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GOSSYPOL AFFECTS PLANT SPERMS ALSO

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Gossypol, a predominant polyphenolic triterpenoid yellow pigment of cotton seed, has been acclaimed to be an effective oral contraceptive in man, monkey, boar, dog, bull, hamster, guinea pig and rat. It decreases sperm count and renders the sperms malformed, immotile and inviable[1–5]. To

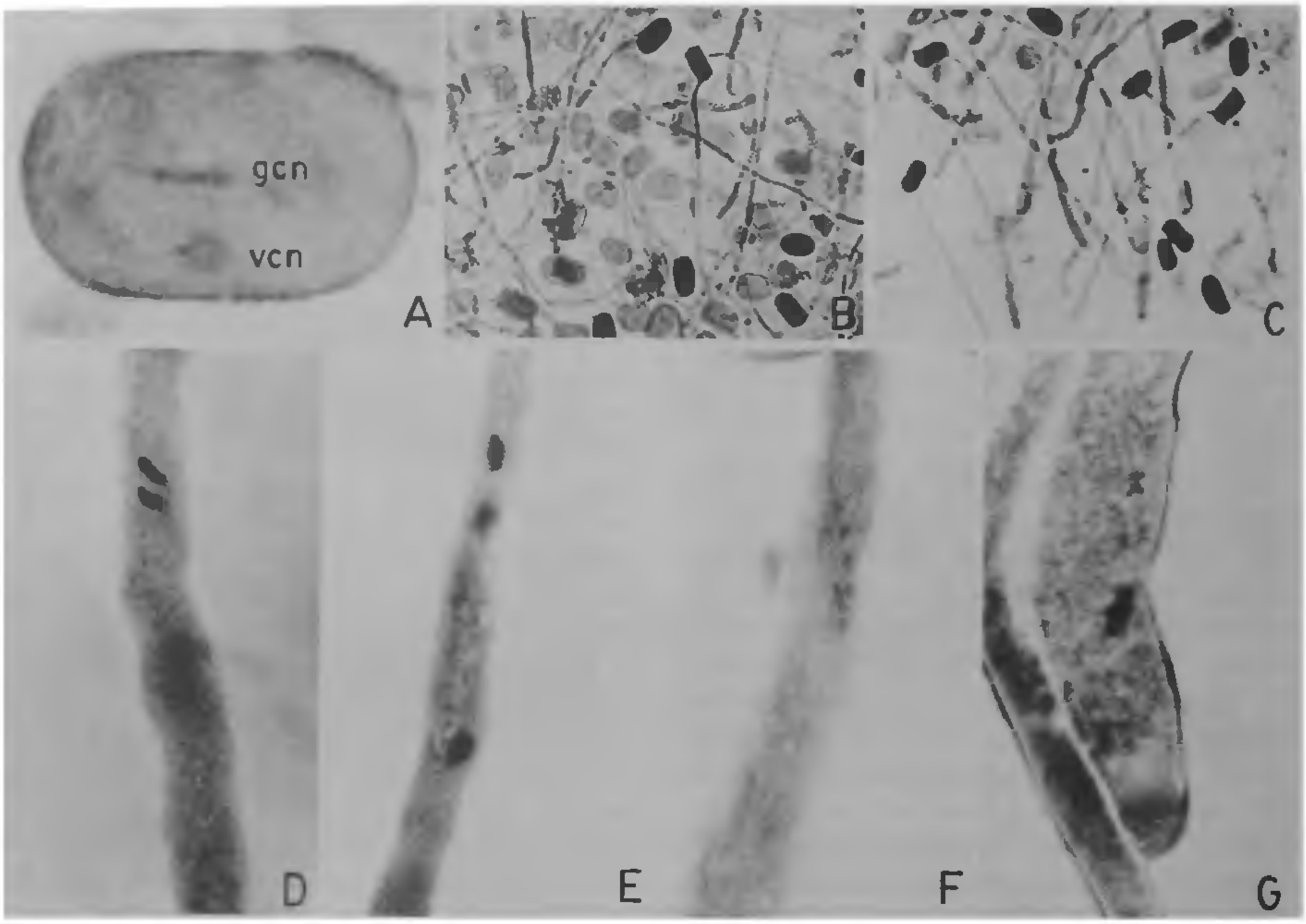

Figure 1A-G. Effects of gossypol on *in vitro* pollen germination, pollen tube growth and male gametogenesis in *Impatiens balsamina*. All but **A** are from sitting drop cultures stained with 1% aceto-orcein. In **D-G** the pollen tube tip is orientated toward the bottom of the page. **A.** Pollen grain showing 2-celled condition; note the globose vegetative cell nucleus (vcn) and the elongate generative cell nucleus (gcn) which is in prometaphase (× 1143); **B, C.** 240-min-old cultures in BM (control) and in BM + 10^{-3} M gossypol. The density of pollen tubes in **B** is an expression of higher per cent pollen germination and greater growth of pollen tubes in the control medium. Several nonopaque grains which have issued pollen tubes were initially opaque. Owing to matting of pollen tubes, only a few tubes can be traced their entire length. Contrast between **B** and **C** is obvious. Several opaque grains have failed to germinate. Also, pollen tubes are shorter than those in **B**, and most pollen tube tips are club-shaped (× 132); **D.** Anaphase of gametogenesis in pollen tube from 90-min-old culture in BM (control) (× 964); **E.** Pollen tube from 240-min-old culture in BM showing telophase of gametogenesis and vegetative cell nucleus close to tube tip (× 964); **F, G.** Pollen tubes from 90-min-old cultures in BM + 10^{-3} M gossypol showing characteristic arrest of male gametogenesis (× 964).

Table 1 *Effects of gossypol on pollen germination, pollen tube growth and male gametogenesis in Impatiens balsamina*

Culture period (min)	Parameter studied	0 (Control)	Concentration of gossypol 10^{-5} M	10^{-4} M	10^{-3} M
30†	Pollen germination†	71.75	63.44	67.58	54.75
	% Inhibition of Pollen germination	0	11.58	5.81	23.69
	d value[a]	na	1.259[ns]	0.642[ns]	2.50**
	Pollen tube length* (μm)	302.8	269.6	264.04	163.8
	% Inhibition of pollen tube elongation	0	10.96	12.80	45.90
90	Pollen tube length* (μm)	451.3	370.4	320.3	195.3
	% Inhibition of pollen tube elongation	0	17.93	29.03	56.73
	%Gametogenesis†*	72.06	71.88	70.28	39.00
	% Inhibition of gametogenesis	0	0.25	2.47	45.88
	d value[a]	na	0.028[ns]	0.278[ns]	4.703**
240	Pollen tube length*** (μm)	546.0	not tested		260.5
	% Inhibition of pollen tube elongation	0	not applicable		52.29
	% Gametogenesis†*†	84.33			90.31
	% Inhibition of gametogenesis	0			(−) 7.09
	d value[a]	na			(−) 1.270[ns]

† In 30 min from culture the generative nucleus remained at prometaphase in all media; hence no data on gametogenesis; †Average per cent of 5 replicates, based on 966–1119 pollen grains per treatment. * Average of 5 means, each mean based on lengths of 30–50 pollen tubes from each treatment in each replicate; *** Average of 6 means, each mean based on lengths of 32–36 pollen tubes from each treatment in each replicate; †* Average of 5 replicates, involving 164–483 pollen tubes per treatment from all the replicates; †*† Average of 6 replicates, involving 801 or 860 pollen tubes per treatment from all the replicates; [a] After Bailey[12]; na, not applicable; ns, not significant at $P \leq 0.05$; ** Highly significant at $P < 0.05$.

investigate whether gossypol similarly affects male gametes of flowering plants, studies were undertaken on pollen cultures of balsam, *Impatiens balsamina* Linn.

Sitting drop cultures of fresh pollen were raised (ca 0.1 mg pollen/60 μl medium/culture) in Brewbaker and Kwack's medium[6] used as the control. Gossypol (Sigma Chemical) was tested at 10^{-5}, 10^{-4} and 10^{-3} M. All cultures were maintained at 30–35°C for 30, 90 or 240 min.

Pollen grains of *I. balsamina* when shed, are 2-celled, and their generative cell is reported to present prometaphase (figure 1A). Two types, opaque and nonopaque pollen grains are formed. The latter fail to germinate. Opaque pollen grains are abundant, rich in starch and germinable. As pollen germination and pollen tube growth proceed the pollen grains gradually decrease in their opacity.

Pollen germination occurred *in vitro* within 10 min from culture. Gossypol inhibited per cent pollen germination as well as pollen tube elongation, at all concentrations tested and at all culture periods (table 1). Increase in gossypol concentration and in culture period caused increased inhibition of tube elongation. In 240-min-old cultures in 10^{-3} M gossypol medium, the inhibition of tube elongation was pronounced and the pollen tube tip became club-shaped (figures 1B,C). Gossypol inhibition of both pollen germination and pollen tube elongation are highly significant at $P<0.05$ (tables 1, 2).

In our experiments male gametogenesis was considered initiated only if the generative cell

Table 2 *Analysis of variance of data on effects of gossypol on pollen tube growth in vitro in Impatiens balsamina*

Source of variation	Culture period (min) 30	90	240
	F value*		
Among concentrations	7.27**	12.07**	250.43**
Within concentrations			

*After Snedecor[13]; ** Highly significant at $P < 0.05$.

progressed to anaphase. The generative cell remained at prometaphase 30 min from culture in all media. In 90-min-old control cultures the generative cell mitosis progressed (figure 1D) and gametogenesis was completed in 72% pollen tubes. If the culture period was prolonged to 240 min, gametogenesis was completed in another 12% of the pollen tubes (figure 1E).

In 90 min treatment with 10^{-3} M gossypol there was ca 46% inhibition of gametogenesis (figures 1F,G). This inhibition is statistically highly significant (table 1). In cultures maintained for 240 min in 10^{-3} M gossypol there was a slight promotion in per cent gametogenesis (table 1), which observation is difficult to explain presently. This duration-dependent differential response is not related to pollen tube growth, because no gametogenesis occurred in 30-min-old control cultures also, although the pollen tube length attained was significantly greater than that in 90– and 240-min-old cultures in 10^{-3} M gossypol medium. The inhibition of gametogenesis in 90-min-old cultures in 10^{-4} M and 10^{-5} M gossypol is not significant. Unlike gossypol, cycloheximide invariably inhibited[7] and chloral hydrate did not inhibit[8] gametogenesis in pollen tubes of *I. balsamina* irrespective of their effect on pollen tube elongation.

Gossypol effects on animal sperms are attributed to its influence on the activity of Ca^{2+} and Mg^{2+} –mediated ATPase[3], lactic dehydrogenase *X*, malate dehydrogenase and glutathione *S*-transferase[5], and on plasma K^+ level[2]. Murthy *et al*[9] demonstrated female antifertility effect of (+) gossypol in albino rats, and electron microscopic study has indicated that gossypol suppresses secretory organelles in human endometrium[10]. To investigate gossypol effects on pollen enzymes and levels of Ca^{2+} and K^+ involved in pollen tube growth and male gametogenesis, and to test the efficacy of L– and D-gossypols as well as the derivatives of gossypol in both male and female gametogeneses in plants would be rewarding avocations. The technique of test tube fertilization[11] may prove valuable to test fertilization ability of the gametes formed in gossypol-treated pollen and ovule cultures. In view of the reports that gossypol affects animal sperms and our present demonstration of its dosage- and duration-dependent inhibition of plant male gametogenesis, the importance of studies on gossypol in gamete biology need hardly be overemphasized.

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