S U M M A R Y

SUMMARY

Plant steroid chemistry is an interesting and intriguing subject because a large number of physiologically and Pharmaceutically important natural products are synthesised in this pathway. Unlike in the animals the plant system harbours multiplicity of sterols. The role of these variety of sterols is obscure. Obtaining biochemical mutants of the steroid pathway should be of great use in unravelling the role played by steroids. Such characterised mutants of steroid pathway is not existant among higher plants. My work was aimed at the use of somatic cell genetic approach to generate such biochemical mutants in steroid pathway in Solanum xanthocarpum.

Mevinolin at extremely low concentrations i.e. 1 to 25 μM inhibited growth of cell suspension culture of S.xanthocarpum. Higher concentrations (50 μM) of mevinolin caused cell cultures to turn brown and subsequently die. The sterol synthesis was inhibited much more by mevinolin than the growth of cell cultures.

Mevinolin inhibited the formation of cell colonies when added to the medium used for cell plating.Agar plated cells were much more sensitive to inhibitory effect of mevinolin

than the cells grown in liquid medium.

Bioassays using cultured leaf discs of S.xanthocarpum showed 50% inhibition of the growth and sterol biosynthesis by 10 μM concentration of mevinolin. A time course study using LD 50 concentration of mevinolin using leaf discs showed within 72 hours of incubation the sterol content of the leaves was reduced to 50% or more with respect to control. The pigment contents of the leaves did not show much inhibition.

In order to identify the site of inhibition by mevinolin experiments were carried out by supplementing the culture medium with some intermediates of isoprenoid pathway such as acetate, mevalonate, squalene and cholesterol. The reversal of growth inhibition of cell culture and cultured leaf discs treated with LD 50 concentration of mevinolin was carried out. Acetate in the form of sodium acetate could not bring about any significant reversal of the growth inhibition at any of the concentrations (100-2000 μM) used. Addition of mevalonate at concentrations ranging from 500 μM - 2000 μM was effective in nearly reversing the inhibition of growth and steroid biosynthesis in the leaf discs. On the other hand 500-4000 μM mevalonate was required to produce such a recovery from the 25 μM mevinolin induced inhibition of growth and sterol synthesis in the cell cultures.

Exogenous squalene was very effective in reversing the mevinolin induced inhibition of growth and sterol biosynthesis. As low concentration as 1.00 μM was effective in reversal of inhibition but higher concentration was inhibitory to the leaf discs. Compared to leaf discs, the cell culture required higher concentration of (1.5 uM) squalene to reverse mevinolin induced inhibition. Exogenous cholesterol could bring about considerable reversal of inhibition of sterol synthesis but the growth inhibition was not reversed in case of leaf discs. This experiment established that mevinolin inhibits the synthesis of mevalonic acid in leaf disc and cell culture thereby inhibiting sterol synthesis and subsequently growth.

Cell lines resistant to LD 50 concentration of mevinolin were isolated using quantitative cell plating technique. Resistant clones developed at LD 50 concentration of mevinolin were cultured in the liquid medium containing the inhibitor. After 10-12 cycles in the selective liquid medium to reduce the escape of the physiological variants.These cell lines were grown for many growth cycles in non selective medium to test the stability of the resistance. These cell lines when challenged to mevinolin, did not show any growth inhibition.

The resistant cell lines after many sub-cultures on the nonselective medium were used for biochemical characterization. The analyses of sterols and steroids of this cell line showed 2 fold increase in the content of the sterols and 2.1 fold increase in the steroidal alkaloid (Solasodine) content.

In order to further characterize this cell line and find out the exact reason for the elevated level of sterols and steroidal alkaloid contents, rate of sterol biosynthesis in this cell line was studied in the presence and absence of inhibitory concentrations of mevinolin. The results indicated faster rate of incorporation of ^{14}C acetate into sterols in the selected cell line.

The relative activity of the rate limiting enzyme HMGR in the selected mevinolin resistant line showed 2.5fold increase in its activity.

Leaf disc assay of the regenerated one month old variant Solanum plants showed resistance to L.D 50 concentration of mevinolin. Analysis of the steroidal contents of these plants showed upto 1.25, 1.34, 1.29 and 1.63 fold more free sterols, steryl esters, steryl glycosides and steroidal alkaloid (solasodine) respectively.