6) were analyzed for isolation and identification of flavonoids. .The presence of quercetin and kaempferol was observed in the seeds as well as in six week old culture of all the three varieties. The total flavonoid content was found to maximum (2.77mg/gdw)in seeds of RG-8 and minimum (1.55mg/gdw) in seeds of Pusa 8-6 variety. Culture of RG8 has high amount of both Kaempferol(2.14mg/gdw) and quercetin 0.82mg/gdw) than GA and Pusa 8-6 varieties. However GA varieties has(2.59mg/gdw) higher amount of flavonoid than Pusa 8-6(1.84mg/gdw).

Keywords: Flavonoids (Kaempferol and quercetin), *Gossypium arboreum* (RG-8) *G.hirsutum* (GA,Pusa8-6).

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INTRODUCTION

Flavonoids are series of related water soluble phenolic glycosides having a common basic structural unit the C_{15} skeleton of flavone or coester of 4-hydroxycinnamic acid. They occur universally in higher plants but are uncommon in cryptogams. They impart colour to flowers and fruits and correlation between flower colour and attraction of insects for pollination is well known [1]. Some of the flavonoids are of pathological significance where as others are physiologically important to animals. Work on flavonoids has been done both in *vivo* [2-10] and *in vitro* tissue culture [11-17]. The seeds and static tissue culture of three varieties of gossypium were estimated for the endogenous level of flavonoids.

MATERIAL AND METHODS

Tissue Culture:

Seeds of three cultivars of cotton were delinted with conc.H_2SO_4, washed thoroughly with water, sterilized for 2-3 minutes in 0.02% mercuric Tissue culture chloride solution, washed with sterilized water and germinated in sterilized Petri dishes. Two to few days after germination, the seedling were cut in 0.5 to 0.1 cm long segments of roots, cotyledons and hypocotyls and were cultured on [18,19] medium, supplemented with different concentration and combination of indole acetic acid(IAA)2,4-dichlorophenoxy acetic acid (2.4-D)and kinetin. The pH-of the medium was adjusted to 5.6-5.8 and solidified with 8% agar. 30-35 ml. of medium was dispensed into 100 ml corning flask and autoclaved for 20 minutes at 18 Ibs and 125 °C. The inoculation was carried out in laminar flow cabinet. Cultures were maintained at 27±°C in dark [20, 21]). Cultures raised on MS medium were transferred to [22] medium and stock cultures were maintained on the medium by frequent sub cultures. Six week cultures were analyzed for estimation of flavonoids.

EXTRACTION PROCEDURE:

Dried weighed and powdered samples of seeds and tissues of all three varieties were soxhlet extracted with 80% hot ethanol [23] on the water bath for 24 hours. The extract was concentrated and the concentrated extract was re-extracted with petroleum ether (40-60°C fraction 1), ethyl ether (fraction II) and ethyl acetate (fraction III)in succession. Fraction III was dried *in vacuo* and the resultant was hydrolyzed with 7% H_2SO_4 for 2 hrs. The mixture was filtered and filterate was dried. Fraction II and Fraction III were used for analysis by thin layer chromatography.

CHROMATOGRAPHY:

Thin layer chromatography (TLC)

The glass plates (20 x 20) coated with silica gel 'G' (0.2-0.3 mm thick and 30 gm / 60 ml distilled water) were dried at room temperature. The dried plates were activated at 100° C for 30 minutes in an oven and cooled at room temperature. Ethyl ether and ethyl acetate fractions' from each sample were separately applied 1 cm above the edge of the plates along with standard reference compounds (Apigenin, Kaempferol, Luteolin, Quercetin and Vitexin). These glass plates were developed in an air tight chromatography chamber containing about 200 ml of solvent mixture of n-butanol, acetic acid and water (4:1:5, upper layer).Several other solvent mixtures such as ethyl acetate saturated with water; acetic acid with water(6:4) and forestal system (acetic acid, concentrated hydrochloric acid and water(10:3:30) were also tried. The solvent mixture n-butanol, acetic acid water(4:1:5)gave the best results. The developed plates on spraying with 5% ethanolic ferric chloride solution ,showed two spots coinciding with that of reference quercetin (bluish gray) other with Kaempferol (brownish).The Rf values were calculated.

Preparative thin layer chromatography (PTLC)

The extract of both the fractions (II and III) were applied on separate plates and developed plates were air dried and visualized under UV light. The florescent spots were scrapped and crystallized with chloroform and were subjected to colorimetric.

Quantitative estimation

The compounds isolated by preparative thin layer chromatography(PTLC) were subjected to colorimeter (for quantitative estimation) by making the volume of elutes up to 10 ml by adding spectroscopic methanol and 3 ml of 0.1 M Aluminum chloride. Optical densities (O.D.) were measured using spectronic 20 colorimeter, set at 440 nm for quercetin and 423 nm for kaempferol against a blank(10 ml. spectronic methanol +3 ml. of 0.1M $AlCl_3$). .Regression curves for quercetin and Kaempferol were separately plotted between their respective concentrations and optical densities which followed Beer's law, and their amount in various samples was determined by comparing with standard regression curves. Five replications of each sample was examined and mean value calculated (S.E. <0.05%).

RESULTS AND DISCUSSION

The callus initiation was observed in excised segments of roots ,cotyledons and hypocotyls but cotyledons yielded a good amount of callus in the shortest time(3-4 weeks).The callus tissue was compact and dark brown in colour. Flavonoid content in all the three varieties of *Gossypium* is reported in **Table 1.** Maximum total flavonoid content was found in seeds of RG-8 (2.77mg/gdw) and minimum in seeds of Pusa 8-6 variety (1.55mg/gdw). The maximum quercetin (0.67mg/gdw) was found in seeds of RG-8 variety and minimum (0.35mg/gdw) in Pusa8-6 variety. Kaempferol was also higher in RG-8(2.10mg/gdw). Seeds of all the three cultivars showed the higher concentration of Kaempferol than quercetin. Kaempferol and quercetin both were confirmed n six week old culture of all the three varieties .Cultures of RG-8 had higher amount of both compounds, Kaempferol (2.14mg/gdw) and quercetin (0.82mg/gdw) than GA and Pusa 8-6 varieties however GA variety has higher amount of flavonol (2.59mg/gdw) than Pusa 8-6 (1.84mg/gdw).

Table: 1. Concentration of flavonoids (mg/gdw) in seeds and tissue cultures of three cotton varieties.

VARIETY	SEEDS			CALLUS TISSUE		
	K	Q	TOTAL	K	Q	TOTAL
GA	1.92	0.50	2.42	2.12	0.47	2.59
RG-8	2.10	0.67	2.77	2.14	0.82	2.96
Pusa 8-6	1.20	0.35	1.55	1.35	0.49	1.84

K=Kaempferol; Q=Quercetin

Kaempferol ,quercetin and iso-rhamnetin with Z-entiobioside have been isolated from *Tribulus pentandrus ,T.terrestris* [24]. From *Passiflora palmeri* 17 flavonoids are reported [25] out of which quercetin , quercetin 7,3-dimethyl ether ,apigenin 7 glucoside isovitaxin are prominent .Free quercetin along with bound kaempferol in leaves and flowers of *Corchorus depressus ,Lycium barbarum* have been reported [26].Flavonoids have also been reported in tissue cultures of *Datura spp*. It can be concluded that these cotton varieties have biosynthetic potential to produce flavonoids in seeds as well as tissue cultures.

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