S U M M A R Y

SUMMARY

The investigations were aimed at the successful establishment of callus and suspension cultures of *Datura metel* L., *Cassia fistula* L. and *Gossypium hirsutum* L. hybrid variety 'Sankar 4' on a chemically defined medium and further to examine the biosynthesis of total polyphenols associated with growth under different cultural parameters. Attempts were also made to study the enzymes related to growth and polyphenol production and their release into the medium.

Studies described in the present investigations clearly established that tissues of the above mentioned plants, originally maintained on coconut milk containing complex media registered higher growth value when transferred to modified Murashige and Skoog's medium (Table 3, Chapter II). Optimal levels of sucrose, auxin (2,4-D) and kinetin for the maximum growth with *Datura* cell suspensions, *Cassia* and cotton tissues were determined. The growth values (Final weight/Initial weight) of the three tissues, as registered after 20 days culture, were 28, 22 and 23 respectively.

The superiority of sucrose over other sugars tested for growth of the tissues and for the maximum production of

total polyphenols was realised from the experiments 4-1 and 4-2. It was further observed that the depletion of the carbohydrate in the medium led to the decrease in the accumulation of polyphenols both in Datura cells and Cassia tissues suggesting that the sucrose formed the limiting factor for polyphenol synthesis. The importance of carbohydrate in the biosynthetic pathway of polyphenol production was further discussed.

Comparison of growth and polyphenol accumulation in tissues grown in liquid and agar media (Experiment 4-8) revealed that both growth and polyphenol production were appreciably higher in the tissues incubated as liquid shake cultures rather than as agar cultures.

The relationship between the inoculum size and volume of the medium on one hand, with growth and polyphenol production on the other, was examined in Experiment 4-9. It was observed that in a fixed volume of the medium, greater amount of polyphenol accumulation and higher growth values were recorded when the inoculum size was low than at higher inoculum sizes. This suggested that growth and polyphenol production were limited by the supply of some essential nutrients in the medium.

The nitrate supply, however, cannot possibly be a main limiting factor for polyphenol production for, as observed in Experiments 4-5 and 4-9, doubling the level of nitrogen source did not enhance polyphenol accumulation. Moreover, of the different nitrogen sources examined, a balanced supply of both potassium and ammonium nitrates was found essential for maximum polyphenol production as well as growth.

The hormonal effects on polyphenol accumulation suggested that, to a limited extent, the polyphenol synthesis could be regulated by auxin and kinetin concentrations in the medium (Experiments 4-3 and 4-4). Though a clear understanding of the action of 2,4-D and kinetin on polyphenol synthesis was not brought out in the present investigation, it was believed that the hormones might trigger the biosynthetic pathways of secondary plant productions through their effects on nucleic acid metabolism.

Examination of the interaction between light and auxin clearly revealed that the inhibitory effects of higher auxin concentration (5×10^{-5}M 2,4-D) on polyphenol production could be reversed by high light intensity

(Experiment 4-10); whereas the light effects on growth at different auxin levels were not stimulatory. However, the inhibitory effect of light on growth was overcome by the presence of GA_3 in the medium as was observed in Experiment 4-11. GA_3 at low concentration (10^{-6}M) enhanced polyphenol accumulation in the tissues both in presence and absence of light. However, at higher concentration ($3x10^{-6}$M) GA_3 supressed the polyphenol production in light as well as in dark. This seemed to indicate that GA_3 effect on polyphenol synthesis was independent of light. In general, it was observed that in cells exposed to high light intensity, the polyphenol accumulation was more as compared to the cells grown in dark.

As mentioned earlier, L-phenylalanine and L-tyrosine are now well recognised intermediate aromatic amino acids in the biosynthesis of phenylpropanoid compounds. When these precursors were tested in Experiments 4-6 and 4-7, L-phenylalanine was found superior to L-tyrosine in enhancing the total polyphenol accumulation in Datura cell cultures. This might be due to the formation of compounds belonging to phenylalanine pool of the biosynthetic pathway which were later converted to cinnamic acid derivatives leading to the formation of several phenolic compounds as already discussed in Chapter VI.

The correlation between morphogenetic changes and polyphenol accumulation in Cassia callus cultures was examined in Experiment 4-12. The observations made on the callus tissues subjected to auxin (IAA)/kinetin interactions clearly showed an inverse relationship between the formation of root primordia and polyphenol accumulation. Further, higher concentrations of auxin and kinetin together produced synergestic effect on growth of the tissues.

The results obtained in the experiments described in Chapters IV and V (Experiment 5-7) showed that the time sequence of periods of highest growth and maximum polyphenol synthesis varied with the tissue. In Datura cultures, maximum polyphenol production was registered in the pre-exponential growth phase; whereas in case of Cassia cultures growth and polyphenol accumulation increased all through the course of culture for 20 days. In cotton tissues, on the other hand, there was a marked lag both in growth and polyphenol production for initial three days, after which both of them enhanced. Of the three tissues examined for polyphenol production Cassia proved the best as it contained highest polyphenols per gram fresh weight.

Studies on peroxidase and IAA oxidase activities in relation to polyphenol synthesis under different cultural parameters revealed a close correlation between peroxidase formation and polyphenol synthesis; there was greater accumulation of polyphenols in tissues with high peroxidase activity. This suggested that polyphenol production could be controlled through the regulation of peroxidase activity. Furthermore, the results also indicated that peroxidase and IAA oxidase activities influenced the level of endogenous auxin thus regulating growth at optimal hormonal concentrations. IAA oxidase activity as observed under different cultural conditions suggested a close relationship with growth rather than with polyphenol production.

The development of PAL activity and total polyphenol production in Cassia callus cultures showed a rough correspondence upto day 9, after which though the polyphenol synthesis continued the PAL activity declined (Experiments 5-8 and 5-9). The early repression of PAL activity might be due to the inhibitory effects of certain phenolic acids accumulated during the course of culture, on the enzyme PAL. A detailed analysis of the different phenolic compounds formed and their repressive effects on PAL enzyme during

growth cycle of the cultured tissues needs to be further investigated to elucidate the importance of PAL in the biosynthesis of phenylpropanoid compounds.

Additional evidence was obtained on the release of enzymes peroxidase and IAA oxidase into the culture medium (Experiments 5-2, 5-4 and 5-6). It was also observed that the release of enzyme was influenced by different nutritional and hormonal levels supplied to the tissues. Progressive changes in peroxidase activities in the tissue and in the medium were highly significant, particularly during the period of maximum polyphenol production (5 to 10 days) in Datura cell cultures. This further supported the view that peroxidase has an important role to play in polyphenol synthesis. The findings of the leaching of enzymes also strengthened the suggestions of earlier workers for exploitation of the plant tissue cultures, particularly suspension cultures, for the extraction and purification of important enzymes on commercial basis.