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SHORT COMMUNICATION

# HORMONAL CONTROL OF STEROID SYNTHESIS IN *SOLANUM XANTHOCARPUM* TISSUE CULTURES

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Abstract-Analysis of tissue cultures of *Solanum xanthocarpum* subjected to the influence of different plant hormones such as 2,4-D, IAA, IBA, kinetin and GA singly and in synergistic combinations showed changes in the steroidal content indicating chemical regulation by auxins.

## INTRODUCTION

APPLICATION of various plant growth regulators to differentiation of tissue cultures *in vitro* has been studied extensively, but very little is known of the influence of such hormones on tissue metabolism. Kaul et al.,[1] studied the influence of various factors on diosgenin production by *Dioscorea deltoidea* callus and suspension cultures, while Furuya *et al.*,[2] and Furuya[3] have examined the effect of certain growth hormones on the control of nicotine synthesis in tobacco callus. The present report concerns the chemical analysis of *Solanum xanthocarpum* (Solanaceae) tissue cultures subjected to the regulating influence of auxins (IAA and IBA), kinetin (kn) and gibberellic acid (GA).

## RESULTS AND DISCUSSION

Previous studies by us[4,5] with *Solanum xanthocarpum* tissues grown *in vitro* have shown the formation of solasonine, β-sitosterol and diosgenin. Analysis of the tissue grown on the control medium showed 0·051% of β-sitosterol and 0·004% of diosgenin. Incorporation of IAA in place of 2,4-D in the medium resulted in the growth of a green and compact tissue but the growth was not sustained beyond 4 weeks. Analysis of the IAA-grown tissues showed a two-fold increase in diosgenin but a reduction in β-sitosterol content. Substitution of 2,4-D by IBA gave results similar to those obtained by using IAA in regard to the growth of tissues as well as the sterol content, except that the synthesis of diosgenin was enhanced further (0·01%). It is noteworthy that no solasodine was present in tissues grown on medium containing IAA or IBA. On the other hand, incorporation of kinetin in conjunction with GA to a medium devoid of 2,4-D caused no appreciable change in the steroidal content as compared to the control, although it resulted in the differentiation of roots and shoots. Table 1 shows the results of the quantitative estimation of the steroids in tissue cultures influenced by the phytohormones.

[1] B. KAUL, S. J. STOHS and E. J. STABA, *Lloydia 32,339 (1960).*
[2] T. FURUYA, H. KOJIMA and K. SYONO, *Chem. Pharm. Bull.* 15, 901 (1967).
[3] T. FURUYA, *Kitasato Arch. Exptl. Med. 41, 47 (1968).*
[4] M. R. HEBLE, S. NARAYANASWAMI and M. S. CHADHA, *Naturwissenschaften 7, 351 (1968).*
[5] M. R. HEBLE, S. NARAYANASWAMI and M. S. CHADHA, *Science* 161, 1145 (1968).

TABLE 1. STEROIDAL CONTENT OF CALLUS TISSUES OF *Solanum xanthocarpum* TREATED WITH DIFFERENT HORMONES

| Treatment | Dry wt. of callus tissues (g) | β-Sitosterol (mg) | Diosgenin (mg) | Solasodine presence (+) absence (-) |
|---|---|---|---|---|
| 2,4-D (1 ppm) (control) | 35 | 17.8 | 1·3 | + |
| IAA (2 ppm) | 25 | 6·0 | 2·0 | – |
| IBA (2 ppm) | 35 | 10·2 | 3.6 | |
| Kn (2 ppm) + GA (1 ppm) | 35 | 12·8 | 1.6 | + |

It is evident from the foregoing that actively growing tissues in culture are influenced by auxins, kinetin and GA in their biosynthetic potential. It is possible that certain functions are reduced or simplified under cultural conditions while others are enhanced, as evidenced by the absence of solasodine and the augmentation of diosgenin with exogenous application of IAA or IBA in the medium. However, changes in tissue morphology did not show concomitant changes in the production of other secondary metabolites to any appreciable extent.

## EXPERIMENTAL

***Callus cultures.*** Stem callus tissues of the plant maintained on a modified Murashige's medium containing coconut milk (10%), inositol (100 ppm), adenine (5 ppm) and 2,4-dichlorophenoxyacetic acid (2,4-D, 1 ppm) for the past 4 years were utilized as control. Tissues grown over a period of 6 months by periodic subculture were pooled together.

***Treatments. 2,4-D*** in the basal medium was substituted by indole-3-acetic acid (IAA, 2 ppm), indolebutyric acid (IBA, 2 ppm), kinetin (kn, 2 ppm) and gibberellic acid, (GA, 1 ppm) singly and in combination. Five-week old calli from each treatment were pooled and used for analysis.

Oven-dried tissues were extracted as outlined in our previous communication.[5] The extracts were chromatographed on silica gel (<0·08 mm) column using benzene-ethyl acetate gradient. Fractions containing β-sitosterol, diosgenin and solasodine were pooled and purified by thin layer chromatography using silica gel G. The compounds thus separated were taken for quantitative estimations. The sterol concentration was determined by the colorimetric technique of Moore and Baumann.[6] The diosgenin content was estimated colorimetrically using Leibermann-Buchard reaction. The colour was developed for 1 hr and absorptivity measured at 575 nm. Since solasodine was present in trace quantities, only qualitative chromatographic evidence was obtained for its presence.

[6] P. R. MOORE and C. A. BAUMANN, *J. Biol. Chem. 195, 615 (1952).*