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The investigation embodied in the present thesis was aimed at: (i) establishment of callus cultures from the anthers of Datura metel Linn., (ii) examination of the nutritional requirements and the effects of various cultural parameters on the growth of cultured tissue, and (iii) elucidation of a clear picture about the physiological and biochemical changes associated with growth.

The callus originated mainly from the connective region of the anther segments. The growth of the callus was rapid on White's medium containing 2.0 mg/l 2,4-D, 10 per cent (v/v) coconut milk and 300 mg/l casein hydrolysate (Experiment 1). Growth of the callus improved markedly when the macroelement salts of Heller's medium were substituted for White's (Experiment 3). Of the auxins tested 2,4-D at 2.0 mg/l supported the highest growth of Datura callus cultures (Experiment 2). Studies on the effect of kinetin alone and in combination with auxin revealed that 0.5 mg/l kinetin promoted maximum growth. At their optimal concentrations kinetin and 2,4-D together supported higher growth than they did individually.

Of the carbohydrates tested, sucrose at 2 per cent was found to be the most suitable energy source. Glucose, fructose, equimolar mixture of glucose and fructose and maltose supported equally poor growth, While starch was found to be the poorest carbon source for Datura callus cultures (Experiment 5). The growth increased with increasing levels of sucrose from 0 to 2 per cent and declined thereafter, though dry weight per cell enhanced with increasing sucrose upto 4 per cent.

The inoculum/volume ratio had considerable influence on growth attained by Datura callus cultures (Experiment 7). In a fixed volume of medium (20 ml), the maximum cell generations and highest increase in cell dry weight occured when the inoculum was low (50 mg). With the increase in inoculum size there was a progressive decline in growth values. At a fixed inoculum, on the other hand, maximum cell generations and the highest increase in cell dry weight were registered in high volume of the medium (60 ml); and with the decrease in volume of the medium there was relative decline in dry weight and in number of cell generations. The relationship observed between the inoculum/volume ratio and the

final growth attained are discussed for their inter-dependence.

Doubling the level of macroelement salts enhanced the growth of the tissues (Experiment 8); implying that the growth of tissues in a fixed volume of the medium was limited by the depletion of one of the essential nutrients from the culture medium. When the concentrations of the individual ions were doubled, enhanced nitrate level reproduced the effect of doubling the whole of the macroelement salts. It was inferred that nitrate was the limiting factor for growth of Datura anther callus. Higher nitrate levels, however, proved to be toxic during subsequent culture passages.

Incorporation of casein hydrolysate in the medium containing nitrate caused marked enhancement in growth of Datura callus cultures (Experiment 9). Addition of molybdenum in minute dose (0.001 mg/l) further promoted the growth (Experiment 10).

Studies with the sugar contents of Datura callus tissues revealed that the sucrose grown tissues showed more of total and bound sugars than free sugars; while

those grown on other carbohydrates contained more of free sugars than bound sugars. The activity of invertase was also higher in the tissues incubated on sucrose medium (Experiment 11). With the increase in concentration of sucrose, there was observed rise in invertase activity, as well as increase in bound sugars over free sugars (Experiment 12). The protein content and glutamic oxaloacetic transaminase activities were more in tissues grown on sucrose as compared with those grown on glucose. (Experiments 14 and 15). The above findings seemed to suggest that higher nitrogen contents and ratio of bound sugars to free sugars probably account for superiority of sucrose over other carbohydrates tested.

Changes in the nitrogen content of Datura callus tissue during the course of culture revealed that the cellular nitrogen per unit dry weight and per cell increased sharply during the first few days of culture when there was little increase in dry weight and total cell number (Experiment 13). When the nitrogen fractions began to decline, growth enhanced rapidly.

Changes in the nitrate reductase and glutamic oxaloacetic transaminase during the course of culture

revealed that both the enzymes were induced in the first 3 days of culture and achieved the peak activity on day 12 before declining (Experiment 16). The pattern of these enzymic activities paralleled the cellular nitrogen content of the callus tissues during the incubation period. The nitrate reductase activity enhanced with increasing levels of nitrate, while the glutamic oxaloacetic transaminase enhanced upto double the level of nitrate in the medium, and declined in presence of four fold nitrate supply, (Experiment 17).

The nitrate reductase and glutamic oxaloacetic transaminase activities in tissues grown on media containing either sodium nitrate, potassium nitrate or calcium nitrate were more or less equal. However, when ammonium nitrate was substituted as a nitrogen source, the nitrate reductase activity was repressed and glutamic oxaloacetic transaminase activity promoted (Experiment 18). Addition of casein hydrolysate in the medium containing nitrate repressed the activity of nitrate reductase, but promoted the glutamic oxaloacetic transaminase. The repression of nitrate reductase and promotion of glutamic oxaloacetic transaminase enzymes became pronounced with

increasing concentration of casein hydrolysate in the medium (Experiment 19). Studies with the effect of molybdenum on nitrate reductase in Datura callus tissues showed that at 0.001 mg/l concentration, the enzyme activity enhanced and at higher levels the activity declined (Experiment 20).

Kinetin and 2,4-D when added either singly or in combination promoted the activity of acid phosphatase (Experiment 21). Kinetin had greater stimulatory effect than 2,4-D at their optimal concentrations.