CHAPTER III

ESTABLISHMENT AND GROWTH OF *DATURA*, COTTON AND *CASSIA* ANTHER CALLUS AND SUSPENSION CULTURES IN COMPLETELY DEFINED MEDIA

ESTABLISHMENT AND GROWTH OF *DATURA*, COTTON AND *CASSIA* ANTHER CALLUS AND SUSPENSION CULTURES IN COMPLETELY DEFINED MEDIA

The experiments described in the present chapter were aimed at successful establishment of the tissue cultures on a chemically defined medium avoiding coconut milk for precise understanding of the physiological and biochemical changes involved in the growth of the tissues.

The callus cultures initiated from the anthers of *Datura metel* L. (Family, Solanaceae) by Rao and Mehta (1968) were maintained in *Datura metel* medium (Table I, Chapter II) supplemented with 10% coconut milk, 2% sucrose and 2 mg/l 2,4-D. Similarly, the callus cultures derived from the anthers of *Cassia fistula* (Family, Caesalpinae), and from a hybrid variety 'Sankar 4' of cotton (*Gossypium hirsutum* Linn. (Family, Malvaceae) were initiated by Bagde and Mehta (unpublished) and maintained on a complex Murashige and Skoog's medium (Table 2, Chapter II). All the three tissues were transferred to modified Murashige and Skoog's medium (modified MS medium) as given in Table 3 (Materials and Methods, Chapter II) and their growth in completely defined medium was examined.

Experiment 3-1 : Establishment of *Datura* Anther Callus and Suspension Cultures on Completely Defined Medium

In order to determine the most suitable synthetic medium for the rapid and continuous growth of *Datura* anther callus which was originally maintained on complex *Datura metel* medium (Table 1, Chapter II), Murashige and Skoog's medium (Table 3, Chapter II) was tested with several modifications. The following growth substances in addition to 2% sucrose were incorporated into the medium singly or in combination:

(i) 2,4-dichlorophenoxyacetic acid (2,4-D),(2.0 mg/l),
(ii) 2,4-D (2.0 mg/l) and kinetin (0.04 mg/l),
(iii) 2,4-D (2.0 mg/l) and kinetin (0.4 mg/l),
(iv) Indoleacetic acid (IAA) (0.5 - 2 mg/l) and kinetin (0.4 mg/l).

During the course of culture for 3 weeks growth of the callus was found to be most rapid on the medium containing 2.0 mg/l 2,4-D and 0.4 mg/l kinetin when compared to the other auxin-kinetin combinations (Fig. 1). On further sub-cultures onto the same medium (i.e. containing 2.0 mg/l 2,4-D and 0.4 mg/l kinetin) the callus maintained vigorous growth. It turned light green in colour and became highly fragile.

Fig. 1. Growth of *Datura metel* L. anther callus on completely defined medium

Inoculum size: 300±10 mg tissue in 40 ml of modified Murashige and Skoog's medium (Table 3, Chapter II).

Photographed after 30 days.

Fig.1

After four passages on the solid medium, the callus pieces were transferred to Erlenmeyer flasks containing liquid medium of the same composition. The flasks were constantly agitated on a horizontal rotary shaker in constant temperature (26±2°C) culture room which was continuously lighted. Measured aliquots of cell suspension were transferred to freshly made liquid medium at intervals of every 3 weeks. The cell suspension thus obtained was used as inoculum for examining the patterns of growth in all the subsequent experiments.

Experiment 3-2 : Growth Curve of Datura Cell Suspensions Cultured in Completely Defined Medium

After several passages on modified Murashige and Skoog's medium (Table 3, Chapter II) measured aliquots of Datura cell suspensions weighing approximately 300±30 mg by fresh weight were transferred to Erlenmeyer flasks containing 40 ml of the freshly made culture medium.

The flasks were continuously agitated on a horizontal rotary shaker in culture room which was constantly lighted and maintained at 26±2°C. Six replicates were harvested at intervals of 5 days up to 20 days for determining growth.

Table 4 : Standard Growth Curve of Datura Suspension Cultures Grown in Synthetic Medium*

Inoculum : 300±30 mg tissue by fresh weight (Dry weight 18.4 mg) in 40 ml of modified Murashige and Skoog's medium (Table 3, Chapter II) supplemented with 2% sucrose, 2 mg/l 2,4-D and 0.4 mg/l kinetin.

Incubation: 20 days in light at 26±2°C

Time (days)	Fresh wt. (mg)	Dry wt. (mg)
0	300±10 (10)	18.4 (2.8)
5	482 (12)	24.0 (4.1)
10	963 (16)	52.0 (3.4)
15	8117 (56)	320.0 (4.2)
20	8402 (48)	350.0 (2.8)

*Data represent average of six replicates.
Figures in the parenthesis represent standard error.

Fig. 2. Progressive changes in growth (Fresh wt.) of *Datura* cell suspensions cultured in completely defined medium (Table 3, Chapter II).

Inoculum size: 300±30 mg tissue in 40 ml medium.

Experimental details as given in Table 4.

Fig. 3. Progressive changes in growth (Dry wt.) of *Datura* cell suspensions cultured in completely defined medium (Table 3, Chapter II).

Inoculum size: 300±30 mg tissue in 40 ml medium.

Experimental details as given in Table 4.

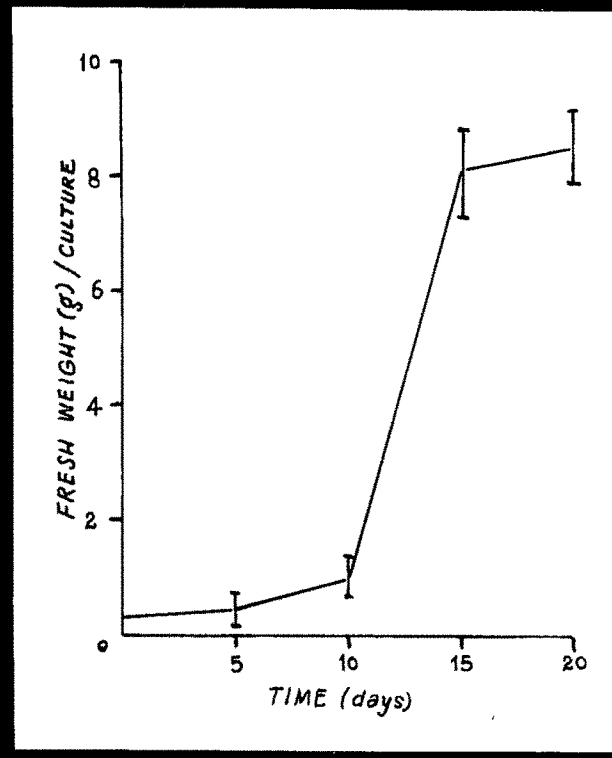
FRESH WEIGHT (g) / CULTURE
10
8
6
4
2
0
5
10
15
20
TIME (days)

Fig. 2

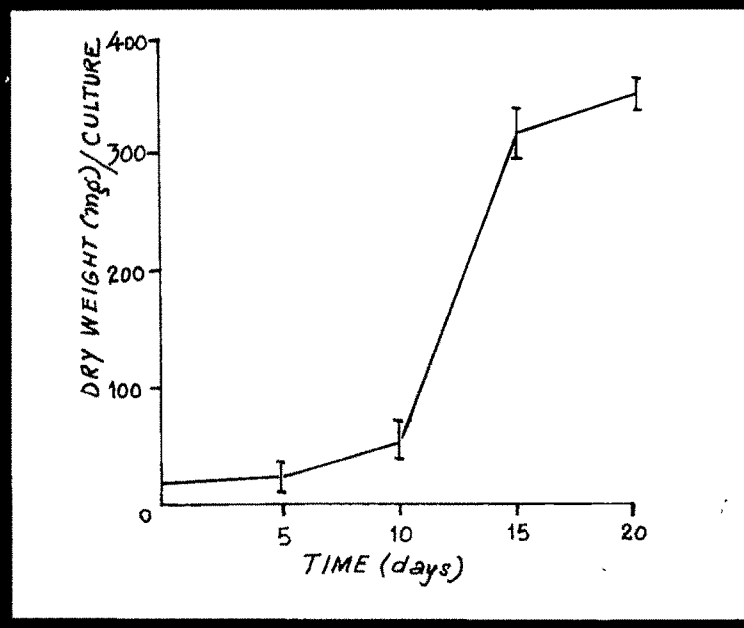
DRY WEIGHT (mg) / CULTURE
400
300
200
100
0
5
10
15
20
TIME (days)

Fig. 3

Fig. 4. Progressive changes in growth (Log Fresh wt. & Log Dry wt.) of *Datura* cell suspension cultured in completely defined medium (Table 3, Chapter II).

Inoculum size: 300±30 mg tissue in 40 ml medium.

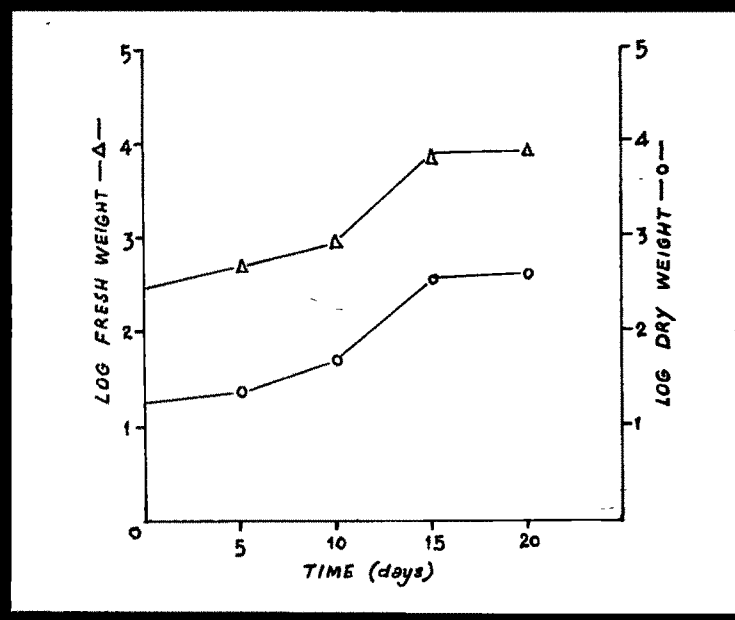

Fig. 4

The growth, measured in terms of fresh and dry weights and plotted against time, showed the usual type of sigmoid curve (Fig. 2 & 3). Data for fresh and dry weights (Table 4) when plotted on semilog basis (Fig. 4) showed that after an initial lag phase growth was rapid between tenth and fifteenth days and after twentieth day increase in growth slowed down. During the course of incubation for 20 days, an overall 28 fold increase in fresh weight and over 19 fold increase in dry weight was recorded.

Experiment 3-3 : Establishment of Cotton and Cassia Callus Cultures on Completely Defined Medium

Callus cultures of cotton and Cassia initiated and maintained on complex (i.e. coconut milk containing) medium (Table 2, Chapter II) were transferred to modified Murashige and Skoog's medium (Table 3, Chapter II) containing 2.0% sucrose and supplemented with the following combinations of auxin and kinetin:

(i) IAA (1-2 mg/l) and kinetin (0.1 mg/l),
(ii) NAA (0.5-2 mg/l) and kinetin (0.4 mg/l),
(iii) 2,4-D (1-2 mg/l) and kinetin (0.4 mg/l).

Fig. 5 & 6. Growth of *Cassia* and Cotton anther callus cultures on completely defined medium (Table 3, Chapter II)

Inoculum size: 200±10 mg tissue in 30 ml medium.

Photographed after 30 days.

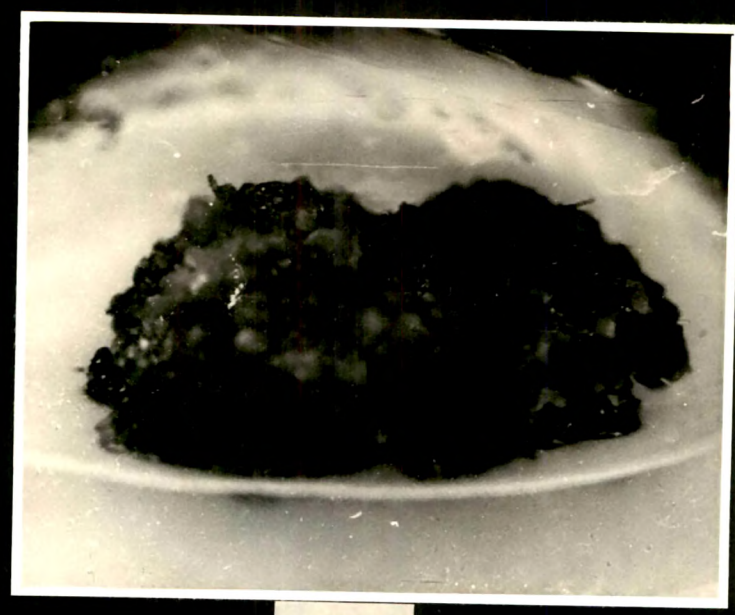

Fig. 5

Fig. 6

During the course of incubation for 4 weeks, growth of both cotton and Cassia tissues was found to be pronounced in the medium containing 2 mg/l 2,4-D and 0.4 mg/l kinetin (Fig. 5 & 6). Cotton tissues were fragile and white in colour when compared to the tissues of Cassia. The latter, however, were dark green showing a greater amount of chlorophyll. On prolonged incubation (i.e. after 6 weeks), the callus turned dark brown in colour. Regular subcultures were made at 3 weeks interval for obtaining sufficient 'clones' of tissues for further experimentation.

Experiment 3-4 : Comparative Study of the Growth of Cassia and Cotton Tissues on Completely Defined Medium

Regularly subcultured tissues of Cassia and cotton, well established on completely defined medium, were used to compare their growth dynamics.

Weighed amount (200±10 mg) of callus tissues was transferred to Erlenmeyer flasks containing 30 ml of culture medium (Table 3, Chapter II). The progress in growth of the callus cultures incubated for 20 days in light at 26±2°C was studied at intervals of 5 days. Six replicates were harvested for the determination of growth.

Table 5 : Growth Curve of Cotton Anther Callus Cultures*

Inoculum : 200±10 mg of tissue (Dry weight: 5.2 mg) in 30 ml of modified Murashige and Skoog's medium (Table 3, Chapter II) supplemented with 2% sucrose, 2.0 mg/l 2,4-D and 0.4 mg/l kinetin.

Incubation: 20 days in light at 26±2°C.

Time (days)	Fresh wt. (mg)	Dry wt. (mg)
0	200±10 (8.2)	5.2 (0.6)
5	375 (16.0)	12.0 (0.8)
10	1251 (28.6)	56.0 (1.2)
15	4621 (54.8)	131.0 (4.8)
20	4559 (62.6)	142.0 (3.6)

*Data represent average of six replicates.
Figures in the parenthesis represent standard error.

Table 6 : Growth Curve of *Cassia* Anther Callus Cultures*

Inoculum : 200±10 mg tissue by fresh weight (Dry weight: 7.6 mg) in 30 ml of modified Murashige and Skoog's medium (Table 3, Chapter II) supplemented with 2% sucrose, 2.0 mg/l 2,4-D and 0.4 mg/l kinetin.

Incubation: 30 days in light at 26±2°C

Time (days)	Fresh wt. (mg)	Dry wt. (mg)
0	200±10 (11.4)	7.6 (0.56)
5	439 (12.0)	21.0 (2.20)
10	1110 (26.0)	51.0 (1.62)
15	2356 (58.2)	104.0 (3.51)
20	4522 (124.0)	172.0 (2.83)
30	5302 (168.2)	192.0 (4.1)

*Data represent average of six replicates.
Figures in the parenthesis represent standard error.

Fig. 7. Progressive changes in growth (Fresh & Dry wts.) of Cotton anther callus cultured on completely defined medium (Table 3, Chapter II).

Inoculum size: 200±10 mg tissue in 40 ml medium.

Other experimental details as given in Table 5.

Fig. 8. Progressive changes in growth (Fresh & Dry wts.) of Cassia anther callus on completely defined medium (Table 3, Chapter II).

Inoculum size: 200±10 mg tissue in 40 ml medium.

Experimental details as given in Table 6.

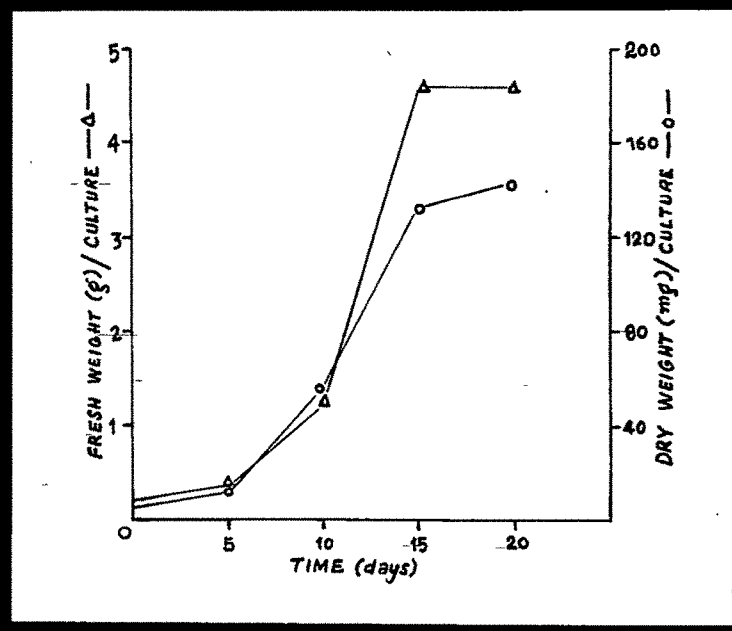
FRESH WEIGHT (g)/CULTURE —Δ—
DRY WEIGHT (mg)/CULTURE —o—
TIME (days)
0
1
2
3
4
5
40
80
120
160
200
5
10
15
20

Fig. 7

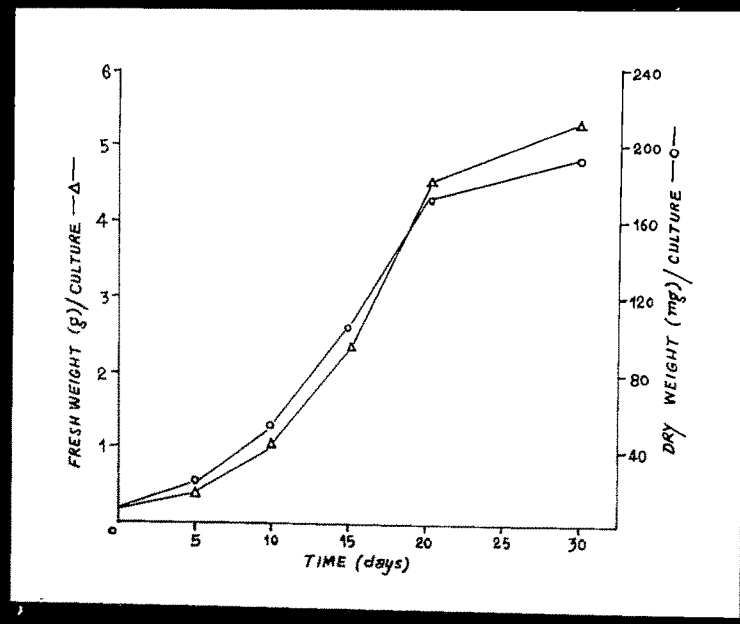
FRESH WEIGHT (g)/CULTURE —Δ—
DRY WEIGHT (mg)/CULTURE —o—
TIME (days)
0
1
2
3
4
5
6
40
80
120
160
200
240
5
10
15
20
25
30

Fig. 8

The growth as measured by fresh and dry weights are presented in Tables 5 and 6. The data plotted against time showed usual sigmoid curve in cotton tissues (Fig. 7); after an initial lag, the growth was rapid from 5 to 15 days after which it slowed down. In the case of Cassia, however, the growth enhanced steadily upto 20 days (Fig.8). During the course of incubation for 20 days overall 22.7 fold increase in fresh weight and 28.4 fold increase in dry weight were recorded in callus cultures of cotton; the corresponding values recorded in Cassia being 22.1 fold and 25.3 fold respectively.

DISCUSSION

It was clear from the experiments that the callus which was originally induced from the excised anthers of Datura, Cassia and cotton (Hybrid variety 'Sankar 4') on coconut milk containing complex medium grew quite satisfactorily when transferred to a completely defined medium.

Of the auxins tested, 2,4-D at 2.0 mg/l concentration in combination with 0.4 mg/l kinetin supported the highest growth of all the three tissues.

When the growth curve of Datura cell suspensions in agitated liquid medium was examined (Experiment 3-2), typical sigmoid curve was observed. After a pronounced lag phase for nearly 5 to 7 days there was rapid increase in growth upto day 15. The highest growth recorded on day 15 registered an overall 28 fold increase in fresh weight and 19.4 fold increase in dry weight.

The growth of the callus cultures of cotton on completely defined medium when plotted against time also showed the usual sigmoid curve. The lag phase was observed for 5 days followed by a prolonged phase of rapid growth which extended upto 15 days. In the case of Cassia tissues, on the other hand, the growth enhanced steadily throughout the culture period of 20 days, not showing a well recognised lag phase. During the course of incubation for 20 days cotton tissues recorded higher growth value (i.e. Final wt/Initial wt) as compared to Cassia tissues.