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HORMONAL EFFECTS ON DIOSGENIN BIOSYNTHESIS AND GROWTH IN *DIOSCOREA DELTOIDEA* TISSUE CULTURES

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Key Word Index—*Dioscorea deltoidea*; Dioscoreaceae; yams; plant tissue cultures; steroidal sapogenins; diosgenin; 25D-spirosta-3,5-diene; plant growth regulators.

Abstract—Analysis of *Dioscorea deltoidea* tissue cultures grown in the presence of 2,4-D, indole-3-butyric acid, isopentenyladenine, benzyladenine and GA singly and in combination showed that the medium with 2,4-D most consistently favored diosgenin production. GA and high benzyladenine concentrations were toxic.

INTRODUCTION

Previous studies have indicated that plant growth regulators may have significant qualitative and quantitative effects on the production of steroids and other compounds in both plants and plant tissue cultures. GAs, auxins or kinetin have increased the steroid content in *Phaseolus aureus* [1] and *Corylus avellana* [2], influenced nicotine production in tobacco callus cultures [3], and doubled production of diosgenin by *Solanum xanthocarpum* tissue cultures [4]. Kaul *et al.* [5] have also examined the effects of cholesterol, 2,4-D, simazine and ascorbic acid on diosgenin production in *Dioscorea deltoidea* tissue cultures.

GA, indole-3-butyric acid (IBA), isopentenyladenine (IPA) and benzyladenine (BA) were added singly and in combinations to the medium of *D. deltoidea* suspension cultures. After the first, third and fourth passages the tissues were analyzed for diosgenin content and growth. Calculations of diosgenin content included the amount of 25D-spirosta-3,5-diene found, as this compound has been found in *Dioscorea* tissue culture extracts [6] and shown to be an artifact of the extraction procedure [7,8].

RESULTS AND DISCUSSION

A one-passage preliminary experiment using these hormones and IAA indicated that diosgenin content could be increased appreciably. The IAA treatments were dropped because the yields in these cultures were generally insufficient.

Growth in all treatments of the final experiment was generally good until the third generation. By then, all cultures except those with 2,4-D (control) were forming large clumps, and the growth rate had decreased. The BA (10 mg/l.) and GA (0·3 mg/l.) media were toxic, and these combinations were discontinued. After the fourth passage, only the controls were growing appreciably.

Diosgenin content averaged 0·50–0·56% for all treatments in all three generations analyzed (Table 1). Media with IBA (10 mg/l.) IPA (0·1 mg/l.) or without cytokinin generally produced above-average yields of diosgenin (0·50–0·72%) in all generations, but in two of the three generations analyzed the control had the highest content (0·82–0·95%). Cultures with BA (10 mg/l.) were significantly lower in diosgenin than with the other treatments.

None of the experimental values were as high as the 2·6% diosgenin content achieved in *D. deltoidea* suspension cultures by the addition of cholesterol and yeast extract to RT-0·1 medium [5], and IBA did not increase diosgenin content to the extent found in *Solanum* tissue cultures [4].

The amount of 25D-spirosta-3,5-diene produced during the extraction process varied from 6–43% of the total diosgenin content. Generally, less diene was found in cultures grown with GA, while treatment without hormones and combinations with IBA (10 mg/l.) produced relatively high amounts. The percentage of artifact showed a general decrease from the first to the fourth generations, the average content dropping from 29 to 11%. Re-extraction of several samples from the first generation showed insignificant changes in diene production, indicating that the difference was not due to changes in technique. Hardman found that fermentation of yam tubers before hydrolysis reduced diene formation, and suggested that the process might be releasing diosgenin from the glycoside [9]. Changes in the storage form of diosgenin may be occurring in tissue cultures, although it has been shown that fermentation of the culture does not increase diosgenin production [10].

Scatter diagrams were plotted and correlation coefficients calculated between generations to determine consistency of hormone effects. Correlation coefficients were positive but low for diosgenin content ($r = 0·5–0·8$) or percent diene ($r = 0·3–0·4$), indicating that treatments yielding high values in one generation tended to produce high values in another, but the correlation was not strong. Diosgenin content was essentially unrelated to the % diene produced in the first and fourth generations ($r = 0·1$ and 0·05, respectively) and only weakly related in the third ($r = 0·27$).

Table 1. Influence of hormones on production of diosgenin in *Dioscorea deltiodea* suspension cultures

Hormone		BA			IPA	
	Concentration	0·00	0·2	10·0	0·1	10·0
		First passage				
IBA	0·00	0·67	0·55	0·46	0·54	0·50
		(38)	(30)	(22)	(17)	(36)
	1·00	0·56	0·46	0·39	0·48	0·47
		(36)	(36)	(26)	(32)	(31)
	10·0	0·66	0·60	0·34	0·58	0·40
		(34)	(26)	(40)	(43)	(22)
GA	0·3	0·47	0·44	0·41	0·40	0·61
		(26)	(17)	(25)	(34)	(14)
Control (RT-0·1) 0·82 (26)						
		Third passage				
IBA	0·00	0·47	0·52	0·31	0·60	0·42
		(38)	(19)	(25)	(18)	(25)
	1·00	0·75	0·55	0·36	0·57	0·33
		(15)	(16)	(17)	(17)	(28)
	10·0	0·71	0·51	0·28	0·75	0·56
		(25)	(26)	(24)	(26)	(25)
GA	0·3	0·70	0·47	0·25	0·64	0·63
		(11)	(16)	(16)	(15)	(12)
Control (RT-0·1) 0·59 (30)						
		Fourth passage				
IBA	0·00	0·61	NS	NS	0·55	0·36
		(12)			(8)	(16)
	1·00	0·55	0·47	NS	0·48	0·26
		(12)	(10)		(9)	(7)
	10·0	0·88	0·62	NS	0·86	0·53
		(9)	(12)		(12)	(12)
GA	0·3	NS	NS	NS	NS	NS
Control (RT-0·1) 0·95 (6)						

Diosgenin content expressed as % total sapogenins; spirosta-3,5-diene content, in parentheses, given as % of total sapogenins. Concentration of hormones expressed as mg/l. of medium. NS—no sample available, RT-0·1....RT medium + 0·1 mg 2,4-D/l.

The foregoing results indicate that these hormones may have a minor role in the regulation of diosgenin production in 4 yr old *D. deltoidea*, cultures, and that they do not affect biosynthesis to the extent found in *S. xanthocarpum* tissue cultures [4]. It is possible that a younger culture would be more responsive. The *Solanum* culture produced about 100 times less diosgenin than *D. deltoidea* and small increases in yield are therefore proportionately greater.

EXPERIMENTAL

Suspension cultures. 4-yr-old *D. deltoidea* Wall. callus cultures growing on RT medium [11] with 1 mg/l. 2,4-D (RT-1·0) were transferred to RT-0·1 suspension medium 3 months before beginning these tests. Subculturing was done every 3 weeks. Cultures were dispersed and undifferentiated. 3 days before subculture into media with other hormones 12-day-old suspension cultures were aseptically washed 2× with RT-0 medium and allowed to grow in RT-0. Five 40-W Sylvania Gro-Lux bulbs and two 25-W incandescent bulbs were mounted 0·75 m above the cultures, yielding about 2100 lx for a 16-hr day.

Treatments. Hormones were added to RT-0 medium. BA (0·2, 10 mg/l.) or IPA (0·1, 10 mg/l.) were combined with either IBA 1·0, 10 mg/l.) or 0·3 mg/l. GA. Each hormone was also used alone in each concentration. Cultures grown with 0·1 mg/l. 2,4-D were used as a control. Three-week old tissue from each treatment was pooled after the first, third and fourth passages and analyzed, except that BA-10 and GA-0·3 treatments were discontinued after the third passage.

Analyses. Tissues were analyzed by a method modified from Kaul [5]. Powdered tissue was refluxed with 20% HCl for 2 hr, washed to neutrality, dried and extracted 16 hr with $CHCl_3$. The extract was silylated with Tri-Sil (Pierce) and analyzed by GLC with FID on a 3% OV-17 column (2 m × 2 mm), with the column at 240°, detector 325°, injector 265° and N_2 at 10 ml/min. Hecogenin was used as an internal standard. The coefficient of variation ($s/\bar{x}\cdot100$) of replicate analyses averaged 6·0%.

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