763 M1Ø = PA
769 Ø SRØ IØ I1 I3
779 19 E R3 ≤ 738
788 AA → 213
794 Halt.

Department of Statistics,
College of Agriculture,
Vellayani, Trivandrum 695 522,
India, *July* 23, 1979.

M. P. ABDURAZAK.

1. Abdurazak, M. P., *Curr. Sci.*, 1980, **49**, 3.
2. Panse, V. G. and Sukhatme, P. V., *Statistical Methods for Agricultural Workers*, Indian Council of Agricultural Research, New Delhi, 1961.

CHOLESTEROL AND BIOSYNTHESIS OF DIOSGENIN BY TUBER-CALLUS OF *DIOSCOREA DELTOIDEA*

SAPOGENINS, including diosgenin, have been reported from tissue cultures of a number of *Dioscorea* species and some other plants[1]. Cholesterol, a steroid precursor and a key intermediate in the biosynthesis of diosgenin has been shown, by using 4-C-^{14} and 26-C-^{14} cholesterol, to be incorporated in diosgenin through callus cultures of *D. deltoidea*[2]. Effect of cholesterol on diosgenin production by seedling-callus of *D. deltoidea* has been studied in detail[3]. However, they[3] reported a decrease in diosgenin production when the cultures were initially fed with cholesterol. On the other hand, initially fed cholesterol has been found to remarkably increase diosgenin production by callus cultures of *Trigonella foenum-graecum*[4] and *D. floribunda, Costus speciosus, Solanum aviculare* and *S. xanthocarpum*[5]. The present authors therefore examined the effect of initially fed cholesterol on diosgenin biosynthesis by tuber-callus of *D. deltoidea*, and the results are reported in this communication.

Callus of young tuber tissue of *Dioscorea deltoidea* Wall., kept on rapidly proliferating (*ca.* 25-fold increase in fresh weight during 50 days) on a modified nutrient agar medium of Schenk and Hildbrandt[6], for more than 2 years, was used as the inoculum. Composition of the culture medium in mg/l, where it differed from SH medium[6] was: 500 NH_4NO_3, 500 meso-inositol, 2 indoleacetic acid (IAA) and 0·5 kinetin (Kn); whereas, 2,4-dichlorophenoxy-acetic acid (2,4–D) remained at the original conc. of 0·5 mg/l. Cholesterol prepared in hot ethanol was added to the medium at 50, 100, 150 and 200 mg/l conc. before autoclaving. Ten replicate cultures of each treatment were incubated under 3 klx fluorescent light for 15 hr daily and at 27° ± 1° C. Sterilization procedure and other cultural conditions were as reported earlier[7].

Callus grown on different treatments was harvested after 60 days of incubation, dried and analysed separately for diosgenin content. Each of the dried tissue samples was refluxed with 5% (v/v) HCl in 70% ethanol for 4 hr. Mixture was filtered and the residual tissue was soxhleted with chloroform and estimated for diosgenin[8].

The acetylated isolated compound gave mp 194°–195° C, the mmp remained undepressed and its ir spectra were superimposable with that of standard diosgenin acetate, all of which confirmed that the compound was diosgenin.

In the control, without any supplement of cholesterol, the diosgenin content was minimum being 1·03%. However, 1·03% diosgenin in tuber-callus is nearly double the content (0·682%) previously reported by Chaturvedi and Srivastava[7] from the same tissue. This increase in diosgenin content is attributable to the composition of the changed nutrient medium. Diosgenin content of callus increased with all levels of cholesterol as compared to the control except at its lowest conc. of 50 mg/l in which the amount of diosgenin remained unchanged. Maximum diosgenin, *i.e.*, 1·88% was biosynthesised at 100 mg/l cholestrol whereas at its higher conc. of 150 and 200 mg/l the diosgenin content of callus progressively decreased being 1·57% and 1·13%, respectively. The present results are in conformity with the reports of stimulatory effect of initially fed cholesterol on diosgenin production by callus cultures of *Trigonella*[4] as also of *D. floribunda, C. speciosus* and *Solanum* spp[5]., but not with the results of Kaul *et al.*[3]. Also, this stimulatory effect of cholesterol is not affected by autoclaving (*cf.* Khanna *et al.*[4]). Addition of cholesterol at any conc. used in the present study did not inhibit callus growth to any appreciable extent. Such observations find support in the results of the studies on tissue culture of *Digitalis mertonensis*[9] where cholesterol promoted callus growth. On the contrary, in previous investigations on callus cultures of *D. deltoidea*[3] and of *Trigonella*[4] cholesterol inhibited callus growth. That is how, such a high content of diosgenin as 2·58%[3] obtained with the use of a combination of cholesterol and yeast extract has been reported to be nullified by the poor growth of cultures of *D. deltoidea* so much so that there has been "..... no appreciable net gain in diosgenin yield per flask ...."[3]. In the light of such observations, the 1·88%[3] diosgenin obtained in the present study by feeding 100 mg/l cholesterol accompanied by prolific growth of callus appears to be quite significant.

We thank Dr. T. N. Khoshoo, Director, National Botanical Research Institute, Lucknow, for the facilities provided.

Tissue Culture Laboratory, National Botanical Research Institute, Lucknow, *August* 18, 1979.

A. R. CHOWDHURY.
H. C. CHATURVEDI.

1. Stohs, S. J. and Rosenberg, H., *Lloydia*, 1975, **38**, 181.
2. —, Kaul, B. and Staba, E. J., *Phytochem.*, 1969, **8**, 1679.
3. Kaul, B., Stohs, S. J. and Staba, E. J., *Lloydia*, 1969, **32**, 347.
4. Khanna, P., Jain, S. C. and Bansal, R., *Indian J. Exp. Biol.*, 1975, **13**, 211.
5. —, Sharma, G. L., Rathore, A. K. and Manot, S. K., *Ibid.*, 1977, **15**, 1025.
6. Schenk, R. U. and Hildebrandt, A. C., *Can. J. Bot.*, 1972, **50**, 199.
7. Chaturvedi, H. C. and Srivastava, S. N., *Lloydia*, 1976, **39**, 82.
8. Sánchez, G. L., Acevedo, J. C. M. and Soto, R. R., *Analyst*, 1972, **97**, 973.
9. Medora, R. S., Tsao, D. P. N. and Albert, L. S., *J. Pharm. Sci.*, 1967, **56**, 67.

ABSCISIC ACID PRODUCTION BY *DIPLOCARPON ROSAE* AND ITS ROLE IN THE DISEASE PRODUCTION

SYNTHESIS of growth regulating substances by plant pathogenic microorganisms has been reported by several workers. A list compiled by Gruen[1] shows that about 80 fungal species including plant pathogens and non-pathogens produce compounds with auxin activity. The synthesis of gibberellin has been extensively studied in *Gibberella fujikuroi* and its imperfect form *Fusarium moniliformae*. The presence of gibberellins in *Agrobacterium tumefaciens* culture has been reported[2]. Ethylene production by microorganisms has been demonstrated with cultures of *Penicillium digitatum*[4], *Ceratocystis fimbriata*[5], and *Pseudomonas solanacearum*[6]. The role of cytokinins in the fasciation disease caused by *Corynebacterium fascians* in *Chrysanthemum*, sweet peas and many other dicotyledon seedlings has been elaborated[7]. A preliminary study to find the cause of severe premature defoliation in rose plants infected by *Diplocarpon rosae* was undertaken.

The culture of *D. rosae* was isolated from the affected leaves of a rose plant collected from the rosery of the University of Agricultural Sciences, Bangalore. The surface sterilized leaf bits were put on the potato dextrose agar medium in the plates and then the culture was purified and its pathogenicity was proved by reinfecting the healthy plants. For isolating the abscisic acid and similar compounds, the fungus was grown in a dialysed sterile 100 ml potato dextrose broth in 250 ml Erlenmeyer flask under stationary conditions. The flasks were incubated for 21 days at 25° ± 2° C. Five flasks were removed at 4, 6, 10, 12, 16 and 21 day intervals and processed. The culture broth along with the mycelial mat was homogenized in a Sorvall Omni-mixer and passed through double layered cheese cloth. The filtrate was centrifuged at 12,000 r.p.m. for 20 min in a refrigerated centrifuge. The supernatant was reduced to 1/4th of its original volume by flash evaporation at 35° C. The concentrated culture filtrate was treated for ABA extraction by following the method of Milborrow[3]. The pH of the filtrate was adjusted to 2·8 (by 1N HCl) and extracted thrice with petroleum ether and these fractions were pooled together and extracted with small volumes of 5% aqueous solution of sodium bicarbonate. The aqueous fraction was adjusted to pH 2·8 with 1 N HCl and extracted again with ether. The petroleum ether fraction was passed through the bed of anhydrous sodium sulphate and dried with compressed air. The samples along with the standard abscisic acid (ABA) were dissolved in petroleum ether and spotted on silica gel TLC plates and the plates were developed in a solvent mixture of benzene : ethyl acetate : acetic acid (50 : 5 : 2, V/V). The developed plates were dried and exposed to UV lamp in a dark room. Similarly, rose plant leaves, healthy and infected by *D. rosae*, were extracted separately by following the same procedure used for *D. rosae* culture and processed accordingly for detecting the presence of ABA.

The growth inhibitory activity of the compound extracted from the culture filtrate was tested by standard wheat coleoptile bioassay method. The coleoptiles were removed individually from the germinated seeds and cut to 5 mm length. Twenty such coleoptiles were placed in small petriplates containing two-fold serial dilutions starting from 500 ppm to 1 ppm of the test compound. The coleoptile lengths were noted after incubation of 12 h.

The dried crude compound obtained from the extract of culture filtrate of *D. rosae* was insoluble in water and white needle-like crystals in appearance. Bright fluorescent spots were observed on TLC plates exposed to short wave UV lamp. Only one fluorescent spot exactly coinciding with that of standard ABA was observed from the samples taken at 4, 5 and 10 day intervals. Whereas the extracts of cultures older than 12 days had three fluorescent spots, only one of the spots coincided with that of standard ABA. The other two had Rf values of 0·54 and 0·91 (Table I). These results indicated that *D. rosae* produced a compound