Influence of Kinetin and Auxins on the Growth and Production of Diosgenin by Costus speciosus (Koen.) Sm. Callus Derived from Rhizome

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Diosgenin has been reported earlier from tissue cultures of Costus speciosus.1-2) Furthermore, the enhancement of diosgenin has been obtained by feeding cholesterol to the undifferentiated suspension cultures of C. speciosus.3) The application of various auxins and cytokinins for inducing organogenetic differentiation in tissue cultures has been extensively studied but very little is known of their influence on biochemical differentiation and the production of secondary metabolites by in vitro cultures of tissues and organs.4-8) There is no previous report on the latter aspect for C. speciosus, which is dealt with in the present communication.

Undifferentiated static callus of Costus speciosus derived from rhizome was maintained for a period of 24 months on RT medium9) by periodic subculturing at 6~8 week intervals. Callus explants were grown in RT medium supplemented singly with 1,3 and 5mg/liter of 1-indoleacetic acid (IAA), a-naphthaleneacetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D) or kinetin. Tissues were harvested after growth periods of 6 and 8 weeks, dried and their growth indices calculated [GI=(final dry weight of tissue-initial dry weight of tissue)/initial dry weight of tissue]. Each of the dried tissue samples was analysed for its diosgenin content.10) Ten replicates were taken in each case. Diosgenin from callus was confirmed by mp, mixed mp (mmp) and IR, with reference to an authentic sample of diosgenin.

An increase in growth of the tissue was observed in the presence of different concentrations of IAA, NAA and 2,4-D, whereas the growth decreased in kinetin during 6 weeks of incubation. The maximum growth took place in 3mg/liter of 2,4-D (3.50) followed by similar concentrations of NAA (2.90) or IAA (2.82). The growth of callus in kinetin (2.00 ~2.17) was even less than the control (2.66). Kinetin has also been reported to decrease growth of Dioscorea deltoidea.5)

The presence of diosgenin in callus was confirmed by TLC (RF 0.71), mp 204~206°C, mmp 204°C and IR spectrum, which were identical with those of the reference diosgenin. The maximum diosgenin content (0.95%) was obtained after 6 weeks of incubation in the tissue grown in the presence of kinetin (5mg/liter), followed by that cultured in IAA (3mg/liter, 0.80%), NAA (1mg/liter, 0.64%), and 2,4-D (1mg/liter, 0.61%). The diosgenin content in the control was 0.76%. The diosgenin content as well as the callus growth decreased after 8 weeks of incubation. On the contrary, cytokinins has been reported to adversely affect diosgenin biosynthesis in callus of D. deltoidea.6,11)

Marshall and Staba11) have reported that growth hormones such as 2,4-D, IAA, isopentenyladenine (2ip), benzyladenine (BA) and gibberellic acid (GA₃) do affect diosgenin production by callus of D. deltoidea, but not to the extent reported by Heble et al.7) for Solanum xanthocarpum. Khanna et al.,8) Brain and Lockwood,12) and Lockwood and Brain13) in Trigonella foenum-graecum tissue cultures have concluded that hormones in the medium greatly influence the synthesis of steroidal sapogenin by the cultured tissue. Our results are in conformity with their findings and suggest that auxins and cytokinins play a regulatory role in sapogenin synthesis.

REFERENCES

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