CHAPTER V

SUMMARY

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Callus tissue derived from the floral buds of Nicotiana tabacum L. var. Anand-2 and from the anthers of Gossypium hirsutum L., cv. Sankar-5 were maintained on MS basal medium supplemented with 2.0 mg/l each of IAA, NAA, KN and 2% sucrose.

Of the auxins tested, maximum growth response of cotton callus was obtained with supplement of IBA (0.2 mg/l) into the MS basal medium. 2,4-D antagonised growth except at low concentrations.

Kinetin as well as BAP at higher concentrations stimulated growth of cotton callus tissues.

GA_3 could bring about growth promotion only at lower concentrations, higher levels being ineffective.

Of all the auxin-cytokinin combinations tested, 2.0 IAA + 1.0 BAP supported maximum growth of cotton callus. Of the auxin-cytokinin-gibberellin interactions 0.2 mg/l NAA + 1.0 mg/l BAP + 25.0 mg/l GA_3 supported the highest growth of the tissues.

Of different carbohydrates tested for growth and accumulation of sugars in callus cultures of cotton, glucose, fructose, sucrose, maltose and starch supported growth. Of

the above 5 carbohydrates tested, fructose at 4% level supported maximum growth. Increasing concentrations of the above 5 carbohydrates increased growth as well as sugar content in the tissue.

Both tobacco and cotton tissues exhibited lag and exponential growth phases. The enzyme activities of invertase, MDH, G-6-PDH and FDPA were high during the lag period and declined with the progress of exponential phase. However, the activity of amylase showed peak values towards the termination of culture period. Accumulation of total and reducing sugars was very high during the initial lag phase and later on declined with the progress of the growth.

There was decline in growth values when tobacco and cotton tissues were grown in the dark. The enzymes GOT, ME and PEPC showed higher activities during lag phase of growth and declined with the progress of exponential growth period.

Auxins, kinetin and GA_3 either stimulated or suppressed the activities of the enzymes amylase, invertase, MDH, G-6-PDH and FDPA. In general, lower IAA concentrations (2.0 mg/l) stimulated the above enzymes, while higher concentrations (5.0 mg/l) inhibited. Contrary to this, higher NAA (2.0 mg/l) promoted all enzyme activities except invertase. Pronounced increase in amylase activity by NAA is notable and hitherto a new report. 2,4-D has inhibited both amylase and invertase activities to a considerable extent.

Higher concentrations of kinetin (2.0 mg/l) promoted amylase, invertase and G-6-PDH; whereas low concentrations (0.04 mg/l) stimulated MDH and FDPA activities.

GA_3 at 100.0 mg/l level promoted amylase, invertase, MDH and FDPA activities quite significantly when compared to 25.0 mg/l level in cotton callus tissues.

While lower concentrations of the glucose, fructose, glucose + fructose, sucrose, maltose and starch stimulated enzyme activities, higher levels (4%) suppressed the activities of invertase, MDH, G-6-PDH and FDPA in general. In cotton, both glycolytic and PP pathways are active together. Suppression of invertase activity by glucose and fructose and glucose + fructose could be attributed to product inhibition or feedback inhibition. Besides this, stimulation of FDPA activity at 2% starch level is notable. Cotton callus tissues could be grown successfully on starch and it was observed that it was getting digested extra-cellularly.

The organogenetic behaviour of tobacco callus was quite interesting. IAA at low levels (0.175 - 0.3 mg/l) invariably induced shoots with 3% sucrose. Higher levels of IAA (0.5 - 2.0 mg/l) favoured root differentiation. Root differentiation could also be achieved by increasing sucrose level (to 6%) in the low IAA (0.3 mg/l) containing medium.

The dramatic difference between organ forming and non-organ forming tobacco callus and proliferating cotton callus tissue was the accumulation of starch prior to organogenesis. The utilization of starch involved enhanced rates of degradation of the metabolite during organogenetic process and this is supported when the starch degrading enzyme amylase was observed.

Activity of invertase also increased in the shoot and root forming tobacco callus when compared to the non-organ forming tissues. Invertase activity in cotton during the corresponding period declined. Presumably the degradation products of starch and free sugars are utilized for organogenetic process since it is a high energy requiring event.

This view was substantiated when the activities of respiratory enzymes in organ forming and non-organ forming tobacco tissues and also cotton were compared. MDH activity was by and large substantially higher during root formation than during shoot formation.

Stimulation of carbohydrate oxidation through the glycolytic pathway at the time of organ initiation is in keeping with the suggested role of starch and the increase in respiratory activity. Pronounced stimulation of PP pathway was also observed during morphogenetic response when compared with unorganised mass of tobacco and cotton

callus tissues. The PPP is a source for producing reducing power (NADPH) for biosynthesis as well as generating precursors for the synthesis for essential cell metabolites.

Also, non-autotrophic CO_2 fixation was markedly higher during organ forming tissues of tobacco as determined by the activities of PEPC, ME and GOT. Presumably, malate might be generating reducing power (NADPH) for reductive biosynthesis in the shoot and root forming callus cultures of tobacco. Thus, contributions of all these metabolic pathways are important for organogenetic processes.

Studies with mannitol as an osmotic agent in tobacco callus tissues revealed interesting facts. It is inferred that sucrose was not acting solely as a source of energy and carbon. The success in partially replacing the sucrose requirement for shoot and root formation with mannitol in osmotically equivalent levels supported the view that part of the sucrose is acting as an osmoticum. Another interesting observation is that the osmotic requirement for root and shoot formation is different. Number of roots increased per callus mass with the increase of mannitol content.

Rifamycin, a specific RNA polymerase inhibitor delayed shoot differentiation, while completely suppressed rhizogenesis in callus tissues of tobacco.

Present studies thus point out the importance of carbohydrate metabolism during organogenesis in tobacco callus tissues and bring out subtle differences between the root and shoot differentiating tobacco callus, on one hand, and between the organ differentiating tobacco and non-differentiating cotton callus on the other.

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