CHAPTER III

RESULTS

Section A - Initiation and establishment of callus cultures of *Nicotiana tabacum* L. var. Anand-2 and *Gossypium hirsutum* L., cv. Sankar-5.

CHAPTER III

RESULTS

The experiments designed and described in the present chapter were aimed at (i) successful establishment of tissue cultures of Nicotiana tabacum L. var. Anand-2, and Gossypium hirsutum L., cv. Sankar-5, (ii) to facilitate better understanding of the physiological and biochemical changes underlying growth as well as organised and unorganised development of these tissues.

Care was taken to minimise the degree of variability in the present experimental set up. The factors taken into consideration included environmental uniformity as well as homogenity, age and size of the experimental materials. In order to confirm and minimise the experimental variation the experiments were repeated twice and the average reading was taken. Furthermore, to reduce variability amongst treatments, and to reduce sampling error, 5 to 6 replicates were harvested at random at a time, pooled together and then analysed.

The experiments were carried out using : (1) the floral bud callus of Nicotiana tabacum L. var. Anand-2 and (2) the anther callus tissues of Gossypium hirsutum L., cv. Sankar-5.

Expt. 1. Initiation and establishment of callus cultures of Nicotiana tabacum.

To initiate callus cultures of tobacco, young flower buds (10-12 mm long) were cut with a scalpel longitudinally and cultured aseptically on MS medium containing 2% sucrose and supplemented with 2.0 mg/l each of IAA, NAA and KN. These flasks were incubated in continuous light at 26±2°C.

Callus developed from the cut ends of the floral parts mainly sepals within 15 to 20 days. The flower buds were completely overgrown by callus mass within 4 weeks. The callus was green in colour and fragile in texture. Such healthy looking callus was maintained by subculturing every 30 days onto fresh MS medium containing 2 per cent sucrose and supplemented with 2.0 mg/l each of IAA, NAA and KN. The stock of callus mass was thus built up for experiments (Fig. 1 A).

Expt. 2. Initiation and establishment of callus cultures of Gossypium hirsutum.

Anthers were isolated aseptically from sterilised floral buds and transferred onto MS medium containing 2 per cent sucrose and supplemented with 2.0 mg/l each of IAA, NAA and KN for the induction of callus tissue.

Callus formation was observed from the anther wall

Fig. 1 A. Tobacco callus. Fig. 1B. Cotton callus.

during the second week in culture. By the end of 4 weeks callus had proliferated enough to engulf the whole anther. The callus tissue thus initiated was creamy white in colour in the beginning. However, it turned lush green during the course of culture and became granular and highly friable. By successive transfers of this callus every 30 days onto freshly made medium of similar composition stocks of tissues were established (Fig. 1 B).

These callus tissues of tobacco and cotton were used during the course of study for their growth requirements, morphogenetic capabilities and also to examine certain key biochemical parameters associated with organogenesis.

Section B - Growth and accumulation of Total and Reducing sugars in callus cultures of *Gossypium hirsutum*.

Growth of plants and their isolated tissues is dependent on the availability of a balanced milieu of nutrient factors and growth substances. As stated in the Introduction (Chapter I) the growth and differentiation of plant cells and tissues is greatly influenced by the cultural conditions including the nutrient status of the medium. Of these, hormones and carbohydrates are essential supplements in the nutrient medium for the successful culture of a host of excised plant tissues and also for the accumulation of different metabolites by these tissues.

Experiments were designed, therefore, to find out the specific hormonal requirement and suitable carbohydrate as energy source for rapid and continuous growth of callus. The accumulation of total and reducing sugars in these cultures was also examined.

To study the hormonal (Section B - I) as well as carbohydrate (Section B - II) influence on growth and the accumulation of total and reducing sugars, callus masses weighing 400±40 mg by fresh weight were incubated on 40 ml agar medium in continuous light at 26±2°C. The callus tissues were transferred onto MS basal medium for 1 week before hormonal and carbohydrate treatments to minimize any carry-over effects. The tissues were harvested at the end of 30 days in culture and analysed for fresh and dry weight increases, and also for the accumulation of total and

reducing sugar contents, both culture wise and on per cent basis.

Section B - I : Hormonal influence

Expt. 3. Influence of auxins

IAA, IBA, NAA and 2,4-D were individually incorporated into the MS basal medium containing 2% sucrose. Each auxin was tested at the concentrations of 0.2, 1.0, 2.0 and 5.0 mg/l. Callus tissues cultured on MS basal medium with 2% sucrose acted as the control.

At the end of incubation period of 30 days, the callus tissues of cotton increased 2.85 and 3.30 folds respectively in fresh and dry weights when cultured on MS basal medium. Accumulation of total sugars was 1.30 mg/culture and 2.47 mg% and that of reducing sugars was 0.98 mg/culture and 1.85 mg% (Table 2).

There was a steady increase of both fresh and dry weights with the increase of IAA concentration in the medium from 0.2 mg/l to 2.0 mg/l. At 5.0 mg/l IAA concentration both fresh and dry weights decreased. slightly. Highest fold-wise increase in fresh weight (over 32 fold) and dry weight (over 21 fold) was registered at 2.0 mg/l IAA. Similarly the reducing sugar content was maximum in tissues grown on

Table : 2. Effect of auxin on growth and accumulation of total and reducing sugars in callus cultures of Gossypium hirsutum.

Inoculum : 400±40 mg callus by fresh weight (16±4 mg dry weight) on 40 ml MS basal medium with 2% sucrose and various auxins at different concentrations.

Incubation: 30 days in continuous light at 26±2°.

Auxin	Concentration mg/l	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars	
				mg/cult.	mg %	mg/cult.	mg %
-	-	1140.0 (±55.3)	52.8 (±8.9)	1.30	2.47	0.98	1.85
IAA	0.2	10641.6 (±423.9)	340.5 (±26.1)	17.23	5.06	8.51	2.50
"	1.0	11602.2 (±431.5)	346.3 (±25.9)	12.71	3.67	7.48	2.16
"	2.0	13072.3 (±510.1)	348.8 (±18.9)	14.20	4.07	12.63	3.62
"	5.0	10381.1 (±398.2)	320.6 (±18.1)	9.39	2.93	6.09	1.90
IBA	0.2	10443.1 (±398.7)	390.1 (±29.6)	27.07	6.94	5.15	1.32
"	1.0	8477.3 (±370.1)	368.5 (±27.4)	14.52	3.94	5.97	1.62
"	2.0	8238.1 (±365.7)	361.8 (±26.5)	27.53	7.61	4.41	1.22
"	5.0	6751.4 (±250.9)	313.7 (±24.8)	17.79	5.67	16.94	5.40
NAA	0.2	11360.4 (±450.1)	340.4 (±18.7)	11.61	3.41	8.58	2.52
"	1.0	10222.1 (±376.5)	337.6 (±22.1)	18.43	5.46	8.04	2.38
"	2.0	7424.0 (±299.7)	307.4 (±20.0)	16.91	5.50	6.64	2.16
"	5.0	9948.5 (±369.6)	320.8 (±23.2)	9.85	3.07	4.46	1.39
2,4-D	0.2	7518.8 (±300.4)	303.9 (±21.6)	12.25	4.03	8.51	2.80
"	1.0	7719.9 (±331.1)	288.6 (±20.3)	19.37	6.71	5.89	2.04
"	2.0	7348.2 (±305.8)	268.6 (±18.2)	15.34	5.71	6.39	2.38
"	5.0	5622.1 (±212.6)	245.2 (±14.3)	9.39	3.83	5.93	2.42

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

2.0 mg/l IAA medium. However, the total sugar accumulated highest in tissues incubated in low (0.2 mg/l) IAA level (Table 2).

Both fresh and dry weights decreased steadily with increasing concentrations of IBA from 0.2 mg/l to 5.0 mg/l. Tissues grown on 2.0 mg/l IBA registered maximum accumulation of total sugars while tissues on 5.0 mg/l IBA showed highest reducing sugar content both on mg/culture and on mg% basis (Table 2). Thus no correlation between growth and sugar accumulation was seen.

Increasing NAA concentration from 0.2 mg/l to 2.0 mg/l decreased growth of the tissue as measured by fresh weight. However, NAA at 5.0 mg/l level again enhanced fresh weight of the tissue. NAA levels tested had no marked effect on the tissue dry weight.

On the other hand, total sugars in the tissue on mg% basis steadily increased from 0.2 mg/l NAA to 2.0 mg/l, but decreased at the higher NAA level (5.0 mg/l). Contrary to this, reducing sugar contents decreased both culture wise and on per cent basis with increasing NAA concentrations. (Table 2).

Low concentration of 2,4-D (0.2 mg/l) supported maximum growth of the tissue. Dry weight of the tissue declined with increasing 2,4-D concentrations from 0.2 to

5.0 mg/l. Similarly, the reducing sugar content on per cent basis was maximum in tissues grown on 0.2 mg/l 2,4-D medium. However, total sugar accumulation was highest at 1.0 mg/l 2,4-D level both on mg/culture and mg% basis (Table 2).

Expt. 4. Influence of cytokinins and gibberellic acid

Kinetin and 6-benzyl-aminopurine (BAP) were used in the concentrations of 0.04, 0.4, 1.0 and 2.0 mg/l, while gibberellic acid (GA_3) was used in the concentrations of 5.0, 25.0, 50.0 and 100.0 mg/l. These hormones were separately incorporated into MS basal medium containing 2% sucrose. The callus tissues of cotton were grown on the above mentioned media for a period of 30 days before analysis for growth and accumulation of sugars.

Increasing concentrations of kinetin (from 0.04 to 2.0 mg/l) increased both fresh and dry weight of the tissue. On the other hand, tissues grown on lower concentration (0.04 mg/l) of kinetin accumulated highest total sugars both on mg/culture and on mg% basis. However, tissues grown on 1.0 mg/l kinetin registered maximum reducing sugars (Table 3).

With increasing concentrations of BAP in the medium from 0.04 mg/l to 1.0 mg/l, there was an increase in fresh as well as dry weights of the tissue like kinetin, maximum growth being recorded at 1.0 mg/l BAP (fresh weight increase

Table : 3. Effect of cytokinin and gibberellin on growth and accumulation of total and reducing sugars in callus cultures of Gossypium hirsutum.

Inoculum : 400±40 mg callus by fresh weight (16±4 mg dry weight) on 40 ml MS basal medium with 2% sucrose and various levels of cytokinins and GA.

Incubation : 30 days in continuous light at 26±2°.

Phyto-hormone	Concen-tration mg/l	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars	
				mg/cult.	mg %	mg/cult.	mg %
-	-	1140.0 (±55.3)	52.8 (±8.9)	1.30	2.47	0.98	1.85
KN	0.04	9895.6 (±381.5)	360.0 (±22.1)	20.56	5.71	7.34	2.04
"	0.4	11690.7 (±465.8)	404.5 (±25.4)	17.03	4.25	6.55	1.62
"	1.0	13285.8 (±582.3)	414.2 (±24.5)	19.68	4.75	8.95	2.16
"	2.0	14699.2 (±591.7)	419.5 (±23.6)	19.93	4.75	6.38	1.52
BAP	0.04	1594.1 (±120.4)	88.5 (± 8.6)	4.39	4.96	2.42	2.74
"	0.4	10778.8 (±381.7)	381.3 (±20.9)	27.64	7.25	16.93	4.44
"	1.0	12942.3 (±399.7)	415.0 (±24.3)	20.58	4.96	11.37	2.74
"	2.0	8520.9 (±276.5)	368.0 (±18.8)	35.11	9.54	20.90	5.68
GA_3	5.0	7160.3 (±308.5)	226.2 (±16.2)	9.61	4.25	4.61	2.04
"	25.0	7563.2 (±300.6)	241.5 (±14.6)	6.64	2.75	2.95	1.22
"	50.0	5283.6 (±165.8)	204.4 (±10.9)	7.32	3.58	4.42	2.16
"	100.0	3577.0 (±140.1)	117.9 (± 8.7)	4.92	4.17	1.46	1.24

Data represents an average of 5 replicates.

Figures in parenthesis represent standard error.

over 32 fold, dry weight increase nearly 26 fold). However, at 2.0 mg/l BAP, while the tissue growth declined the total and reducing sugar content registered peak values (Table 3).

At 25.0 mg/l GA_3 level the increase in fresh and dry weights of the tissue was slightly higher than at 5.0 mg/l. Fold-wise increases of the tissue grown on 25.0 mg/l GA_3 were 18.91 and 15 respectively on fresh and dry weight basis. However, both fresh and dry weights decreased steadily with the increasing conc. of GA_3 from 25.0 mg/l to 100.0 mg/l. On the other hand, the total and reducing sugar content in the callus was high at low GA_3 concentration (5.0 mg/l) and with further increase in GA_3 level, the sugar contents declined (Table 3).

Expt. 5. Influence of auxin-cytokinin interaction

To study the effect of auxins and cytokinins in combination the conc. of kinetin was kept constant at 2.0 mg/l, while that of auxins was varied from 0.2 to 2.0 mg/l. Highest increase in fresh and dry weight (24.3 and 22.8 folds respectively) was obtained on IAA (2.0 mg/l) + KN (2.0 mg/l) medium. When IAA was replaced by other auxins, growth values declined in the order of NAA, IBA and 2,4-D.

Both total and reducing sugars showed maximum values

on IAA (2.0 mg/l) + KN (2.0 mg/l) combination which also supported highest callus growth. Accumulation of sugars declined when IAA was replaced by IBA or NAA or 2,4-D (Table 4).

In yet another experiment, auxins in a range of 0.2 to 2.0 mg/l were tested in combination with BAP (1.0 mg/l). IAA (2.0 mg/l)+ BAP (1.0 mg/l) combination supported maximum fresh and dry weights of the tissues out of the four combinations tested; whereas 2,4-D (0.2 mg/l) + BAP (1.0 mg/l) resulted in the lowest growth values.

Though 2,4-D + BAP combination reduced callus growth it favoured highest accumulation of total and reducing sugars in the tissue. Sugar accumulation declined when IAA, NAA or IBA were used along with BAP (Table 4).

Expt. 6. Influence of auxin-gibberellic acid and auxin-cytokinin-gibberellic acid interactions

The effect of different concentrations and combinations of auxin + GA, auxin + kinetin + GA and auxin + BAP + GA on growth and accumulation of total and reducing sugars are presented in Table 5.

Throughout the experiment, the concentration of the GA was kept constant (25.0 mg/l). GA when combined with NAA

Table : 4. Effect of auxin-cytokinin interaction on growth and accumulation of total and reducing sugars in callus cultures of Gossypium hirsutum.

Inoculum : 400±40 mg callus by fresh weight (16±4 mg dry weight on 40 ml MS basal medium with 2% sucrose and different levels of auxin, cutokinin combination.

Incubation : 30 days in continuous light at 26±2°.

Hormonal combination	Hormonal concentration (mg/l)	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars	
				mg/cult.	mg %	mg/cult.	mg %
IAA + KN	2.0+2.0	9725.1 (±381.4)	365.3 (±13.7)	21.92	6.00	17.32	4.94
IBA + KN	0.2+2.0	7963.7 (±236.9)	314.5 (±12.1)	17.83	5.67	16.35	5.20
NAA + KN	0.2+2.0	9399.6 (±389.5)	362.6 (±12.8)	16.32	4.50	11.24	3.10
2,4-D+KN	0.2+2.0	6713.1 (±200.0)	299.1 (±10.7)	14.30	4.78	10.05	3.36
IAA + BAP	2.0+1.0	10803.9 (±399.4)	376.1 (±16.6)	35.09	9.33	16.17	4.30
IBA + BAP	0.2+1.0	9472.7 (±361.7)	344.1 (±13.5)	15.49	4.50	11.56	3.36
NAA + BAP	0.2+1.0	8604.7 (±314.9)	303.7 (±11.8)	17.22	5.67	12.21	4.02
2,4-D+BAP	0.2+1.0	5468.1 (±216.6)	254.8 (±10.2)	25.61	10.05	14.47	5.68

Data represents an average of 5 replicates.
Figures in the parenthesis represent standard error.

Table : 5. Effect of auxin-gibberellin, and auxin-cytokinin-gibberellin interactions on growth and accumulation of total and reducing sugars in callus cultures of Gossypium hirsutum.

Inoculum : 400±40 mg callus by fresh weight (16±4 mg dry weight) on 40 ml MS basal medium with 2% sucrose and different levels of auxin, gibberellin and auxin, cytokinin, gibberellin combination.

Incubation : 30 days in continuous light at 26±2°.

Hormonal combination	Hormonal concentration (mg/l)	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars	
				mg/cult.	mg%	mg/cult.	mg%
IAA+GA_3	2.0+25.0	7010.4 (±215.7)	281.2 (±11.5)	22.33	7.94	12.09	4.30
IBA+GA_3	0.2+25.0	4737.8 (±175.9)	211.3 (± 9.7)	8.92	4.22	6.04	2.86
NAA+GA_3	0.2+25.0	8689.5 (±301.2)	324.9 (±13.4)	19.49	6.00	13.06	4.02
2,4-D+GA_3	0.2+25.0	3110.7 (±144.9)	163.2 (± 8.1)	6.89	4.22	4.28	2.62
IAA+KN+GA_3	2.0+2.0+25.0	10791.8 (±381.6)	354.6 (±12.2)	18.12	5.11	12.84	3.62
IBA+KN+GA_3	0.2+2.0+25.0	9694.5 (±379.5)	344.3 (±13.0)	13.57	3.94	11.98	3.48
NAA+KN+GA_3	0.2+2.0+25.0	9802.7 (±365.8)	312.1 (±11.7)	18.73	6.00	17.23	5.52
2,4-D+KN+GA_3	0.2+2.0+25.0	10931.5 (±400.1)	321.4 (±12.2)	15.36	4.78	14.27	4.44
IAA+BAP+GA_3	2.0+1.0+25.0	15114.1 (±600.3)	392.0 (±17.7)	16.54	4.22	11.68	2.98
IBA+BAP+GA_3	0.2+1.0+25.0	11162.4 (±409.8)	322.2 (±12.4)	17.37	5.39	13.86	4.30
NAA+BAP+GA_3	0.2+1.0+25.0	15710.2 (±602.6)	452.3 (±19.9)	25.65	5.67	18.82	4.16
2,4-D+BAP+GA_3	0.2+1.0+25.0	7982.8 (±233.4)	258.3 (±11.2)	17.07	6.61	12.24	4.74

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

(0.2 mg/l) yielded good growth when compared to the other auxins, minimum growth being registered in presence of 2,4-D.

Total and reducing sugar accumulation was highest on IAA + GA medium, next in order being on NAA + GA and IBA + GA media. 2,4-D + GA combination resulted in inhibition of total and reducing sugar accumulation (Table 5).

Of the four combinations of auxin + kinetin + GA incorporated into MS basal medium, maximum growth values were supported by 2.0 mg/l IAA + 2.0 mg/l KN + 25.0 mg/l GA, followed closely by IBA + KN + GA, 2,4-D + KN + GA and then NAA + KN + GA.

Accumulation of total and reducing sugars was maximum on NAA + KN + GA medium, where callus growth was least, followed by IAA + KN + GA, 2,4-D + KN + GA and then IBA + KN + GA (Table 5).

When kinetin was replaced by BAP, growth on fresh and dry weight basis was higher on NAA + BAP + GA medium, actual fold-wise increases being 39.3 and 28.3 respectively. Growth values declined with IAA + BAP + GA, IBA + BAP + GA and lowest values were recorded again on 2,4-D + BAP + GA medium.

However, accumulation of total and reducing sugars was maximum on 2,4-D + BAP + GA, followed by NAA, IBA and IAA containing media (Table 5).

Summary : Of the four auxins tested for their influence on growth and accumulation of total and reducing sugars, IAA and IBA showed marked influence when compared to NAA and 2,4-D. While IAA (2.0 mg/l) supported maximum fresh weight of the tissue, IBA (0.2 mg/l) registered maximum dry weight.

Maximum accumulation of total sugars on mg% basis was found on 2.0 mg/l IBA and that of reducing sugars on 5.0 mg/l IBA. Except in case of IBA, the auxin levels which supported highest dry weight of the tissue also favoured maximum reducing sugar content (Expt. 3).

Of the two cytokinins tested, KN supported higher growth than BAP. However, BAP was more effective than KN in supporting maximum accumulation of sugars both on culture and mg% basis. Higher kinetin concentrations supported maximum growth, while lower concentrations supported sugar accumulation. Contrary to this, lower BAP concentration registered maximum growth, and higher BAP concentration resulted in maximum accumulation of sugars.

Lower concentration of GA (5.0 mg/l) was more favourable for accumulation of total and reducing sugars, while higher GA concentration (25.0 mg/l) supported maximum growth values (Expt. 4).

Of the four auxins tested in combination with kinetin,

IAA + KN yielded maximum fresh and dry weight and also higher accumulation of sugars. Likewise IAA + BAP combination also favoured tissue growth when compared to the other auxins. When 2,4-D was combined either with KN or with BAP, there was marked decline in growth values both on fresh and dry weight basis. However, 2,4-D enhanced sugar accumulation in tissues when used along with BAP (Expt. 5).

When GA was combined with IAA, IBA, NAA and 2,4-D, growth values were higher on IAA + GA medium. Among auxin + kinetin + GA combinations, growth was highest on IAA + KN + GA medium. However, replacement of KN by BAP has resulted in highest growth values on NAA + BAP + GA medium. Whenever 2,4-D was combined either with GA or KN + GA or BAP + GA, there was a pronounced decline in growth value.

Of all the 12 combinations, accumulation of total sugars was higher on IAA + GA medium on mg% basis, while reducing sugars was higher on NAA + KN + GA medium (Expt. 6).

Section B - II : Carbohydrate influence

To study the influence of carbohydrates on growth and accumulation of sugars, the medium used was MS medium containing 0.2 mg/l NAA + 1.0 mg/l BAP + 25.0 mg/l GA_3.

Expt. 7. Effect of monosaccharides

Six monosaccharides glucose, fructose, galactose, arabinose, mannose and sorbose were separately tested each at 3 concentrations 1, 2 and 4%. There was a progressive increase in fresh and dry weight of the tissue with increasing glucose and fructose level in the medium. Glucose at 4% level supported over 36 fold increase in fresh and dry weights of the tissue. But fructose (4% level) supported better growth than glucose, with 40 and 47 fold-wise increases respectively on fresh and dry weight basis (Table 6).

With the increasing concentrations of glucose and fructose, there was also an increase in the accumulation of both total and reducing sugars on mg/culture as well as mg% basis.

Other monosaccharides tested - galactose, arabinose, mannose and sarbose did not support callus growth at all, at the concentrations used.

Table : 6. Effect of different monosaccharides on growth and accumulation of total and reducing sugars in callus cultures of Gossypium hirsutum.
Medium : MS + 0.2 NAA + 1.0 BAP + 25.0 GA_3.
Inoculum : 400±40 mg callus by fresh weight (16±4 mg dry weight) on 40 ml medium with different levels of monosaccharides.
Incubation : 30 days in continuous light at 26± 2°.

Mono-saccharide	Concen-tration gm %	Fresh weight mg./cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars	
				mg/cult.	mg %	mg/cult.	mg %
Glucose	1.0	7185.0 (±229.1)	174.6 (±9.5)	5.94	3.40	2.25	1.29
"	2.0	13390.6 (±504.8)	373.1 (±13.6)	10.19	2.73	4.81	1.29
"	4.0	14484.8 (±529.7)	588.9 (±24.8)	42.40	7.20	28.38	4.82
Fructose	1.0	6473.6 (±191.2)	181.5 (±8.2)	6.17	3.40	3.54	1.95
"	2.0	14773.2 (±589.6)	394.6 (±16.4)	9.47	2.40	7.22	1.83
"	4.0	16036.2 (±682.5)	750.4 (±30.8)	46.00	6.13	41.95	5.59
Galactose	1.0	1637.7 (±80.0)	65.7 (±4.9)	3.55	5.40	3.29	5.00
"	2.0	966.7 (±59.8)	49.7 (±3.6)	2.19	4.40	1.44	2.89
"	4.0	906.9 (±76.5)	65.5 (±5.1)	4.72	7.20	3.98	6.08
Arabinose	1.0	848.6 (±73.1)	32.9 (±2.7)	0.79	2.40	0.44	1.33
"	2.0	492.8 (±52.3)	25.8 (±3.0)	1.58	6.13	1.25	4.86
"	4.0	440.9 (±41.4)	31.3 (±3.4)	3.63	11.60	2.88	9.21
Mannose	1.0	453.5 (±42.8)	25.2 (±3.2)	1.03	4.07	0.78	3.09
"	2.0	385.6 (±39.9)	45.2 (±5.0)	2.62	5.80	2.31	5.10
"	4.0	797.8 (±29.3)	56.5 (±14.1)	22.39	8.73	16.90	6.59
Sorbose	1.0	356.3 (±39.4)	19.6 (±3.8)	0.98	4.98	0.63	3.20
	2.0	555.1 (±33.7)	28.2 (±4.5)	1.64	5.81	1.13	4.00
	4.0	380.8 (±37.2)	27.5 (±3.8)	2.19	7.95	1.61	5.84

Data represents an average of 5 replicates.
Figures in the parenthesis represent standard error.

Expt. 8. Effect of di- tri- and polysaccharides

Three disaccharides sucrose, maltose and lactose and one each of tri- and polysaccharides raffinose and starch were tested.

Sucrose, maltose supported growth but lactose did not support among the disaccharides tested. As was noticed earlier with monosaccharides, increasing concentrations of sucrose and maltose enhanced fresh and dry weights of the tissues as well as accumulation of sugars. Over 40 fold increase in fresh and dry weights of the tissues was noticed when 4% sucrose was incorporated. Replacement of sucrose by maltose at the same concentration resulted in 37 and 43 fold increases in fresh and dry weights respectively (Table 7).

The trisaccharide raffinose (pentahydrate) failed to support any growth of the tissues at the concentrations tested. Cotton callus tissues, however, could utilize starch (polysaccharide) as a source of carbon. As noticed earlier, increasing concentrations of starch increased both growth and sugar accumulation. Tissues grown on 4% starch medium showed over 27 and 21 fold increases respectively on fresh and dry weight basis (Table 7).

Expt. 9. Effect of sugar alcohols

Three sugar alcohols myo- inositol, mannitol and

Table : 7. Effect of different, di, tri, and polysaccharides on growth and accumulation of total and reducing sugars in callus cultures of Gossypium hirsutum.

Medium : MS + 0.2 NAA + 1.0 BAP + 25.0 GA_3.

Inoculum : 400±40 mg callus by fresh weight (16±4 mg dry weight) on 40 ml medium with different levels of di, tri and polysaccharides.

Incubation : 30 days in continuous light at 26±2°.

Carbo-hydrate	Concen-tration gm %	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars	
				mg/cult.	mg %	mg/cult.	mg %
Sucrose	1.0	8140.5 (±250.4)	189.5 (±10.2)	4.55	2.40	2.08	1.10
"	2.0	13710.2 (±560.7)	452.3 (±22.9)	19.90	4.40	6.06	1.34
"	4.0	16306.5 (±642.3)	645.6 (±33.4)	30.54	4.73	20.72	3.21
Maltose	1.0	6599.4 (±300.6)	157.6 (±12.5)	2.32	1.47	1.89	1.20
"	2.0	12431.0 (±468.7)	340.7 (±25.9)	9.30	2.73	4.94	1.45
"	4.0	14610.0 (±582.3)	685.4 (±42.6)	49.35	7.20	34.00	4.96
Lactose	1.0	385.6 (±37.9)	18.7 (±3.4)	1.06	5.68	0.73	3.88
"	2.0	460.7 (±43.1)	25.5 (±3.6)	1.71	6.71	1.08	4.23
"	4.0	863.7 (±72.9)	53.6 (±5.0)	4.41	8.23	3.94	7.36
Raffinose	1.0	547.7 (±32.4)	25.1 (±3.2)	1.16	4.64	0.83	3.29
"	2.0	591.6 (±36.9)	36.3 (±3.7)	2.36	6.51	1.66	4.57
"	4.0	519.5 (±31.5)	38.0 (±4.0)	3.16	8.32	2.91	7.67
Starch	1.0	4979.8 (±200.0)	125.4 (±6.3)	2.67	2.13	1.49	1.19
"	2.0	10568.9 (±420.2)	264.0 (±11.5)	7.21	2.73	3.01	1.14
"	4.0	11160.8 (±441.1)	341.6 (±13.8)	18.45	5.40	10.86	3.18

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

Table : 8. Effect of sugar alcohols on growth and accumulation of total and reducing sugars in callus cultures of Gossypium hirsutum.

Medium : MS + 0.2 NAA + 1.0 BAP + 25 GA_3.

Inoculum : 400±40 mg callus by fresh weight (16±4 mg dry weight) on 40 ml medium with different levels of sugar alcohols.

Incubation : 30 days in continuous light at 26±2°.

Sugar alcohol	Concentration gm %	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars mg/cult.	Total Sugars mg %	Reducing Sugars mg/cult.	Reducing Sugars mg %
Myo-inositol	1.0	513.6 (±40.0)	24.5 (±6.2)	0.59	2.40	0.15	0.61
"	2.0	379.3 (±32.6)	21.9 (±4.0)	0.26	1.20	0.10	0.45
"	4.0	301.1 (±30.2)	22.9 (±3.8)	1.19	5.20	0.59	2.57
Mannitol	1.0	375.1 (±33.4)	16.1 (±3.0)	0.34	2.13	0.13	0.78
"	2.0	397.5 (±36.8)	19.1 (±3.5)	0.56	2.91	0.23	1.23
"	4.0	397.9 (±35.1)	20.0 (±3.7)	1.18	5.89	0.43	2.17
Sorbitol	1.0	304.9 (±30.1)	11.6 (±2.5)	0.25	2.13	0.10	0.85
"	2.0	276.9 (±24.3)	15.2 (±2.9)	0.32	2.13	0.15	1.00
"	4.0	242.5 (±22.7)	14.6 (±2.9)	0.60	4.13	0.30	2.07

Data represents an average of 5 replicates.
Figures in the parenthesis represent standard error.

sorbitol were used separately but none of them could support growth of cotton callus tissues at the concentrations used. Callus tissues on these media either turned dark brown or black during the course of culture (Table 8).

Summary : Of the six monosaccharides tested for their influence on growth and accumulation of sugars, only glucose and fructose supported healthy callus growth. Other monosaccharides failed to support any growth. With the increase in glucose or fructose level in the medium, there was a marked increase in fresh and dry weights of the tissue as well in the accumulation of sugars (Expt. 7).

Of the three disaccharides tested, only sucrose and maltose supported growth, whereas lactose failed completely to support growth. The tri-saccharide raffinose also could not support growth of cotton callus, while polysaccharide starch proved to be a good source of carbon.

Increasing concentrations of sucrose, maltose and starch resulted in increase of fresh and dry weights of the tissues as well accumulation of total and reducing sugars both on culture and mg per cent basis (Expt. 8).

Three sugar alcohols myo-inositol, mannitol and sorbitol could not support growth of cotton callus tissues (Expt. 9).

Section C - I : Physiological studies with Amylase, Invertase, MDH, G-6-PDH, and FDPA and total and reducing Sugars and total starch during growth of callus tissues of tobacco and cotton.

Present study was undertaken in an attempt to examine the role of phytohormones in growth and the physiological and biochemical changes associated therewith. For both tobacco and cotton tissues, the same medium was used which supported rapid and maximum growth without any decline in growth values on prolonged culture. The medium used was MS basal fortified with 2 mg/l IAA, 2 mg/l NAA and 2 mg/l KN and 2% sucrose (Standard medium).

The results obtained are presented under the following heads :

(i) Studies with callus tissue of N. tabacum during its growth on Standard medium.

(ii) Studies with callus tissue of G. hirsutum during its growth on Standard medium.

Expt. 10. Studies with callus tissues of N. tabacum during its growth on Standard medium.

Fresh callus weighing 300±30 mg was inoculated onto 40 ml of the standard medium and the culture vessels were incubated in continuous light at 26±2°C. Periodical observations were made during its growth and the results are documented below.

(a) Growth :

Growth measured as increase in fresh and dry weights

is illustrated in Fig. 2 A and Table 9. Growth of tobacco tissue on fresh weight basis increased linearly upto day 6 with 2.5 fold increase during this period. The fresh weight increased from the day 6 to 9 was over 3 fold and thereof till day 18, growth of the tissue was rather slow. During this period the increase in fresh weight of the tissue was almost double. From day 18 onwards growth was very rapid giving a maximum yield of over 10 grams of fresh tissue. The total fold-wise increase during 30 days was 33.7 on fresh weight basis.

On dry weight basis growth of the callus tissues exhibited a period of slow growth during initial 6 days, followed by exponential growth till day 18, total fold-wise increase being 17.36 during this 18 day period. The dry weight almost doubled during days 18 to 30. The overall fold-wise increase in dry weight was 40.50.

(b) Total and reducing sugar accumulation :

There was a rapid uptake of sugars from the medium during the initial culture period. Total sugars on mg% basis were 18.39 during day 3 and there was a gradual depletion to 3.28 by day 30. On mg/culture basis the maximum accumulation was seen on day 21, the value being 18.64. Reducing sugars on mg% basis was maximum on day 3, the value being 15.00 and then it started declining. On

Table : 9. Growth, accumulation of total and reducing sugars, starch and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Nicotiana tabacum

Medium : MS + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l kinetin + 2% sucrose (Standard medium).

Inoculum : 300 ±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Total Starch		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	300.0 (±30.0)	12.0 (±3.0)	0.39	3.28	0.35	2.90	1.05	8.75	0.26	0.29	0.31	0.35	1.69	0.88	1.05	1.17	1.50	1.67
3	576.0 (±22.8)	42.5 (±6.1)	7.82	18.39	6.38	15.00	4.05	9.56	0.57	0.18	0.56	0.20	5.50	1.91	5.50	1.95	14.98	5.31
6	734.7 (±38.6)	44.6 (±4.0)	6.81	15.27	5.80	13.00	5.00	11.20	0.47	0.14	1.07	0.32	14.63	3.35	9.20	2.72	14.69	4.35
9	2411.2 (±48.1)	98.7 (±7.3)	11.84	12.00	8.58	8.69	12.94	13.11	2.07	0.32	2.50	0.38	39.80	3.02	33.75	5.18	84.39	12.96
12	3528.1 (±98.8)	133.3 (±8.6)	13.82	10.37	11.48	8.61	14.12	10.59	1.85	0.16	6.38	0.57	63.25	2.93	31.75	2.81	123.48	10.94
15	4194.8 (±120.0)	174.1 (±10.0)	14.99	8.61	8.76	5.03	19.22	11.04	3.20	0.12	3.55	0.13	53.59	1.72	37.75	1.05	138.43	5.16
18	4516.3 (±128.0)	208.4 (±9.5)	15.42	7.40	10.23	4.91	26.53	12.73	2.69	0.12	10.46	0.45	71.78	1.97	36.13	1.57	171.62	7.45
21	6268.4 (±214.0)	299.2 (±12.4)	18.64	6.23	11.97	4.00	29.29	9.79	5.19	0.18	4.90	0.14	144.08	2.42	72.09	2.05	344.76	9.82
30	10129.4 (±443.9)	486.1 (±16.3)	15.94	3.28	14.10	2.90	42.53	8.75	8.68	0.29	10.52	0.35	57.49	0.88	35.45	1.17	50.65	1.67

Data represents an average of 5 replicates.

Figures in parenthesis represent standard e

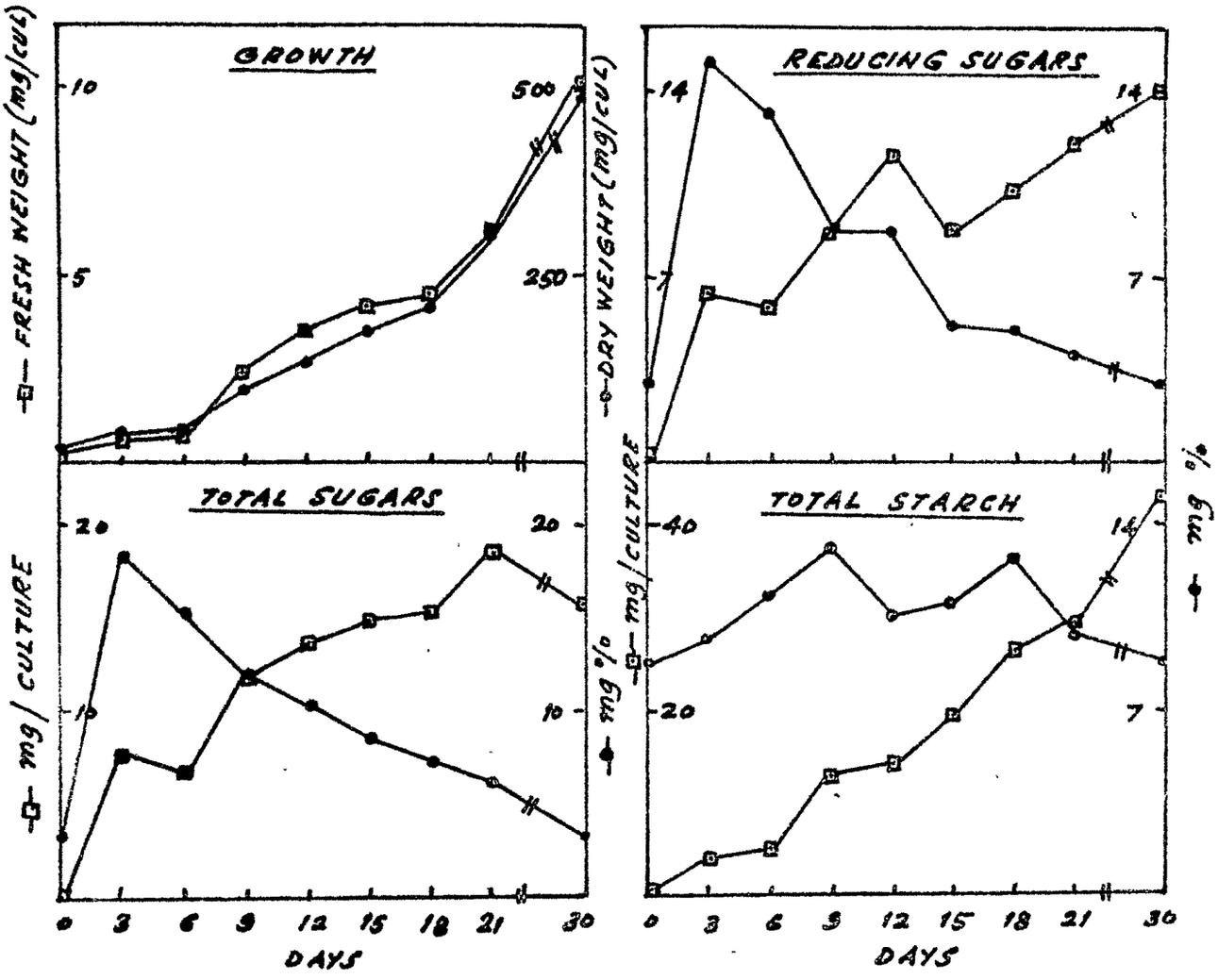

FIG. 2A (REF. TABLE NO. 9)

day 30, it was only 2.90. Per culture basis, the maximum level of 14.10 was attained on day 30 (Fig. 2 A, Table 9).

(c) Total starch :

Starch accumulated in the tissues from days 0 to 9, when it reached the peak value of 13.11 mg%. Then it declined on day 12 and slowly rose to 12.73 mg% by day 18. By day 30 it dropped to 8.75 mg%. But on mg/culture basis maximum yield was registered on day 30, the actual value being 42.53 (Fig. 2 A, Table 9).

(d) Amylase :

Amylase activity assayed every three days in cell free extracts and illustrated in Fig. 2 B and Table 9, showed that the total activity remained rather slow during the first 6 days in culture. Between days 6 and 9 it increased sharply. Again between days 18 and 30 there was another spurt reaching a peak value on day 30.

The specific amylase activity declined to mere 0.14 during initial 6 days of culture. Thereafter it increased reaching the maximum on day 9. There was a sharp fall in the activity by day 12 and this continued till day 21. During days 21 to 30, there was again a rise in its activity.

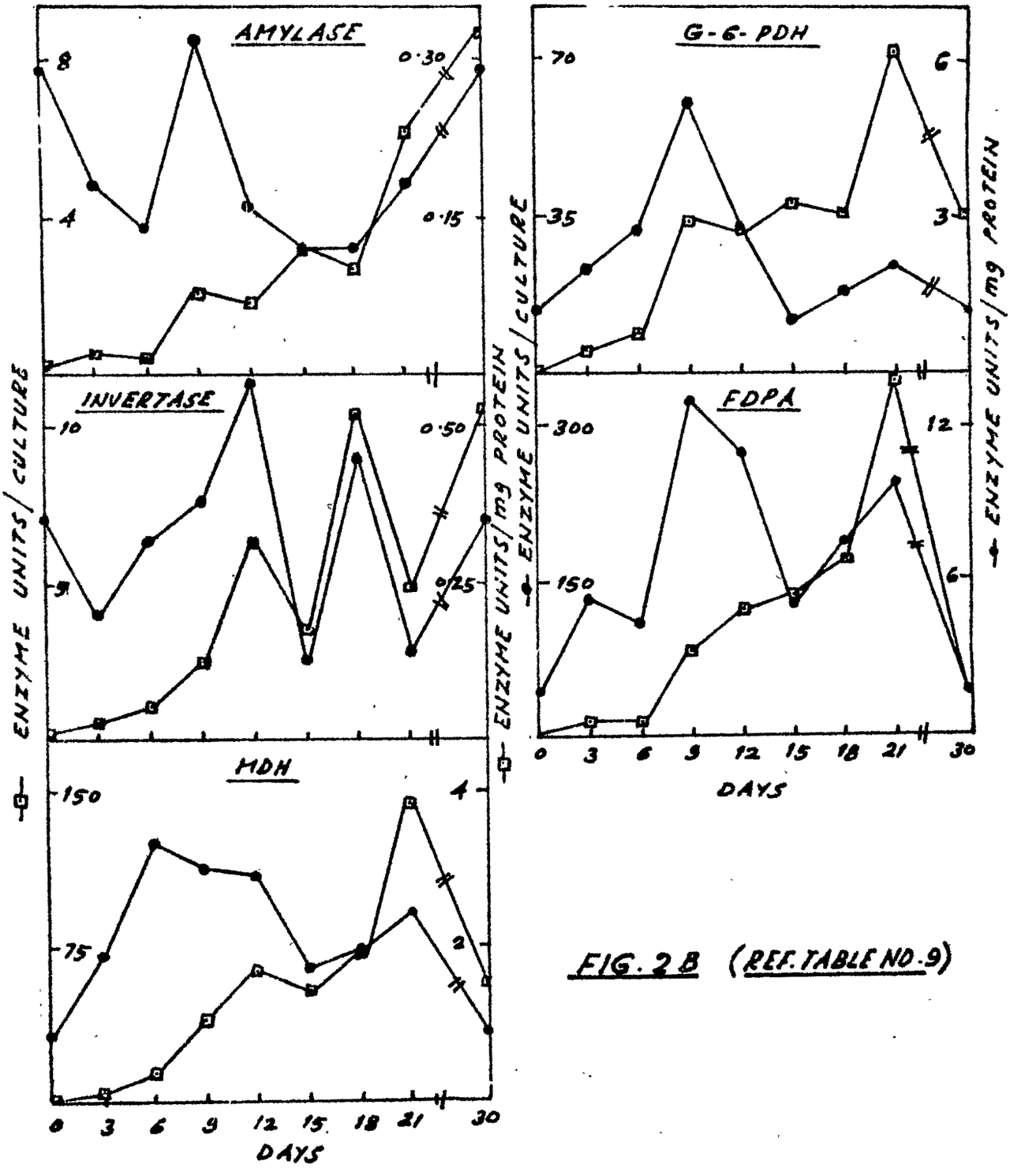

FIG. 2 B (REF. TABLE NO. 9)

(e) Invertase :

Total invertase activity increased rapidly and linearly during the first 12 days of culture (Fig. 2 B, Table 9). It declined on day 15 and shot up sharply by day 18. After an another drop on day 21, it reached the peak value on day 30.

The specific activity of invertase decreased during initial 3 days, followed by a slow and steady increase till day 12 when the peak activity was observed. Then it declined to a mere 0.13 by day 15 and another increase was registered between days 15 and 18. After a drop on day 21 another rise was registered on day 30.

(f) M D H :

Fig. 2 B and Table 9 illustrate progressive changes in MDH activity.

The total activity increased very rapidly during the initial 12 days in culture attaining its first peak value on that day. After a slightly decreased activity by day 15, it increased sharply to attain its second peak value on day 21. After day 21 the activity declined till day 30.

There was a linear increase in the specific activity of MDH between days 0 and 6, attaining the peak value on day 6. The enzyme activity declined between days 6 and 15, but increased again to attain its second peak value on day 21.

Between days 21 and 30 the enzyme activity declined to its value as of day 0.

(g) G-6-P D H :

Progressive changes in total and specific G-6-PDH activity are illustrated in Fig. 2 B and Table 9.

There was a sharp increase in total activity of G-6-PDH between days 0 and 9 reaching its first peak value. After a very slight decrease during days 9 to 12 it increased rapidly till day 21, attaining its second peak. Between days 21 and 30, the activity declined.

The specific activity of G-6-PDH increased linearly during the initial 9 days of culture. It attained a peak value on day 9 and then started declining till day 15. Following a marginal increase till day 21, it declined till day 30 to its value as of day 0.

(h) F D P A :

The progressive changes in the enzyme activity as illustrated in Fig. 2 B and Table 9 showed a sharp and rapid increase from day 0 to day 21 reaching its peak value on day 21. Thereafter the total activity declined till day 30.

The specific activity of FDPA increased rather sharply till day 3 followed by a marginal decrease till day 6. It

enhanced again between days 6 and 9, attaining its peak value by day 9. From days 9 to 15 the activity dropped only to be followed by a linear increase till day 21. Thereof it declined till day 30 to its value as of day 0.

Expt. 11. Studies with callus tissues of G. hirsutum during its growth on Standard medium.

Fresh callus tissue (400±40 mg) was inoculated onto 40 ml of the standard medium and the culture vessels were incubated in continuous light at 26±2°C. Periodical observations were made during its growth and the results are documented below.

(a) Growth :

Callus growth measured as increase in fresh and dry weights is represented in Fig. 3 A and Table 10.

On fresh weight basis callus growth increased linearly upto day 6. Rapid increase in fresh weight occurred during days 6 to 18 (over 14 fold). Thereafter growth of the tissue was little slow till day 21. From day 21 onwards growth was very rapid giving an yield of over 12 grams of fresh tissue. The total fold-wise increase during 30 days was 31.25 on fresh weight basis. Total fold-wise increase in dry weight during 30 day culture period was 22.33. In comparison, tobacco tissue registered 33.70 and 40.50 fold increases by fresh and dry weight basis respectively.

Table : 10. Growth, accumulation of total and reducing sugars, starch and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 2 mg/l IAA + 2 mg/l + 2 mg/l NAA + 2 mg/l kinetin + 2% sucrose (Standard medium).

Inoculum : 400±40 mg fresh tissue.

Incubation : at 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Total Starch		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg.%	mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.06	12.90	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	4.00	6.67
3	502.7 (±42.8)	21.1 (±3.9)	6.33	30.00	2.87	13.60	2.63	12.48	1.23	0.98	3.86	2.13	12.86	10.24	12.20	9.71	22.33	17.60
6	648.0 (±56.0)	27.1 (±4.2)	9.76	36.00	8.87	32.72	2.99	11.04	3.16	1.81	4.73	1.59	15.03	8.59	3.88	2.22	29.20	16.67
9	1449.9 (±99.2)	53.5 (±6.1)	9.10	17.00	3.17	5.92	4.88	9.12	7.07	2.44	6.82	0.72	46.98	9.53	15.95	3.24	84.09	17.06
12	2451.6 (±106.5)	96.9 (±7.3)	14.00	14.45	5.52	5.70	11.38	11.74	18.21	2.20	9.18	0.81	112.77	10.00	35.79	3.17	69.03	10.70
15	3462.5 (±121.8)	127.2 (±9.0)	14.41	11.33	7.02	5.52	17.78	13.98	17.63	2.55	16.96	1.53	133.65	12.06	34.63	3.13	131.57	11.86
18	5672.8 (±199.7)	190.5 (±10.8)	16.17	8.49	7.75	4.07	24.77	13.00	43.56	2.84	11.71	1.00	306.33	20.00	51.06	3.33	124.80	8.15
21	6921.2 (±300.0)	219.6 (±13.2)	12.45	5.67	6.32	2.88	30.48	13.88	49.71	2.66	26.71	1.43	207.64	11.11	152.30	8.15	166.11	8.89
30	12499.5 (±480.7)	356.8 (±19.8)	23.19	6.50	11.70	3.28	46.03	12.90	84.09	2.24	5.45	0.29	179.99	4.80	91.67	2.44	125.00	6.67

Data represents an average of 5 replicates.

Figures in parenthesis represent standard error.

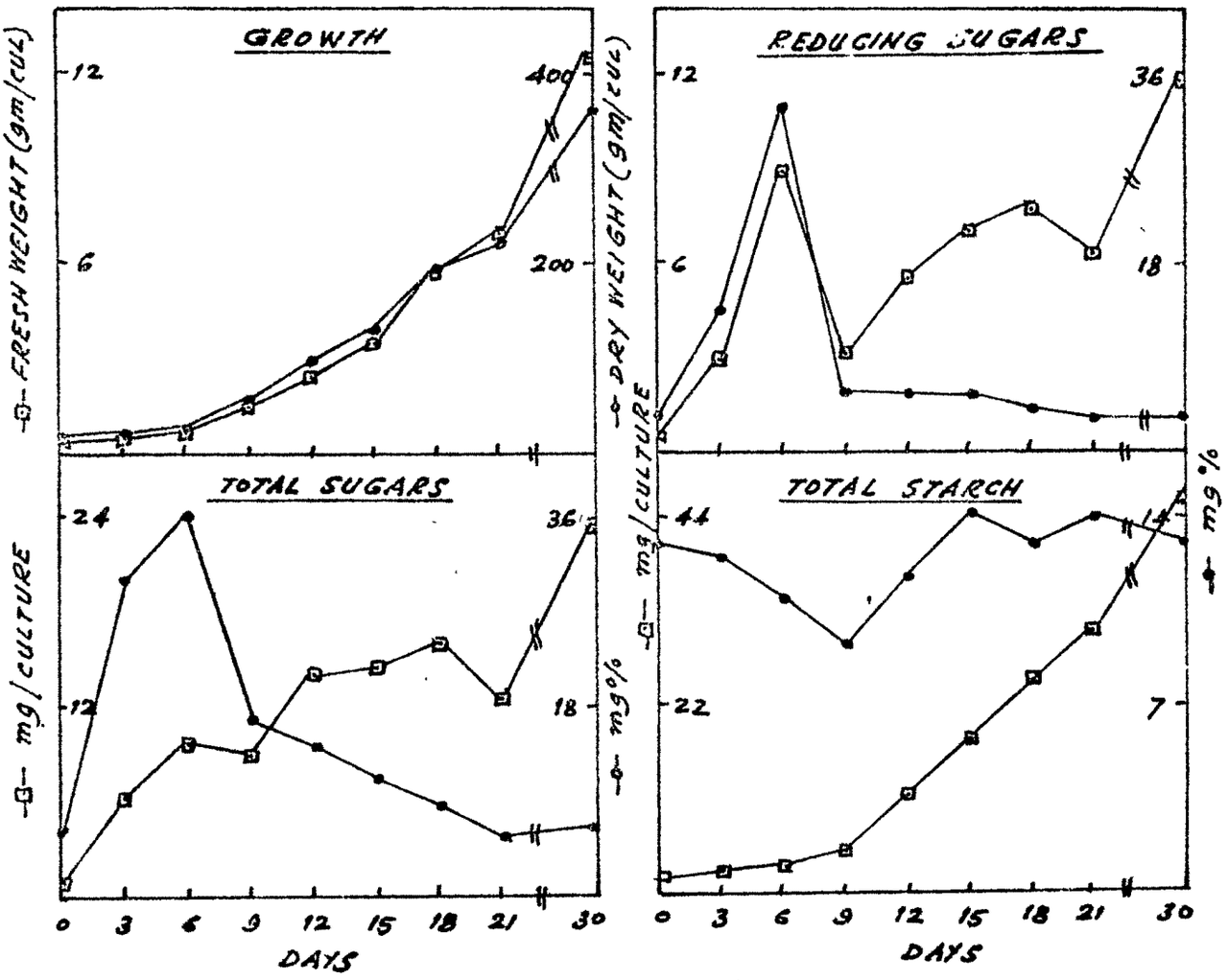

FIG. 3A (REF. TABLE NO. 10)

(b) Total and reducing sugar accumulation :

Changes in total and reducing sugar accumulation in cotton callus grown on standard medium are presented in Fig. 3 A and Table 10.

Total and reducing sugars accumulated from day 0 till day 6, on mg% basis, the increases being over 5 and 10 fold respectively. Thereafter they decreased till day 30. On mg/culture basis both the total and reducing sugars showed peak values on day 30.

There was a more accumulation of total and reducing sugars during the early days of culture in cotton callus when compared with the tobacco tissue.

(c) Total Starch :

Variation in total extractable starch content is illustrated in Fig. 3 A and Table 10.

Total starch on mg% basis decreased linearly from 12.90 to 9.12 mg% between days 0 and 9. From day 9 till day 15 it increased to a peak value of 13.98 mg%. From day 15 onwards it remained more or less stable till day 21, and thereof decreased by day 30 in culture. On mg/culture basis total starch increased linearly and very rapidly from day 0 till day 30 (over 22 fold) when the peak value was reached.

There was no marked difference in starch accumulation between the tobacco and cotton callus tissues.

(d) Amylase :

The total and specific amylase activity of cotton callus tissue is illustrated in Fig. 3 B and Table 10.

The total enzyme activity declined from 2.69 units to 1.23 units by day 3. Thereafter it increased very rapidly and sharply till day 30 to reach a peak value of 84.09 units. Total fold-wise increase was 31.26.

The specific activity of amylase declined by 2.29 fold by day 3. Between days 3 and 9 it increased from 0.98 units to 2.44 units. Though peak value of 2.84 units was registered on day 18, specific activity of amylase remained more or less stable from day 9 till day 30.

The total amylase activity in tobacco tissue reached a peak on day 30 like in cotton, the value being 8.68 units. Specific activity of amylase in tobacco exhibited a peak on day 9, the value being 0.32 units. On the other hand, in cotton the specific activity demonstrated a peak on day 18, the value being 2.84. However, the fold-wise increases in both were very similar. The total activity was higher in tobacco but the specific activity was slightly better in cotton.

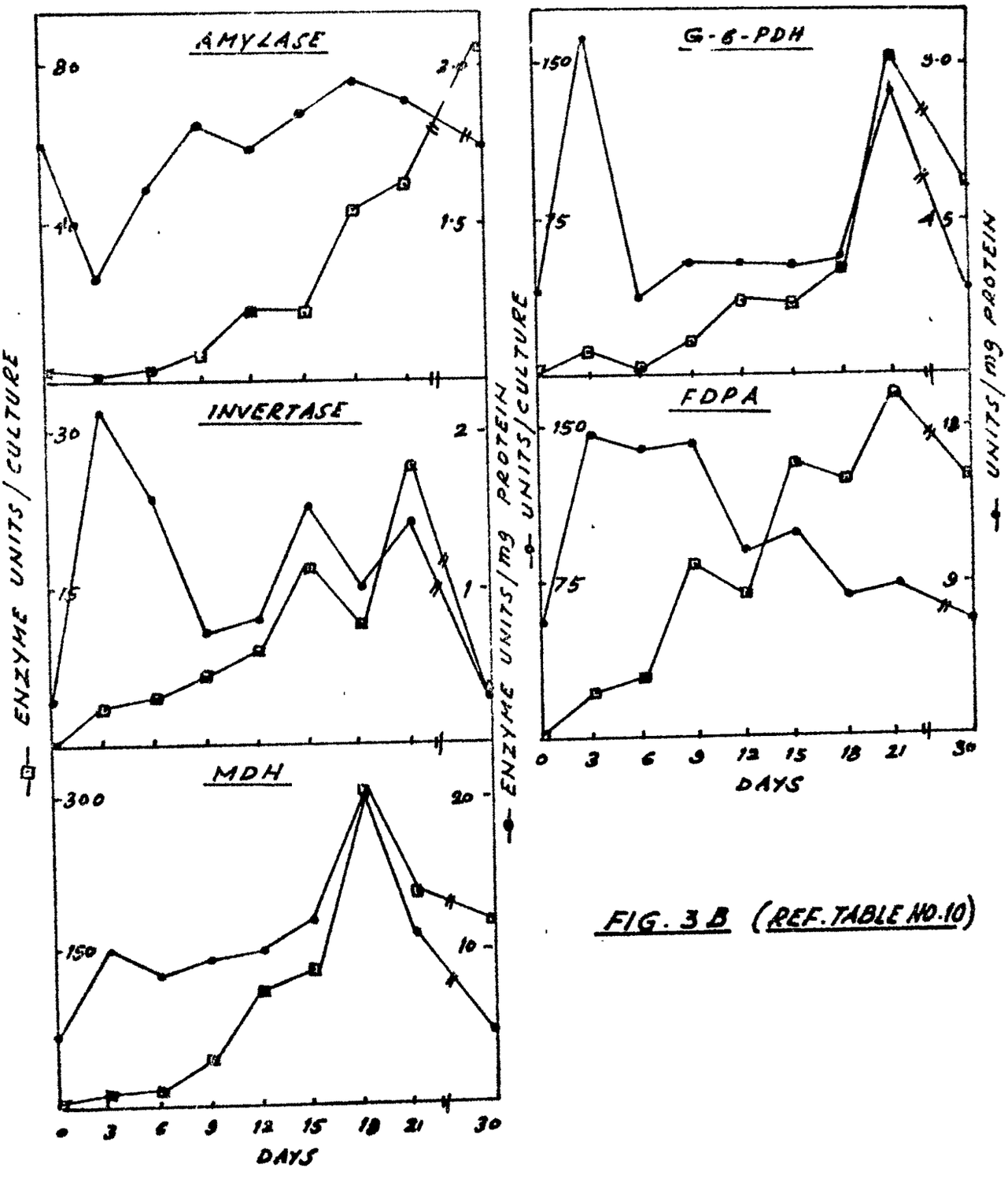

FIG. 3 B (REF. TABLE NO. 10)

(e) Invertase :

Fig. 3 B and Table 10 illustrate total and specific activity of invertase in cotton tissues.

The total invertase activity increased rapidly from day 0 till day 15. It declined on day 18 and again shot up sharply by day 21 to reach a peak. From 26.71 units on day 21 it declined to 5.45 units by day 30.

The specific invertase activity increased very sharply to reach a peak during the first three days in culture. Fold-wise increase during this period was 7.34. Between days 3 and 9 it was on decline. Again the activity increased from 0.72 units to 1.53 units by day 15 in culture. After fluctuating, the specific activity declined to reach its original value of 0.29 units by day 30.

In tobacco total invertase activity reached a peak on day 30, the fold-wise increase being 33.94; whereas in cotton the total invertase activity reached a peak on day 21, the fold-wise increase being 157.12. The specific activity of invertase in tobacco tissue registered a peak on day 12, the fold-wise increase being 1.63 while in cotton peak activity was observed on day 3, with 7.34 fold increase.

(f) M D H :

Fig. 3 B and Table 10 illustrate progressive changes in

MDH activity in cotton callus cultures.

The total MDH activity increased very sharply from 5.75 units to 306.33 units by day 18 to reach a peak value. Between days 18 and 30 it was on decline from 306.33 units to 179.99 units.

The specific activity of MDH increased 2.13 folds during the first 3 days of culture. Then it slightly declined by day 6. Thereof the specific activity of MDH increased in a linear fashion till day 18 to reach a peak value of 20.00 units. Between days 18 and 30 the activity dropped to 4.80 units of its original value.

The total activity of MDH in tobacco showed a peak on day 21 with 85.25 fold increase. Cotton callus showed the peak activity on day 18 with 53.27 fold increase. While peak (3.81 fold) increase in specific activity of MDH was registered on day 6 in tobacco, the cotton callus showed peak value (4.17 fold) on day 18.

(g) G-6-P D H :

The total G-6-PDH activity increased 4.16 folds during the first three days in culture and then dropped sharply by day 6. Thereafter the activity shot up to 152.30 units to reach a peak by day 21. Between days 21 and 30 the activity declined to 91.67 units (Fig. 3 B, Table 10).

The specific activity of G-6-PDH showed double peaked developmental pattern. Activity increased 3.98 folds during the first three days to reach a first peak. Then it declined sharply from 9.71 units to 2.22 units by day 6. After a slight increase by day 9, enzyme activity was more or less stable till day 18. Between days 18 and 21 the activity increased from 3.33 units to 8.15 units to register the second peak. Thereof it declined to 2.44 units by day 30 (Fig. 3 B, Table 10).

In tobacco peak activity of G-6-PDH on unit/culture basis was observed on day 21 with 68.66 folds increase; and that of cotton was also observed on day 21 but with 51.98 folds increase. G-6-PDH on unit/mg protein basis registered a peak in tobacco on day 9 with 4.43 folds increase, while that of cotton demonstrated double peaks, one on day 3 with 3.98 folds and another on day 21 with 3.34 folds increase.

(h) F D P A :

Fig. 3 B and Table 10 illustrate progressive changes in the total and specific activity of cotton callus.

The total FDPA activity increased rapidly from day 0 till day 9, from 4.00 units to 84.09 units. After some decline to 69.03 units by day 12, the activity shot up till day 21 to reach a peak value of 166.11 units. Between days 21 and 30 activity dropped to 125.00 units.

The specific activity of FDPA demonstrated a peak value during the first three days in culture. Activity remained almost steady from day 3 till day 9. Then it declined from 17.06 units to 10.70 units and remained again almost steady till day 21 and thereafter declined to reach its original value of 6.67 units by day 30.

While nearly 230 folds increase in FDPA activity was noticed in tobacco callus with the peak on day 21, in cotton callus enzyme FDPA exhibited peak on day 21 with just 41.53 folds increase. On unit protein basis tobacco callus registered peak on day 9 with 6.55 fold increase while that of cotton registered a peak on day 3 with 2.64 fold increase.

Summary :

Some correlations could be made out clearly between different phases of growth and the specific activities of amylase, invertase, MDH, G-6-PDH and FDPA.

Sugar accumulation was very rapid both in tobacco and cotton callus tissues during the initial days of culture and slowly they were depleted from the tissues towards the end of culture period. Total starch content on mg% basis started accumulating during the days 0 to 9 in tobacco in contrast to the cotton tissue where the highest accumulation occurred on day 15. Thereof during late exponential and post-exponential phases of growth the content declined in both the tissues.

Throughout the growth period, except on day 9, specific amylase activity declined in tobacco tissues. On the other hand, the amylase activity declined during lag phase of growth in cotton but during the exponential and post-exponential phases the activity remained more or less stable. Specific invertase activity increased during lag phase, and declined during exponential and post-exponential phases in both tobacco and cotton. The activity of MDH per unit protein increased sharply during lag phase of growth in both the tissues. During the remaining period of growth in tobacco MDH activity was on decline. Unlike in tobacco, the activity was almost steady during the exponential phase in cotton callus. With the onset of early post-exponential phase it reached a peak and declined thereof. The total G-6-PDH activity per unit protein increased during the lag period and attained its peak value during early exponential phase in tobacco. Later on the enzyme activity declined; whereas in cotton the enzyme activity showed a double peaked developmental pattern. First peak was attained during the lag phase of growth and the second peak was observed during early post-exponential phase. Similar to tobacco, FDPA activity on unit protein basis shot up during early lag phase in cotton. While in tobacco the peak activity was recorded during the exponential phase, the activity in cotton declined throughout the exponential and post-exponential phases of growth.

Section C - II : Physiological studies with GOT, ME and PEPC and total and reducing sugars during growth of callus tissues of tobacco and cotton in the dark.

In the present study, the role of dark fixation of CO_2 which utilizes phosphoenolpyruvate derived from carbohydrate as a substrate is examined during growth of tobacco and cotton callus tissues. To examine the physiological changes associated with growth in callus tissues of tobacco and cotton grown in the dark, the following parameters were studied :
(a) Growth, (b) Total and reducing sugar accumulation,
(c) Glutamic-oxalic transaminase, (d) Malic enzyme and
(e) Phosphoenolpyruvate carboxylase.

Weighed amount of callus masses were inoculated onto 40 ml of MS basal + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l KN + 2% sucrose medium. The comparative study of the tobacco and cotton tissues is presented under the following heads :

(i) Studies with callus tissue of N. tabacum during its growth on standard medium in the dark.

(ii) Studies with callus tissue of G. hirsutum during its growth on standard medium in the dark.

Expt. 12. Studies with callus tissue of N. tabacum during its growth on standard medium in the dark.

From the culture vessels incubated at 26±2°C in continuous dark, five replicate flasks were harvested every third day and analysed for growth, sugar accumulation and enzymes. The results are described below.

(a) Growth :

Growth measured as increase in fresh and dry weights and is illustrated in Fig. 4 and Table 11.

Growth on both fresh and dry weight basis exhibited an identical pattern. Growth was rather slow during the first 15 days. Thereafter it increased at a faster rate. Total fold-wise increases in fresh and dry weights after 30 days of culture were over 17 and 14 respectively.

(b) Total and reducing sugar accumulation :

During the first 3 days of culture there was a rapid accumulation of total and reducing sugar content on mg% basis. Thereafter the sugar content slowly decreased till the termination of culture. On mg/culture basis peak values were attained on day 18 (Fig. 4, Table 11).

(c) G O T :

Progressive changes of total and specific GOT activities are illustrated in Fig. 4 and Table 11.

While the total GOT activity increased 4 fold during the days 0 and 3; its activity on unit/mg protein basis decreased during this initial 3 days. Similarly during the days 3 and 6 although the total activity decreased, there was a linear

Table : 11. Growth, accumulation of total and reducing sugars and progressive changes in the activity of GOT, ME and PEPC in callus cultures of Nicotiana tabacum

Medium : MS + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l kinetin + 2% sucrose (standard medium).

Inoculum : 300±30 mg fresh tissue.

Incubation : At 26±2°C in total darkness.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		G O T		M E		P E P C	
			mg/cult.	mg %	mg/cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	300.0 (±30.0)	12.0 (±3.0)	0.39	3.28	0.35	2.90	8.40	8.24	-	-	-	-
3	633.2 (±44.6)	30.3 (±5.1)	4.93	16.27	3.78	12.48	32.93	1.29	12.66	0.36	12.66	0.36
6	870.2 (±49.8)	45.2 (±6.4)	6.42	14.21	5.40	11.95	16.71	4.17	8.70	0.22	10.44	0.26
9	1108.6 (±100.3)	51.8 (±5.2)	4.86	9.39	3.73	7.21	31.04	7.78	13.30	0.33	19.96	0.50
12	1180.3 (± 98.7)	54.7 (±5.9)	4.72	8.62	3.80	6.95	56.65	6.32	9.44	0.11	11.80	0.13
15	2056.4 (±187.9)	81.0 (±9.3)	6.05	7.47	3.54	4.37	125.44	13.86	8.23	0.09	20.56	0.23
18	2645.1 (±192.5)	112.7 (±10.1)	7.66	6.80	5.64	5.00	66.66	4.94	21.16	0.16	31.74	0.22
21	3216.9 (±200.0)	120.5 (±8.8)	6.15	5.10	4.92	4.08	176.93	15.28	3.22	0.23	32.17	0.23
30	5164.2 (±256.8)	171.9 (±12.8)	5.50	3.20	3.51	2.04	144.60	8.24	-	-	-	-

Data represents an average of 5 replicates.

Figures in parenthesis represent standard error.

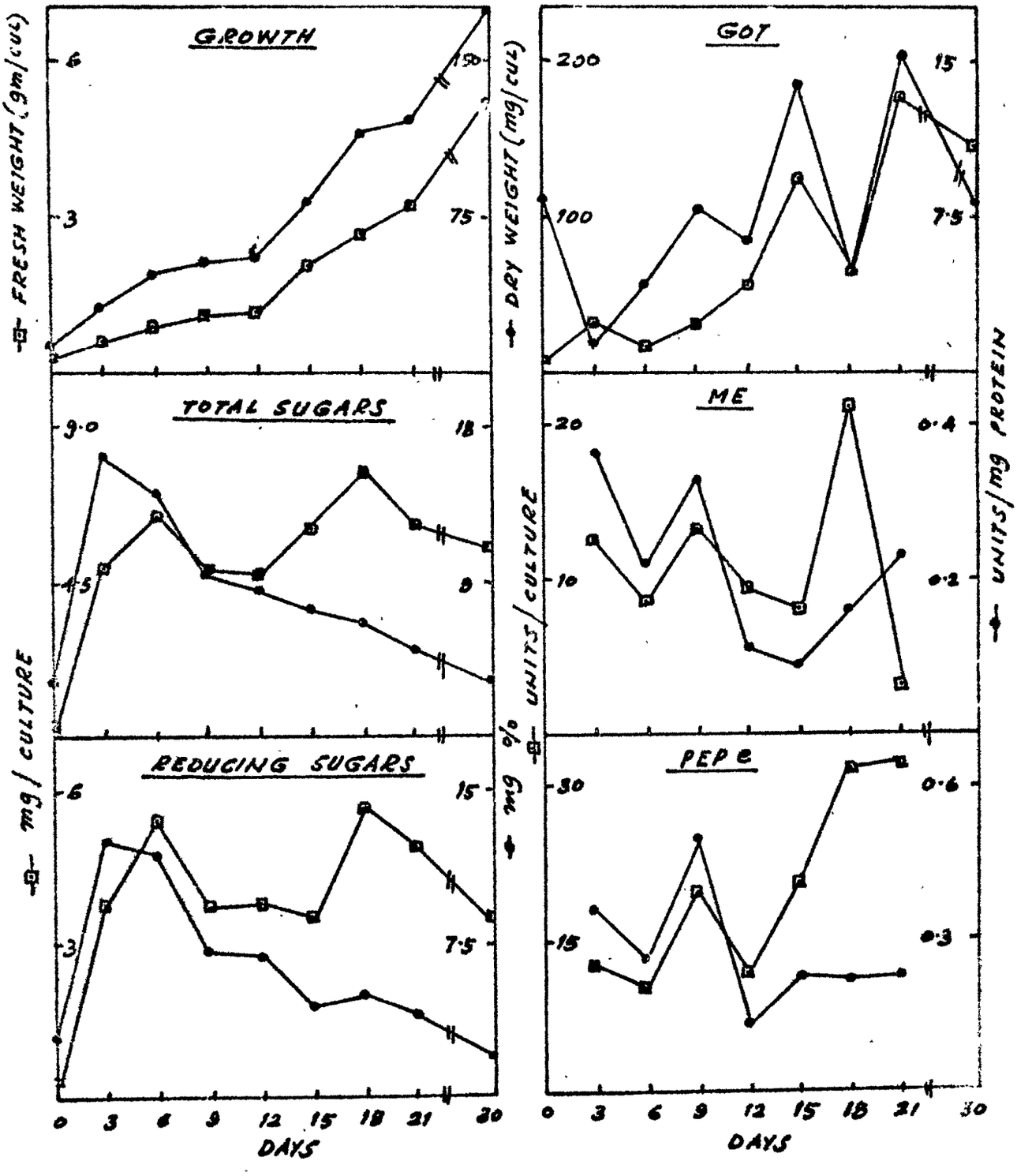

FIG. 4 (REF. TABLE NO. 11)

increase in specific activity between days 3 and 9. Total and specific activity of GOT increased rapidly to reach its peak value on the day 21. Between days 21 and 30 the enzyme activity decayed both on unit/culture and on unit/mg protein basis.

(d) M E :

Progressive changes in malic enzyme activity during the growth of tobacco callus tissue are presented in Fig. 4 and Table 11.

Enzyme activities on day 0 could not be detected. The total and specific activity of ME followed a similar trend till day 18 in culture. Total activity reached the peak value on day 18 and then dropped very sharply between days 18 and 21; on the other hand, the specific activity showed peak value at the onset of culture period on day 3. Though specific activity dropped very sharply between days 9 and 15, thereof a gradual increase was noticed till day 21. Activity could not be detected thereafter.

(e) P E P C :

Progressive changes in total and specific activities of PEPC are illustrated in Fig. 4 and Table 11.

The total and specific activities of PEPC could not be detected on day 0. Total enzyme activity reached 12.66 units on day 3 and after fluctuations till day 12, the activity increased till day 21 to attain a peak of 32.17 units. On the other hand, the specific activity showed the peak value on day 9 and declined thereof and it could not be detected after day 21.

Expt. 13. Studies with callus tissue of G. hirsutum during its growth on standard medium in the dark.

From the culture vessels incubated in continuous dark, five replicate flasks were harvested for the measurement of growth and sugars; and determination of enzyme activities. The results are documented below.

(a) Growth :

Growth measured as increase in fresh and dry weights is illustrated in Fig. 5 and Table 12.

Growth on fresh weight basis increased 13.87 folds during the entire culture period. Fold-wise increase in dry weight during the corresponding period was over 13. This value was much lesser in comparison with light grown tissues. Also, these growth values for cotton were 3.3 and 0.8 folds lower than those of tobacco callus growth on fresh and dry weights.

Table : 12. Growth, accumulation of total and reducing sugars and progressive changes in the activity of GOT, ME and PEPC in callus cultures of Gossypium hirsutum

Medium : MS + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l kinetin + 2% sucrose (standard medium)

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in total darkness.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		G O T		M E		P E P C	
			mg/cult.	mg %	mg/cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	3.61	4.00	-	-	-	-
3	703.8 (±59.6)	34.6 (±5.4)	11.49	33.21	9.10	26.31	40.82	21.48	12.70	6.68	40.80	21.47
6	1236.6 (±87.5)	43.2 (±6.8)	11.65	26.97	8.10	18.75	37.73	7.14	8.29	1.57	18.71	3.54
9	1166.5 (±82.3)	48.0 (±6.7)	7.36	15.33	5.95	12.39	43.16	11.56	9.33	2.50	23.33	6.25
12	1949.7 (±112.9)	98.3 (±11.4)	12.77	13.26	9.96	10.34	103.33	10.39	19.49	1.96	27.30	2.75
15	2418.3 (±169.5)	98.6 (±12.1)	11.40	11.56	7.77	7.88	246.67	28.33	9.67	1.33	19.35	2.22
18	3615.4 (±201.7)	112.7 (±12.9)	9.51	8.44	6.64	5.89	90.39	8.33	-	-	28.92	2.67
21	4478.0 (±215.3)	172.2 (±16.9)	10.99	6.38	8.61	5.00	53.74	4.45	-	-	35.82	2.96
30	5547.8 (±244.7)	216.1 (±19.5)	6.57	3.04	3.28	1.52	50.11	4.00	-	-	-	-

Data represents an average of 5 replicates.

Figures in parenthesis represent standard error.

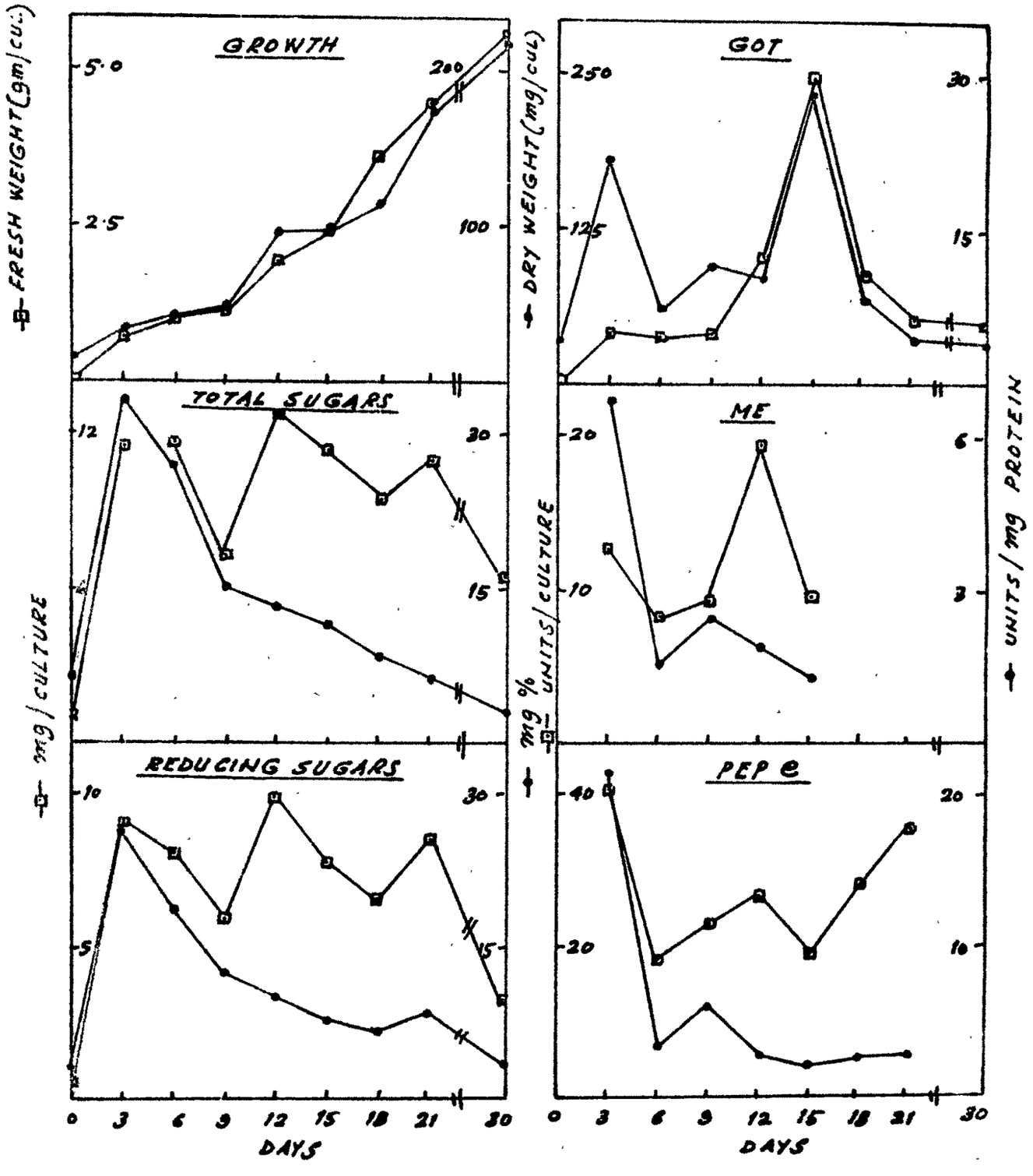

FIG. 5 (REF. TABLE NO. 12)

(b) Total and reducing sugar accumulation :

Changes in total and reducing sugar accumulation is illustrated in the Fig. 5 and Table 12.

Total sugars on mg% basis increased more than 5 folds during the initial 3 days of culture. Thereof it declined slowly and steadily till day 30. Reducing sugars increased more than 8 folds on mg% basis during the first 3 days of culture and declined thereof till day 30.

In comparison with tobacco, the accumulation on mg% basis, of both the total and reducing sugars was higher on any given day in cotton.

(c) G O T :

Progressive changes of total and specific GOT activities are illustrated in Fig. 5 and Table 12.

The total activity of GOT increased till day 15 to attain a peak value with over 68 folds rise. The specific activity also showed its peak value on day 15 with about 7 fold increase only. Between days 15 and 30 activity on both the accounts was on decline.

The total and specific activities of GOT in tobacco showed peak values on day 21, whereas in cotton tissues peak activities were attained much earlier (on day 15). The specific activity

of GOT was always several folds higher in cotton than that of tobacco except on day 21.

(d) M E :

Progressive changes in malic enzyme activity are presented in Fig. 5 and Table 12.

The enzyme activity could not be detected on day 0 like that of tobacco callus tissue. Total activity of ME increased till day 12 to reach a peak value of 19.5 units. Between days 12 and 15 activity declined and could not be detected further. On the other hand, the specific activity of ME attained peak on day 3 with over 6.68 units; but declined by day 15, and thereof activity could not be detected.

While malic enzyme activity could be detected till day 21 in tobacco, it could not be detected after day 15 in cotton tissues. Between days 3 and 15 higher specific activities of ME were noticed in cotton than in tobacco.

(e) P E P C :

Progressive changes of total and specific activities of PEPC in cotton callus tissues are illustrated in Fig. 5 and Table 12.

Activity of PEPC could not be detected on day 0 in cotton

tissues like that in tobacco callus. Total and specific activities of PEPC attained peak values on day 3 with over 40 and 21 units respectively. After fluctuating till day 21, the activity could not be detected later on.

The specific activity of PEPC was higher on any day in cotton when compared with that in tobacco tissue; and especially on day 3, it was nearly 60 folds higher in cotton.

Summary :

In both tobacco and cotton, growth values were lesser when compared to that of light grown tissues. However, growth was better in tobacco than that of cotton.

The total and specific activities of GOT attained peak values much earlier i.e., on day 15 in contrast to day 21 of tobacco. Except on day 21, the specific activity of GOT was several folds higher in cotton than that of tobacco.

Activity of malic enzyme could not be detected in cotton tissues later than day 15, while in tobacco it could be detected till day 21. Between days 3 and 15 higher specific activity of malic enzyme in cotton tissues was noticed than that of tobacco.

PEPC exhibited peak value on day 3 in cotton callus on unit/mg protein basis, while in tobacco the peak value was attained on day 9. The specific activity of PEPC was several folds higher in cotton in comparison with that of tobacco tissue.

Section D : Hormonal effect om growth and development of Amylase, Invertase, MDH, G-6-PDH and on total and reducing sugars in callus cultures of cotton.

It is a tacit assumption that the regulation of plant growth and differentiation has its origin partly in changes in the pattern of enzyme activities. Every growth substance can apparently under appropriate conditions regulate the synthesis of RNA and probably also the synthesis of DNA, and in turn cell division. Effects on DNA polymerase have also been demonstrated for two growth substances. Secondly, every growth substance can regulate the activity of one or more hydrolytic enzymes.

Present study was undertaken in an attempt to elucidate the role of phytohormones in growth and organogenesis if any and the physiological and biochemical changes associated therewith in callus cultures of cotton. The following parameters were studied : (a) Growth, (b) Total and reducing sugar accumulation, (c) Amylase, (d) Invertase, (e) M D H, (f) G-6-P D H, and (g) F D P A. Regulatory effect of phytohormones on the developmental pattern of the above enzymes was studied in the present endeavour.

Results in this section are presented under following captions :

(i) Effect of 2 mg/l and 5 mg/l IAA on growth and development of above mentioned enzymes and on accumulation of sugars.

(ii) Effect of 0.2 mg/l and 2 mg/l NAA on growth and

development of enzymes mentioned above and on accumulation of sugars.

(iii) Effect of 0.2 mg/l and 5 mg/l 2,4-D on growth and development of enzymes mentioned above and on accumulation of sugars.

(iv) Effect of 0.04 mg/l and 2 mg/l kinetin on growth and development of enzymes mentioned above and on accumulation of sugars.

(v) Effect of 25 mg/l and 100 mg/l GA_3 on growth and development of enzymes mentioned above and on accumulation of sugars.

Callus tissues weighing 400±40 mg were used as inoculum in 40 ml of the experimental medium. The culture vessels were incubated in continuous light at 26±2°C for 30 days. Five replicates were harvested at the interval of 5 days for the measurement of growth and sugar contents as well as for the analysis of enzymes.

Expt. 14. Effect of 2 mg/l and 5 mg/l IAA on growth and development of Amylase, Invertase, MDH, G-6-PDH and FDPA and on total and reducing Sugars in cotton callus cultures.

MS basal media containing 2% sucrose and supplemented with 2 mg/l and 5 mg/l IAA were used. These are referred to in the text as medium A and medium B respectively.

(a) Growth :

Growth measured as increase in fresh and dry weight is presented in Tables 13, 14.

The growth of cotton callus on medium A was better than on medium B. On the low IAA (2.0 mg/l) medium the fresh and dry weights increased over 32 and 21 folds respectively; while in presence of high IAA (5.0 mg/l), fresh and dry weight increases were over 25 and 20 folds respectively. On both the media growth was very slow during the initial 5 days after which it increased rapidly.

(b) Total and reducing sugar accumulation :

Changes in total and reducing sugar accumulation are presented in Tables 13, 14.

Total sugars on mg% basis increased dramatically almost by 10 and 9 folds during the initial 5 days of culture. Thereof sugar content declined continuously till day 30. Reducing sugars also followed a similar trend. On mg% basis they increased more than 7 folds on both the media in the first 5 days and thereof reducing sugar content declined steadily.

(c) Amylase :

The development of amylase activity is presented in Tables 13, 14.

Table : 13. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 2.0 mg/l IAA + 2% sucrose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	4.00	6.67
5	495.3 (±43.2)	19.9 (±3.8)	12.87	64.66	4.93	24.80	2.52	1.24	1.65	1.04	15.06	9.50	11.06	6.98	17.83	8.78
10	1432.9 (±97.5)	60.3 (±5.2)	14.27	23.66	3.67	6.08	4.95	2.36	1.94	1.16	25.22	6.83	21.97	6.33	96.00	16.34
15	2582.2 (±103.7)	106.5 (±6.4)	14.55	13.66	6.05	5.68	13.38	1.13	26.35	2.22	65.59	17.47	49.06	5.59	113.62	9.57
20	5474.5 (±220.3)	143.1 (±11.2)	7.87	5.50	4.44	3.10	26.71	1.96	46.18	1.83	181.75	20.75	36.42	3.88	248.47	13.04
25	10498.0 (±410.5)	296.8 (±22.0)	13.86	4.67	6.00	2.02	66.17	2.52	39.30	1.50	233.06	12.33	48.98	2.59	167.97	8.89
30	13072.3 (±510.1)	348.8 (±18.9)	14.20	4.07	12.63	3.62	87.94	1.35	74.41	1.14	410.47	8.72	74.07	1.57	202.23	4.72

Data represents an average of 5 replicates.

Figures in parenthesis represent standard error.

Table : 14.1 Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 5 mg/l NAA + 2% sucrose.

Inoculum :400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	4.00	6.67
5	490.1 (±40.7)	21.6 (±4.2)	12.53	58.00	5.05	23.36	1.82	0.86	1.18	0.89	11.86	8.96	7.68	5.80	21.56	10.00
10	1769.0 (±62.3)	76.6 (±10.0)	13.79	18.00	4.66	6.08	7.67	1.20	11.86	1.86	32.55	5.11	38.33	6.02	95.53	15.00
15	4049.0 (±158.5)	144.7 (±13.1)	19.77	13.66	6.48	4.48	20.12	1.31	32.18	2.09	134.43	15.09	33.74	3.79	153.86	10.00
20	5728.6 (±221.9)	210.2 (±14.9)	8.77	4.17	4.52	2.20	37.32	1.71	22.11	1.29	214.25	20.78	36.28	3.52	263.52	12.11
25	10097.7 (±401.6)	314.6 (±17.8)	9.97	3.17	6.23	1.98	61.50	1.79	19.10	0.69	214.07	11.78	30.29	1.67	141.37	7.78
30	10381.1 (±398.2)	320.6 (±18.1)	9.39	2.93	6.09	1.90	52.94	1.31	35.67	0.88	186.86	4.62	27.61	0.68	124.57	3.08

Data represents an average of 5 replicates.

Figures in parenthesis represent standard error.

On medium A total amylase activity after fluctuating slightly during initial phase of growth, started ascending and attained its peak on day 30 with a fold-wise increase of over 32. However, specific amylase activity developed to its peak value of 2.52 units on day 25 and dropped thereof towards the termination. On the other hand, on medium B the total and specific activities of amylase progressed rapidly till day 25 to register peak values. Total amylase activity till day 20 was higher on medium B than on A, while specific activity, except on day 15, was higher on medium A.

(d) Invertase :

The development of invertase activity is presented in Tables 13, 14.

Total invertase activity both on media A and B showed its peak value on day 30. Medium A exhibited significantly higher activity of invertase than medium B except on days 10 and 15. Specific invertase activity also on both the media exhibited peak value on day 15 and thereof the activity was on decline. On any given day, the specific invertase activity on medium A was marginally higher than on medium B.

(e) M D H :

Progressive changes of MDH activity are presented in Tables 13, 14.

Total MDH activity on medium A increased rapidly and substantially from day 0 till day 30; while on medium B total MDH activity exhibited its peak on day 20. Total activity of MDH between days 10 and 20 was appreciably higher on medium B than A, but thereon it was more on medium A. On the other hand, the specific MDH activity followed a similar developmental pattern in both the media. Peak values were attained on day 20 with almost same fold-wise increase. However, specific MDH activity was marginally higher on all days on medium A than on B.

(f) G-6-P D H :

Progressive changes of enzyme activity are presented in Tables 13, 14.

Total G-6-PDH activity on medium A developed rapidly to attain its peak value by day 30 with a fold-wise increase of over 25. Specific activity, on the other hand, increased almost 3 fold during the first 5 days of culture period to attain its peak. Thereon the activity decayed linearly till day 30. In contrast, on medium B the total and specific activities increased over 13 and 2.5 folds to attain peak values by day 10 after which the activity declined. By and large, the specific activity of G-6-PDH remained higher on medium A than on medium B.

(g) F D P A :

Progressive changes of FDPA activity are presented in Tables 13, 14.

Total FDPA activity on medium A increased rapidly by over 62 fold during day 0 and day 20 to attain its peak. Thereafter the activity declined sharply by day 20, but rose again by day 30. On medium B the activity increased nearly 66 fold between day 0 and 20 to register its peak value and declined thereof till day 30.

The specific activity of FDPA on media A and B showed peak values on day 10. Though the activity fluctuated, it declined in both the media towards the end of culture period.

Specific activity of FDPA was slightly higher on medium A than on medium B, except on days 5 and 15.

Expt. 15. Effect of 0.2 mg/l and 2 mg/l NAA on growth and development of Amylase, Invertase, MDH, G-6-PDH and FDPA and on total and reducing Sugars in cotton callus cultures.

MS basal media containing 2% sucrose and supplemented with 0.2 mg/l and 2 mg/l NAA were used. These are referred to in the text as medium A and medium B respectively.

(a) Growth :

Growth measured as increase in fresh and dry weights is presented in Tables 15, 16.

Growth was superior on medium A (0.2 mg/l NAA) than on medium B (2.0 mg/l NAA). Fold-wise increases on fresh weight basis were 28.4 and 18.6 respectively on media A and B. On dry weight basis total increases were over 21 and 19 folds respectively during 30 day culture period. Fresh weight was almost 7 folds higher on medium A, while dry weight showed hardly any difference.

(b) Total and reducing sugar accumulation :

Progressive changes in the accumulation of total and reducing sugars are presented in Tables 15, 16.

Total and reducing sugars on mg% basis accumulated during the first five days on media A and B and thereon declined till day 30. On mg/culture basis peak values were attained on day 30 in both the cases.

There was no marked difference in sugar accumulation between the two media.

(c) Amylase :

Progressive changes of amylase activity are presented in Tables 15, 16.

Table : 15. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 0.2 mg/l NAA + 2% sucrose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	4.00	6.67
5	731.7 (±44.6)	33.9 (±4.3)	4.30	12.67	3.81	11.25	3.57	1.04	2.36	1.20	19.02	7.65	10.24	4.12	36.60	14.71
10	1080.9 (±48.8)	51.0 (±6.2)	5.19	10.17	4.24	8.31	4.88	1.16	7.46	1.77	25.51	5.90	12.97	4.28	45.40	10.77
15	1650.3 (±64.5)	92.3 (±7.8)	8.61	9.33	7.06	7.65	6.25	0.62	9.65	1.96	19.80	2.00	41.26	4.70	137.00	10.61
20	3215.5 (±130.0)	149.3 (±10.4)	5.15	3.45	4.63	3.10	18.90	1.34	20.98	1.69	96.47	6.82	85.74	6.06	141.50	10.00
25	5989.9 (±233.7)	256.2 (±16.5)	8.74	3.41	5.87	2.29	39.57	1.57	47.61	1.81	129.38	4.91	77.87	2.95	180.00	6.83
30.	11360.4 (±450.1)	340.4 (±18.7)	11.61	3.41	8.58	2.52	49.20	0.92	124.38	1.67	86.34	1.15	204.49	2.73	287.60	5.30

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

Table : 16. Growth, accumulation of total and reducing sugars and progressive changes in the activity of amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 2 mg/l NAA + 2% sucrose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	4.00	6.67
5	627.5 (±52.7)	29.7 (±4.8)	3.02	10.17	2.27	7.65	4.38	1.52	0.61	0.54	18.55	6.35	15.27	7.18	52.70	24.70
10	1559.1 (±62.5)	67.3 (±5.6)	4.60	6.83	3.16	4.69	9.82	1.62	6.01	0.90	30.56	7.90	21.85	3.59	46.80	17.70
15	2353.4 (±84.9)	102.2 (±7.8)	4.75	4.65	3.81	3.73	13.55	1.60	2.08	0.25	48.01	5.67	38.44	4.54	108.30	12.78
20	3822.9 (±150.6)	167.5 (±12.3)	6.42	3.83	4.99	2.98	25.72	3.36	9.17	0.70	110.86	14.50	22.94	3.00	122.30	15.99
25	6257.4 (±242.3)	261.0 (±17.2)	9.40	3.60	6.26	2.40	39.94	3.15	7.38	0.59	141.42	11.30	27.11	2.17	112.60	9.00
30	7424.0 (±299.7)	307.4 (±20.0)	16.91	5.50	6.64	2.16	58.49	2.02	22.56	0.78	138.09	4.65	44.54	1.54	215.30	7.44

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

Total enzyme activity on both the media followed the same developmental pattern. Activity increased rapidly and continuously till day 30 to attain peak values with 18 and 21.7 fold increases. On any given day the total amylase activity was higher on medium B than on A.

The specific amylase activity on medium A dropped considerably by day 15 and recovered partially by day 25. Contrary to this, the specific enzyme activity on medium B remained stable between days 5 to 15 and then attained a peak on day 20. Thereof the activity decayed. On any given day the specific activity was considerably higher on medium B than on medium A.

(d) Invertase :

The invertase activity, total and specific, is presented in Tables 15, 16.

Total enzyme activity on media A and B developed rapidly and continuously from day 0 till day 30 to attain peak values with more than 731 and 132 fold increases respectively. On any given day the total invertase activity was several folds higher on medium A than on B.

The specific invertase activity on medium A increased nearly 7 folds to attain its peak by day 15 and remained more or less steady thereafter. On the other hand, on medium B the specific activity after increasing 3 fold by day 6 fluctuated

till the end of culture period. By and large, the specific invertase activity was higher on medium A than on medium B.

(e) M D H :

Total and specific MDH activity is presented in Tables 15, 16.

Enzyme activity on unit culture basis increased rapidly to attain peak values on day 25 with over 22 and 24 folds on media A and B respectively. Activity dropped between days 25 and 30.

The specific activity of MDH on medium A increased 1.6 fold to reach its peak value by day 5. The activity decayed between days 5 and 15 but developed fast during subsequent 5 days. However, the activity declined between days 20 and 30. On medium B, the specific MDH activity showed its peak value on day 20 with over 3 fold increase and dropped between days 20 and 30.

MDH activity on unit culture and unit protein basis, on medium A was not as high as on medium B except on day 5.

(f) G-6-P D H :

Progressive changes in total and specific activities of G-6-PDH are presented in Tables 15, 16.

On both the media total enzyme activity increased rapidly

to attain peak values on day 30 with over 70 and 15 folds respectively. Between days 15 and 30 total activity was several folds higher on medium A than on B.

The specific activity of G-6-PDH on medium A developed slowly but linearly to attain its peak value on day 20, but decayed thereof till day 30. In contrast, on medium B, the specific G-6-PDH activity showed its peak value on day 5 and declined thereafter. Except on day 5, the specific activity was higher on medium A when compared with that on medium B.

(g) F D P A :

Total and specific FDPA activities are presented in Tables 15, 16.

The increase in total FDPA activity on medium A was rapid and steep from day 0 till day 30, total fold-wise increase being over 64. On medium B, peak activity was observed on day 30 with over 53 fold increase. Except on days 5 and 10 the total enzyme activity was higher on medium A than on B.

On both the media peak specific activity was noticed on day 5, the actual values being 14.71 and 24.70 respectively. Between days 5 and 30 the activity was on decline continuously and linearly in both the media. By and large, the specific FDPA activity was higher on any given day on medium B than on A.

Expt. 16. Effect of 0.2 mg/l and 5 mg/l 2,4-D on growth and development of Amylase, Invertase, MDH, G-6-PDH and FDPA and on total and reducing Sugars in cotton callus cultures.

MS basal media containing 2% sucrose and supplemented with 0.2 mg/l and 5 mg/l 2,4-D were used. These are referred to in the text as medium A and medium B respectively.

(a) Growth :

(a) Growth measured as increase in fresh and dry weights is presented in Tables 17, 18.

Both on fresh and dry weight basis growth was better on medium A than on B. Fold-wise increases on fresh weight basis were 18.8 and 14.1 respectively on media A and B. On dry weight basis total increases were 19.0 and 15.3 folds respectively.

(b) Total and reducing sugar accumulation :

Progressive changes in the accumulation of total and reducing sugars are presented in Tables 17, 18.

Both total and reducing sugars on mg% basis accumulated rapidly during the initial 5 days in culture and thereof declined steadily till the end of culture period. However, accumulation on mg% basis was more on medium A than on B especially between days 5 and 15.

Table : 17. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 0.2 mg/l 2,4-D + 2% sucrose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	4.00	6.57
5	623.9 (±50.0)	27.7 (±4.7)	6.56	23.67	4.24	15.31	1.53	0.91	2.14	1.07	11.73	6.96	27.50	16.33	39.90	23.69
10	1248.4 (±49.9)	52.3 (±5.4)	5.45	10.42	3.76	7.19	3.88	0.68	1.30	0.23	33.71	5.87	66.60	8.12	89.90	14.43
15	2022.2 (±81.3)	82.7 (±6.5)	6.89	8.33	3.43	4.15	5.15	0.58	1.01	0.11	26.69	3.00	84.90	9.54	141.60	15.91
20	3702.1 (±144.5)	145.1 (±10.2)	7.86	5.42	3.55	2.45	18.85	0.84	12.26	0.47	142.16	9.37	224.60	14.80	177.70	11.71
25	6880.5 (±261.7)	262.2 (±18.8)	15.52	5.92	4.90	1.87	29.82	0.94	17.73	0.56	195.41	6.17	247.70	7.16	123.90	3.92
30	7518.8 (±300.4)	303.9 (±21.6)	12.25	4.03	8.51	2.80	36.68	1.36	26.61	0.98	138.35	5.11	125.30	4.68	140.70	3.00

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

Table : 18. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 5 mg/l 2,4-D + 2% sucrose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.25	2.69	2.24	0.17	0.25	5.75	4.80	2.93	2.44	4.00	6.67
5	536.7 (±39.4)	24.0 (±3.9)	3.44	14.33	2.05	8.54	1.32	0.91	3.41	1.77	17.60	12.15	21.80	15.06	26.84	16.52
10	1441.1 (±53.6)	58.1 (±5.2)	4.02	6.92	2.22	3.82	4.72	0.61	9.15	1.18	37.18	8.78	66.30	8.01	86.50	11.12
15	1901.4 (±70.5)	78.2 (±6.0)	4.63	5.92	2.13	2.73	6.22	0.64	6.78	0.48	34.99	3.61	66.70	6.67	91.26	9.41
20	3726.7 (±136.8)	136.3 (±9.4)	5.79	4.25	2.28	1.67	18.97	0.91	15.70	0.83	108.82	5.21	176.40	8.45	205.58	14.64
25	5483.2 (±210.1)	220.6 (±13.4)	9.00	4.08	4.13	1.87	23.76	0.99	17.74	0.74	141.47	5.86	82.30	3.41	142.60	5.91
30	5622.1 (±212.6)	245.2 (±14.3)	9.39	3.83	5.93	2.42	31.86	..06	21.41	0.78	122.56	4.44	76.83	2.79	168.66	6.12

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

(c) Amylase :

Progressive changes of amylase activity are presented in Tables 17, 18.

Total enzyme activity on media A and B declined slightly by day 5. Thereof on both the media the activity developed linearly and rapidly to attain peak values on day 30.

The specific activity of amylase also followed the similar developmental pattern on both the media. However, no significant difference in specific activity was observed between the two treatments.

(d) Invertase :

Invertase activity, total and specific, is presented in Tables 17, 18.

Though total enzyme activity decayed in both the media by day 15, it revived rapidly to reach peak values on day 30 with over 156 and 125 fold increases in media A and B respectively. Total activity was higher on medium B than on A except on day 30.

The specific activity also on both the media increased during the initial 5 days in culture to attain peak values. After declining between days 5 and 15, it developed linearly till day 30. Except on day 30, the specific activity was higher on medium B than on A.

(e) M D H :

Progressive changes in the total and specific activities of MDH are presented in Tables 17, 18.

Total MDH activity on media A and B showed peak values on day 25 with over 34 and 24 fold increases respectively. Total activity was more on medium B till day 15 and thereafter medium A supported higher MDH activity.

On medium A, peak specific activity was registered on day 20 with a total fold-wise increase of nearly 2 and the activity dropped thereof rather rapidly. On the other hand, on medium B, the specific MDH activity demonstrated its peak value on day 5 with 2.53 fold increase. Though the enzyme activity decayed by day 15, it staged a comeback by day 20 and then remained steady. Specific MDH activity was more on medium B till day 15 and thereafter the activity was higher on medium A.

(f) G-6-P D H :

Total and specific activities of G-6-PDH are presented in Tables 17, 18.

Total enzyme activity on medium A increased substantially from day 0 till day 25 to attain its peak value with over 84 folds. On medium B, on the other hand, the activity increased over 60 folds to register its peak value on day 20.

The specific activity of G-6-PDH on both the media

demonstrated peak values on day 5 and then decayed. On medium A, both total and specific activities of G-6-PDH were higher on any given day than on medium B.

(g) F D P A :

Progressive changes in the total and specific activities of FDPA are presented in Tables 17, 18.

FDPA on unit culture basis exhibited peak values on day 20 with over 44 and 51 fold increases respectively on media A and B. On both the media enzyme activity declined significantly thereof.

On both the media specific FDPA activity attained peak values on day 5 with 3.55 and 2.78 fold increase and declined thereof. Total as well as specific enzyme activities were higher on medium A till day 15 and thereafter higher activities were noticed on medium B than on A.

Expt. 17. Effect of 0.04 mg/l and 2 mg/l kinetin on growth and development of Amylase, Invertase, MDH, G-6-PDH and FDPA and on total and reducing Sugars in cotton callus cultures.

MS basal medium containing 2% sucrose and supplemented with 0.04 mg/l and 2 mg/l kinetin were used. These are referred to in the text as medium A and medium B respectively.

(a) Growth :

Growth measured as increase in fresh and dry weights is presented in Tables 19, 20.

Fold-wise increases on fresh and dry weight basis on medium A were 24.7 and 22.5, while on medium B they were 36.8 and 26.2 respectively. Obviously growth was higher on medium B than on A.

(b) Total and reducing sugar accumulation :

Variation in the accumulation of total and reducing sugars are presented in Tables 19, 20.

Total sugars on mg% basis increased 7.9 and 9.9 folds by day 5 on media A and B respectively. Thereafter they declined very rapidly on both the media till the end of culture period. On mg/culture basis peak values were observed on day 30 on both the media.

Reducing sugars also followed a similar pattern.

(c) Amylase :

Progressive changes of amylase activity are presented in Tables 19, 20.

Total enzyme activity on medium A slightly declined by day 5 and thereafter developed sharply till day 25 to reach

Table : 19. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 0.04 mg/l Kinetin + 2% sucrose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	4.00	6.67
5	557.7 (±42.9)	25.0 (±4.5)	12.92	51.66	7.74	30.96	2.01	1.09	1.49	0.74	16.51	10.22	10.38	5.67	29.00	23.64
10	1536.2 (±61.1)	59.5 (±6.7)	16.07	27.00	4.62	7.76	4.00	0.65	10.04	1.45	41.17	10.72	11.27	2.93	82.95	13.50
15	2984.6 (±116.8)	119.1 (±8.0)	12.70	10.66	2.67	2.24	7.78	0.48	36.66	2.09	94.31	15.80	24.87	4.17	228.77	12.81
20	6284.8 (±245.9)	206.6 (±13.4)	8.62	4.17	2.38	1.15	27.24	1.20	29.57	1.31	207.37	12.98	56.56	3.69	238.82	10.56
25	8323.5 (±321.7)	348.5 (±18.4)	13.35	3.83	3.94	1.13	54.78	1.52	24.62	0.85	136.31	9.12	39.41	2.80	133.18	8.89
30	9895.6 (±381.5)	360.0 (±22.1)	20.56	5.71	7.34	2.04	50.38	1.00	24.51	0.80	209.83	8.33	26.38	1.99	178.12	7.20

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

Table : 20. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 2.0 mg/l kinetin + 2% sucrose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in contiruous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	4.00	6.67
5	547.5 (±41.3)	26.8 (±4.7)	17.03	64.66	7.65	28.56	2.77	1.97	1.98	1.37	15.26	8.54	11.73	8.46	17.52	10.67
10	1639.1 (±60.9)	68.4 (±8.0)	16.18	23.66	4.43	6.48	5.82	1.31	18.83	1.98	28.85	5.50	32.78	6.29	91.79	17.92
15	3384.8 (±133.7)	113.1 (±7.9)	6.41	5.67	3.27	2.89	10.49	1.36	26.51	2.98	75.82	8.30	64.31	7.34	234.08	16.87
20	9007.5 (±360.5)	290.2 (±15.8)	15.03	5.18	6.44	2.22	58.69	2.41	30.99	2.37	200.50	12.36	27.02	1.62	246.33	16.43
25	13083.0 (±520.4)	387.8 (±21.3)	19.70	5.08	10.70	2.76	68.36	2.97	46.29	1.75	240.73	11.25	30.24	1.13	196.25	11.63
30	14699.2 (±591.9)	419.5 (±23.6)	19.93	4.75	6.38	1.52	71.71	2.99	30.37	1.62	376.30	10.77	19.31	0.48	232.18	11.50

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

its peak value with over 20 fold increase. On the other hand, on medium B, total activity developed unabated from day 0 till day 30 to attain the peak with over 26 fold increase.

The specific amylase activity on medium A was lesser on any day than that on day 0. Though a drop in the activity was observed by day 10 on medium B, it started increasing slowly thereof till day 30 to reach a peak value of 2.99 units. Amylase activity on unit culture and on unit protein basis was considerably higher on medium B than on A all through the culture period.

(d) Invertase :

Invertase activity, total and specific, is presented in Tables 19, 20.

Rapid development in total enzyme activity ensued between days 0 and 15 to attain its peak value of over 215 fold increase. Activity decayed linearly between days 15 and 30. On the other hand, total invertase activity on medium B increased sharply over 272 folds between days 0 and 25 to register its peak value.

The specific invertase activity reached its peak values on day 15 in both the media, registering 7.2 and 10.3 fold increases respectively. Thereafter activity decayed in both the media. Total and specific activities of invertase were higher on medium B than on A.

(e) M D H :

The MDH activity, total and specific, is presented in Tables 19, 20.

Total enzyme activity on media A and B registered peak values on day 30 with over 36 and 65 fold increases respectively.

Specific MDH activity on medium A increased to 15.80 units by day 15 to attain the peak value. Thereof activity decayed till day 30. On medium B, the peak enzyme activity was observed on day 20, the actual value being 12.36 units. Marginal decay in enzyme activity ensued between days 20 to 30. Till day 20, total and specific MDH activity was higher on medium A, and thereof it was markedly higher on medium B.

(f) G-6-P D H :

Total and specific activities of G-6-PDH are presented in Tables 19, 20.

Total enzyme activity on medium A attained its peak value on day 20 with 19.3 fold increase and activity decayed rather rapidly thereof. On medium B, total activity developed very sharply and registered its peak on day 15 with 22 fold rise, followed by decline till the end of culture period.

The specific G-6-PDH activity on media A and B showed peak values on day 5 with 2.3 and 3.5 fold increases

respectively. Activity declined, however, in both the media till the end of culture period. Total and specific G-6-PDH activity was many times higher on medium B till day 15, but thereafter it was higher on medium A on both the counts.

(g) F D P A :

Progressive changes in the total and specific activities of FDPA are presented in Tables 19, 20.

Total enzyme activity exhibited peak value on day 20 on both the media with a fold-wise increase of 59.7 and 61.6 respectively.

Specific FDPA activity demonstrated its peak value on day 5 in medium A with over 3.5 fold increase. Enzyme activity dropped continuously from day 5 till day 30 along a linear gradient. On the other hand, in medium B the enzyme activity progressed from day 0 till day 10 to attain a peak with about 2.7 fold increase. Continuous but slow decay in activity ensued between days 10 and 30. Both total and specific activities of FDPA were higher on medium B (except on day 5) than on A.

Expt. 18. Effect of 25 mg/l and 100 mg/l GA_3 on growth and development of Amylase, Invertase, MDH, G-6-PDH and FDPA and on total and reducing Sugars in cotton callus cultures.

MS basal medium containing 2% sucrose and supplemented

with 25 mg/l and 100 mg/l GA_3 were used. These are referred to in the text as medium A and medium B respectively.

(a) Growth :

Growth measured as increase in fresh and dry weights is presented in Tables 21, 22.

Fold-wise increases on fresh and dry weight basis on medium A were 18.9 and 15.1, while on medium B they were 8.9 and 7.4 respectively. Growth was many times higher on medium A than that on medium B.

(b) Total and reducing sugar accumulation :

Variation in the accumulation of total and reducing sugars are presented in Tables 21, 22.

Both total and reducing sugars on mg% basis accumulated rapidly during initial 5 days on both the media and thereafter declined till day 30. More accumulation of sugars was observed on medium B than on A during the initial 5 days.

(c) Amylase :

Progressive changes of amylase activity are presented in Tables 21, 22.

Total enzyme activity followed the same developmental trend

Table : 21. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 25 mg/l GA_3 + 2% sucrose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	4.00	6.67
5	454.9 (±38.7)	18.0 (±3.8)	8.94	49.66	3.86	21.44	1.62	1.78	1.70	1.87	12.74	14.00	8.19	9.00	16.83	18.50
10	1174.1 (±44.9)	48.3 (±5.2)	16.10	33.33	6.22	12.88	3.27	1.11	1.22	0.42	34.52	11.76	9.39	5.20	44.62	15.00
15	1839.7 (±76.5)	78.8 (±6.9)	8.40	10.66	4.98	6.32	5.69	0.86	8.42	1.27	64.39	9.72	36.79	5.56	86.47	13.06
20	3245.7 (±122.3)	122.8 (±9.4)	11.46	9.33	7.32	5.96	17.11	2.30	4.74	0.73	77.90	12.00	17.31	2.67	94.13	14.50
25	5318.3 (±201.4)	188.3 (±12.0)	7.21	3.83	3.74	1.99	34.64	2.04	4.84	0.28	117.00	6.88	49.64	2.92	138.28	8.13
30	7563.2 (±300.6)	241.5 (±14.6)	6.64	2.75	2.95	1.22	50.89	1.73	7.37	0.25	222.36	7.54	63.03	2.74	237.40	9.74

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

Table : 22. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 100 mg/l GA_3 + 2% sucrose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.29	5.75	4.30	2.93	2.44	4.00	6.67
5	403.6 (±36.9)	20.0 (±3.9)	12.47	62.33	5.29	26.44	1.83	1.92	0.59	0.67	6.46	7.28	1.08	1.22	22.60	25.45
10	856.9 (±46.4)	33.5 (±4.4)	10.27	30.66	6.35	18.96	3.39	1.69	4.75	2.52	21.25	11.94	9.14	4.70	49.70	26.36
15	1287.0 (±49.3)	56.7 (±5.1)	11.15	19.66	7.03	12.40	7.84	2.77	8.48	2.61	44.79	15.82	11.15	3.91	43.76	15.79
20	2088.3 (±77.2)	85.5 (±6.9)	4.28	5.00	2.47	2.89	14.99	2.98	15.23	2.70	74.37	14.96	13.92	2.42	104.42	18.63
25	2203.5 (±83.0)	100.5 (±7.3)	5.53	5.50	3.78	3.76	15.30	3.96	16.78	1.49	45.39	8.39	14.41	1.70	139.46	8.97
30	3577.0 (±40.1)	117.9 (±8.5)	4.92	4.17	1.46	1.24	26.44	2.85	21.51	1.52	113.03	8.31	28.62	1.75	143.08	9.86

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

on both the media. Activity increased sharply and substantially till day 30 to attain peak values with over 18 and 9 fold increases respectively on media A and B.

The specific amylase activity on medium A registered its peak on day 20 with 2.30 units. Decline in activity ensued between days 20 to 30. On medium B, the specific activity slowly dropped by day 10 and then started rising up till day 25 to register its peak value with 3.96 units. Total activity was higher on medium B between days 0 to 15 and thereafter it was significantly higher on medium A. The specific activity, on the other hand, was considerably higher on medium B than on A on all the days.

(d) Invertase :

Total and specific invertase activity is presented in Tables 21, 22.

After initial fluctuations, the total enzyme activity developed fast till day 15 on medium A with over 49 fold increase. The activity declined, however, thereof. On medium B, the total invertase activity progressed unabated till day 30 recording over 126 fold increase.

The specific invertase activity on medium A reached its peak value on day 5 with over 6 fold increase and declined thereof. Following rapid increase in the enzyme activity, on

medium B, the peak value was found on day 20 with over 9 fold increase. Activity dropped by day 25 and remained steady thereof. Except on day 5, both the total and specific invertase activities were several folds higher on medium B than on A.

(e) M D H :

The MDH activity, total and specific, are presented in Tables 21, 22.

Peak value was reached on day 30 as a result of sharp and continuous increase in the total activity of MDH on medium A, fold-wise increase being over 38. On medium B also the total activity developed very rapidly till day 30 to attain a peak with over 19 fold increase.

The specific MDH activity registered its peak value on day 5 on medium A with a fold-wise increase of 2.92. On medium B the specific MDH activity progressed linearly till day 15 to attain a peak with 3.30 fold increase. Total MDH activity was several folds higher on medium A than on B on any given day; whereas the specific MDH activity was higher on medium A only on day 5. Thereof, higher activity was noticed on medium B.

(f) G-6-P D H :

The progressive changes in total and specific G-6-PDH activities are presented in Tables 21, 22.

On both media A and B peak in total enzyme activity was observed on day 30 with over 21 and 9 fold increases respectively.

The specific G-6-PDH activity on medium A registered its peak value on day 5 with about 3.69 fold increase. Activity declined by day 20 and remained almost steady till day 30. On medium B the specific activity reached its peak on day 10 with 1.93 fold increase. Continuous decay in activity ensued between days 10 and 25 and remained steady thereof. Total and specific activities of G-6-PDH were many folds higher on medium A than on B during the entire course of culture.

(g) F D P A :

Total and specific activities of FDPA are presented in Tables 21, 22.

On both the media, total enzyme activity increased sharply to reach peak values on day 30 with over 59 and 35 folds respectively.

The specific FDPA activity on medium A showed peak value on day 5 (3 fold increase), while on medium B peak was observed on day 10 (3.95 fold increase). By and large, total and specific activities of FDPA were higher on medium B than on A.

Summary :

Growth was higher on low IAA (2 mg/l) medium when compared to high IAA containing medium (5 mg/l). By and large, specific activities of the enzymes amylase, invertase, MDH, G-6-PDH and FDPA were higher on 2 mg/l IAA medium than on 5 mg/l IAA medium.

Growth was superior on 0.2 mg/l NAA than on 2 mg/l. However, amylase and MDH activities were markedly higher on 2 mg/l NAA than on 0.2 mg/l NAA medium. On the other hand, the invertase activity on both the counts was higher on low (0.2 mg/l) NAA medium. While the specific G-6-PDH activity was higher on medium containing low NAA (0.2 mg/l), the FDPA specific activity was higher on 2 mg/l NAA containing medium.

Low 2,4-D containing medium (0.2 mg/l) favoured better growth than high 2,4-D (5 mg/l). While no significant difference in specific amylase activity was noticed between the above two 2,4-D media, total and specific invertase activities were, on the whole, higher on medium containing 5 mg/l 2,4-D. MDH activity on unit protein basis showed higher activities on high (5 mg/l) 2,4-D medium till day 15 and thereafter significantly higher activities were noticed on low 2,4-D medium (0.2 mg/l). G-6-PDH on both the counts demonstrated higher activities on low 2,4-D medium. FDPA

showed higher activities on low 2,4-D till day 15, but thereafter considerably higher activities were noticed on high 2,4-D containing medium.

Higher kinetin level (2 mg/l) in the medium favoured superior growth. Specific amylase, invertase and FDPA exhibited considerably higher activities on medium containing 2 mg/l kinetin than on 0.04 mg/l kinetin containing medium. Till day 20, higher activity of MDH on both the counts was observed on medium with low kinetin and thereof it was higher on medium with high kinetin. Total and specific G-6-PDH activity was significantly higher on medium with high kinetin till day 15, but thereafter it was higher on medium containing low kinetin.

Low GA (25 mg/l) supported higher growth in comparison with high GA (100 mg/l); but higher GA containing medium favoured considerably higher activities of the enzymes amylase, invertase, MDH and FDPA on unit protein basis. However, low GA (25 mg/l) containing medium showed many fold higher activity of G-6-PDH on both the counts during the course of culture.

Section E : Effect of carbohydrates on growth and development of Amylase, Invertase, MDH, G-6-PDH and FDPA and on total and reducing sugars in callus cultures of cotton.

Hormones and carbohydrates are essential supplements in the nutrient medium for the successful culture of isolated plant tissues and cells. Carbohydrate nutrition and the effect of different carbon sources on organogenetic development has been described in the earlier Chapter, Introduction. The role of carbohydrate in cultured plant cells is not only to provide an energy source, but also to act as a specific regulatory agent presumably recognized in cells by virtue of a specific feature of its structure (Jeffs and Northcote,1967).

In present study, the regulatory effect of different carbohydrates on growth and development of enzymes Amylase, Invertase, MDH, G-6-PDH and FDPA is examined in callus cultures of cotton.

Results in this section are presented under following captions :

(i) Studies with callus tissues of G. hirsutum cultured on 2 mg/l each of IAA, NAA and KN supplemented with 1, 2 and 4% glucose.

(ii) Studies with callus tissues of G. hirsutum cultured on 2 mg/l each of IAA, NAA and KN supplemented with 1, 2 and 4% fructose.

(iii) Studies with callus tissues of G. hirsutum cultured on 2 mg/l each of IAA, NAA and KN supplemented with 0.5% glucose + 0.5% fructose; 1% glucose + 1% fructose; and 2% glucose + 2% fructose.

(iv) Studies with callus tissues of G. hirsutum cultured on 2 mg/l each of IAA, NAA and KN supplemented with 1 and 4% sucrose.

(v) Studies with callus tissues of G. hirsutum cultured on 2 mg/l each of IAA, NAA and KN supplemented with 1, 2 and 4% maltose.

(vi) Studies with callus tissues of G. hirsutum cultured on 2 mg/l each of IAA, NAA and KN supplemented with 1, 2 and 4% starch.

Callus tissues weighing 400±40 mg were used as inoculum in 40 ml of the experimental medium. The culture vessels were incubated in continuous light at 26±2°C for 30 days. Five replicates were harvested at the interval of 5 days for the measurement of growth and sugar contents as well as for the analysis of enzymes.

Expt. 19. Studies with callus tissues of G. hirsutum cultured on 2 mg/l each of IAA, NAA and KN supplemented with 1, 2 and 4% glucose.

In the experimental medium glucose was incorporated as a source of carbon at 1, 2 and 4% levels. The media are referred to in the text as medium A, medium B and medium C respectively.

(a) Growth :

Growth, expressed as increase in fresh and dry weights

is presented in Tables 23, 24, 25.

Growth on fresh and dry weight basis was stimulated with increase of glucose content into the medium. Fold-wise increases in fresh weight were over 20, 31 and 41 on media A, B and C respectively. On dry weight basis the fold-wise increases were over 16, 23 and 48 respectively.

(b) Total and reducing sugar accumulation :

Progressive changes in the accumulation of total and reducing sugars are presented in Tables 23, 24, 25.

With increasing concentrations of glucose, total and reducing sugars on mg% basis also increased. Total and reducing on mg% basis accumulated rapidly during initial 5 days in culture and declined steadily thereafter till the termination of culture period. Reducing sugars followed similar pattern on the three media.

(c) Amylase :

The development of amylase activity is presented in Tables 23, 24, 25.

The total enzyme activity on media A, B and C increased rapidly to reach its peak value on day 30 with over 16, 31 and 44 folds rise respectively.

While there was no significant difference in the specific

Table : 23. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l kinetin + 1% glucose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	4.00	6.67
5	1368.6 (±44.5)	49.4 (±4.8)	14.16	28.67	6.64	13.44	1.58	0.39	6.09	1.48	30.66	7.47	15.51	3.78	76.60	28.66
10	2356.4 (±83.2)	71.2 (±6.0)	13.86	19.47	2.58	3.63	8.14	1.08	17.67	2.34	55.61	7.38	71.50	9.48	108.40	17.38
15	4024.3 (±160.4)	124.1 (±7.3)	16.05	12.93	5.04	4.06	19.63	1.63	7.07	1.00	146.48	14.13	84.50	7.00	80.50	16.67
20	5675.4 (±221.7)	173.9 (±11.2)	14.38	8.27	8.23	4.73	29.92	1.95	14.12	0.92	162.32	10.59	41.60	2.72	193.00	12.60
25	7435.6 (±300.2)	187.7 (±12.4)	10.27	5.47	2.91	1.55	34.92	1.95	27.08	0.88	217.12	7.92	96.70	3.17	252.60	9.27
30	8108.4 (±320.5)	257.3 (±16.8)	9.26	3.60	5.89	2.29	44.47	2.79	10.60	0.52	283.79	14.00	64.90	2.20	229.50	12.80

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

Table : 24. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l kinetin + 2% glucose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein.
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	4.00	6.67
5	1427.1 (±53.2)	67.0 (±5.1)	10.18	15.20	5.30	7.92	3.98	0.61	8.82	1.35	25.12	4.83	57.10	8.70	149.80	22.82
10	4147.6 (±160.5)	123.1 (±9.2)	17.57	14.27	15.88	4.90	20.24	0.96	21.75	1.03	110.33	5.22	177.00	8.37	165.90	7.84
15	7040.4 (±280.9)	206.9 (±16.4)	28.14	13.60	25.22	6.19	25.39	1.20	15.16	0.72	278.80	13.20	122.00	5.78	307.90	16.00
20	9490.2 (±370.8)	311.1 (±25.6)	21.15	6.80	13.75	4.42	57.80	1.69	24.46	0.72	210.68	6.17	259.40	7.59	170.80	5.00
25	11043.2 (±442.6)	357.2 (±28.9)	15.25	4.27	8.86	2.48	62.58	1.23	27.10	0.42	366.63	7.22	169.30	3.33	209.80	4.13
30	12780.9 (±510.2)	368.2 (±28.6)	15.72	4.27	9.50	2.58	85.98	2.69	15.08	0.47	416.66	13.04	89.50	2.80	268.40	6.40

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

Table : 25. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l kinetin + 4% glucose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	4.00	6.67
5	891.4 (±50.0)	67.5 (±5.6)	16.29	24.13	7.79	11.54	3.70	1.22	7.75	2.56	21.75	4.18	28.50	9.40	46.40	15.31
10	2375.3 (±82.7)	162.1 (±12.3)	30.91	19.07	16.08	9.92	5.38	0.64	6.44	0.77	55.11	4.56	52.90	6.29	84.10	10.00
15	4595.0 (±181.9)	199.5 (±15.1)	28.47	14.27	4.95	2.48	11.28	0.77	6.36	0.43	153.47	10.44	68.90	4.69	238.90	16.25
20	8715.4 (±344.2)	429.9 (±32.4)	78.54	18.27	39.12	9.10	37.35	2.14	3.58	0.21	66.24	3.80	81.60	7.67	296.30	7.00
25	12020.8 (±480.5)	617.9 (±48.7)	75.82	12.27	56.23	9.10	65.93	1.37	3.39	0.07	367.84	7.16	340.60	6.99	312.50	6.34
30	16532.6 (±667.3)	768.1 (±61.2)	94.25	12.27	80.88	10.53	118.73	1.56	3.82	0.05	562.11	7.39	209.41	2.95	429.04	6.96

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

amylase activity in media A and B, the enzyme activity on medium C was always lower than that of day 0. Peak enzyme values were observed on day 30 in media A and B with 1.25 and 1.20 fold increases respectively.

(d) Invertase :

Progressive changes in total and specific invertase activity are presented in Tables 23, 24, 25.

While the total enzyme activity on media A and B showed peak values on day 25 with over 159 fold increase, on medium C the peak was registered on day 5 with over 45 fold increase. Activity on medium C declined thereafter. Total activity was higher on medium B than on A and C. When the activity was compared between A and C, it was higher several folds on medium A than on C except on day 5.

The specific invertase activity attained its peak value on day 10 with 8.1 (medium A) and 8.8 (medium C) fold increases, while on medium B peak value was noticed on day 5 with 4.7 fold increase. Activity on all the three media decayed thereafter till day 30. The specific invertase activity was higher on all the days on medium A than on B and C. When media B and C were compared enzyme activity was many folds higher on medium B.

(e) M D H :

The MDH activity, total and specific, is presented in Tables 23, 24, 25.

The total enzyme activity on all the three media registered its peak value on day 30 with over 49, 72 and 97 fold increases respectively.

Both the media A and B showed double peaked developmental pattern. The first peak was attained on day 15 with 14.1 and 13.2 units, while the second peak was observed on day 30 with 14 and 13 units respectively. On medium C, however, peak value was attained on day 15 with 10.4 units and the activity decayed sharply by day 20 and picked up thereafter. The specific MDH activity was higher on medium A than on B and C. On medium B, the activity was higher than on medium C allthrough the course of culture.

(f) G-6-P D H :

The G-6-PDH activity, total and specific, is presented in Tables 23, 24, 25.

The total enzyme activity on media A and C demonstrated its peak value on day 25 with 33 and 116.3 fold increases, while on medium B the peak was observed on day 20 with 88.5 fold increase. Total activity was several folds higher on medium B than on A. Between media B and C activity was higher on medium B till day 20 and thereafter it was higher on medium C.

The specific G-6-PDH activity on medium A demonstrated

its peak on day 10 with 9.5 units, whereas media B and C demonstrated peak values on day 5 with 8.7 and 9.4 units. The enzyme activity, however, decayed on all the three media by day 30 in culture. The specific activity was higher on medium B than on A and C except between days 10 to 15. When media A and C were compared it was higher on medium C except between days 10 to 15.

(g) F D P A :

The FDPA activity, total and specific, is presented in Tables 23, 24, 25.

The total enzyme activity on medium A demonstrated its peak value on day 25 with over 63 fold increase, medium B exhibited peak activity on day 15 with over 76 fold increase, while on medium C, enzyme activity increased unabated and also substantially till day 30 with over 107 fold increase.

While media A and B showed peak values on day 5 with 28.7 and 22.8 units respectively, medium C registered its peak on day 15 with 16.3 units. The specific activity declined thereof on all the three media. The specific FDPA activity was significantly higher on medium A than B and C on any given day. When medium B was compared with medium C, the activity was higher on C except on day 5.

Expt. 20. Studies with callus tissues of G. hirsutum cultured on 2 mg/l each of IAA, NAA and KN supplemented with 1, 2 and 4% fructose.

In the experimental medium fructose was incorporated at 1, 2 and 4% levels and the media are referred to in the text as medium A, medium B and medium C respectively.

(a) Growth :

Growth, expressed as increase in fresh and dry weights, is presented in Tables 26, 27, 28.

On fresh weight basis, fold-wise increases were 15, 29 and 39 respectively on media A, B and C. On dry weight basis, fold-wise increases were 11, 23 and 47 respectively. Obviously, growth increased with increasing fructose level in the medium.

(b) Total and reducing sugar accumulation :

Progressive changes in the accumulation of total and reducing sugars are presented in Tables 26, 27, 28.

Total and reducing sugars on mg% basis accumulated very rapidly during early days in culture and then started declining steadily till the termination of culture period. Total and reducing sugars on both per culture and mg% basis were highest on medium C followed by B and A.

Table : 26. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l kinetin + 1% fructose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	4.00	6.67
5	728.3 (±38.7)	26.9 (±3.9)	1.65	6.13	1.15	4.29	3.42	1.84	4.03	0.99	26.51	6.50	24.03	5.89	46.60	14.40
10	2219.4 (±83.2)	66.9 (±4.8)	3.21	4.80	1.16	1.73	10.42	1.57	13.71	2.06	52.82	7.93	63.70	9.60	119.90	18.00
15	3129.4 (±121.5)	97.8 (±7.6)	2.87	2.93	1.86	1.90	18.40	1.96	17.33	1.85	85.75	9.13	184.60	19.70	59.50	6.50
20	4374.1 (±163.7)	113.4 (±8.8)	6.20	5.47	2.15	1.90	22.27	2.04	5.16	0.87	115.48	10.56	93.30	8.50	113.70	10.40
25	5642.0 (±230.0)	163.8 (±12.3)	7.32	4.47	5.08	3.10	36.76	2.04	12.15	0.87	185.06	6.25	127.90	11.10	95.90	5.30
30	6052.8 (±240.4)	174.3 (±12.5)	7.44	4.27	4.69	2.69	30.81	1.89	10.17	0.53	58.11	3.00	90.80	4.70	90.80	4.70

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

Table : 27. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l kinetin + 2% fructose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	4.00	6.67
5	847.5 (±46.3)	45.8 (±3.7)	7.14	15.60	4.41	9.63	2.49	1.34	4.68	0.90	30.34	16.27	24.41	7.73	54.20	29.10
10	2734.7 (±105.9)	109.0 (±8.2)	8.86	8.13	6.40	5.87	10.36	0.82	19.95	1.59	62.35	14.50	133.10	10.60	188.70	15.00
15	5328.9 (±207.4)	195.9 (±15.6)	10.72	5.47	7.89	4.03	28.10	2.40	13.73	1.17	155.60	13.27	122.60	10.60	165.20	14.10
20	7331.9 (±290.1)	238.5 (±17.5)	13.83	5.80	7.39	3.10	34.44	1.47	16.36	0.83	211.16	10.67	132.00	6.70	168.60	11.50
25	9833.0 (±388.7)	353.8 (±28.4)	22.89	6.47	11.50	3.25	40.82	1.15	37.95	0.80	308.76	7.14	409.50	9.70	186.80	7.30
30	11628.0 (±451.3)	368.4 (±28.0)	17.68	4.80	7.74	2.10	65.89	1.77	11.09	0.24	181.40	4.88	124.00	3.30	209.30	5.60

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

Table : 28. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l kinetin + 4% fructose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.00 (±40.0)	16.00 (±4.00)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.20	5.75	4.80	2.93	2.44	4.00	6.67
5	727.1 (±38.9)	57.5 (±5.1)	14.33	24.93	6.43	11.18	1.12	0.23	3.71	0.81	15.85	3.89	32.50	8.00	54.00	13.80
10	1702.5 (±62.6)	136.3 (±10.7)	18.54	13.60	13.13	9.63	5.57	0.61	10.52	1.14	37.80	4.11	84.00	9.10	81.70	8.90
15	3814.4 (±146.4)	260.2 (±20.3)	49.62	19.07	29.09	11.18	12.48	0.56	19.46	0.87	47.30	2.10	153.80	6.80	144.90	6.40
20	6103.6 (±241.7)	400.9 (±33.5)	151.82	37.87	56.85	14.18	18.87	0.97	5.15	0.31	197.76	10.13	109.90	5.60	183.10	9.40
25	10432.2 (±420.1)	575.1 (±45.2)	78.21	13.60	57.05	9.92	36.04	0.96	6.15	0.16	433.98	11.56	139.10	3.70	260.80	6.90
30	15453.8 (±670.4)	760.6 (±61.9)	138.96	18.27	103.97	13.67	69.78	1.00	11.00	0.20	207.08	3.00	113.30	2.00	200.90	3.60

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

Table : 30. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l kinetin + 1% glucose + 1% fructose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	8.00	6.67
5	938.4 (±43.7)	57.1 (±4.6)	9.98	17.47	5.50	9.63	2.52	0.38	8.41	1.60	4.13	0.99	76.60	14.58	42.40	7.69
10	3427.4 (±139.9)	119.9 (±9.1)	8.96	7.47	3.86	3.22	8.93	0.80	28.03	1.24	134.35	5.94	193.10	8.54	245.60	18.00
15	6009.1 (±240.2)	229.5 (±17.4)	23.25	10.13	17.21	7.30	18.57	1.41	19.47	1.47	206.71	15.64	220.30	16.66	72.10	5.44
20	11812.8 (±470.5)	366.8 (±29.7)	47.43	12.93	22.12	6.03	38.66	1.49	19.84	0.76	408.72	15.73	279.60	11.76	158.60	5.91
25	13316.3 (±524.9)	389.2 (±30.8)	36.86	9.47	29.19	7.50	70.21	1.65	25.44	0.60	420.80	9.88	199.70	4.69	240.20	8.12
30	14096.0 (±561.8)	391.5 (±31.6)	29.25	7.47	21.10	5.39	71.66	1.59	14.64	0.33	310.11	6.88	56.40	1.25	267.80	5.94

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

Table : 31. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l kinetin + 2% glucose + 2% fructose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	4.00	6.67
5	629.5 (±47.2)	46.3 (±5.1)	9.20	19.87	5.02	10.85	1.95	0.55	6.21	1.76	3.40	0.97	39.20	11.12	41.60	11.80
10	1085.4 (±49.9)	78.5 (±6.3)	20.30	25.86	10.72	13.66	3.75	0.52	7.92	1.11	23.23	3.24	64.00	6.93	59.70	8.33
15	2470.4 (±100.6)	182.6 (±14.7)	24.83	13.60	16.62	9.10	10.71	1.06	8.23	0.81	45.46	4.49	63.40	6.26	44.50	4.39
20	4273.2 (±162.5)	244.3 (±20.4)	44.63	18.27	22.23	9.10	19.84	0.68	8.49	0.58	88.03	6.06	126.80	8.73	72.60	5.00
25	8246.2 (±322.8)	420.9 (±33.8)	87.55	20.80	38.30	9.10	32.73	1.13	6.98	0.27	303.46	11.50	123.80	4.69	66.00	2.50
30	10280.0 (±403.4)	534.0 (±45.6)	98.95	18.53	63.65	11.92	48.28	1.16	16.47	0.30	242.61	5.76	212.50	5.04	236.40	5.61

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

(c) Amylase :

The development of amylase activity is presented in Tables 26, 27, 28.

Total enzyme activity exhibited its peak value on day 25 on medium A with over 13 fold increase, whereas media B and C recorded their peak values on day 30 with over 24 and 26 folds rise respectively. On the whole, total activity was higher on medium B than A and C. When media A and C were compared, higher activities were noticed on medium A except on day 30.

While there was no marked difference in the specific amylase activity on media A and B, the enzyme activity on medium C was always lower than that of day 0. On media A and B peak values were observed on days 20 and 15 with 2.0 and 2.4 units respectively. The specific amylase activity was higher on medium A than on B except on day 15.

(d) Invertase :

Progressive changes in total and specific invertase activity are presented in Tables 26, 27, 28.

While tissues on media A and C exhibited their peak total activity on day 15 with over 102 and 114 fold increase, that of medium B recorded its peak value on day 25 with over 223 fold increase. The enzyme activity was higher many folds on medium B than on A and C except on day 15. When medium A was

compared with medium C, enzyme activity was higher on the whole on medium A except on day 15.

On all the three media, the specific invertase activity demonstrated its peak value on day 10 with over 7, 5.5 and 4 folds increase respectively. The specific invertase activity was considerably higher on medium A than on B and C.

(e) M D H :

The MDH activity, total and specific, is presented in Tables 26, 27, 28.

The total enzyme activity on media A, B and C demonstrated peak values on day 25 with over 32,53 and 75 fold increases respectively. Total activity was many folds higher on medium B than on A. When medium B was compared with medium C, it was many folds higher on medium B till day 20 and thereof it was higher on medium C. When medium A was compared with C, it was higher on medium A till day 15 and thereafter it was higher on medium C.

The specific MDH activity on medium A recorded its peak value on day 20, actual value being 10.6 units, whereas medium B exhibited its peak value on day 5 with 16.3 units, while on medium C activity demonstrated its peak on day 25 with 11.6 units. By and large, the specific MDH activity was higher on medium B than on A and C.

(f) G-6-P D H :

The G-6-PDH activity, total and specific, is presented in Tables 26, 27, 28.

With a fold-wise increase of 63 and 52, total G-6-PDH exhibited peak values on day 15, respectively on media A and B, whereas on medium B, the peak value was recorded on day 25 with a fold-wise increase of over 139. Total activity was several folds higher on the whole on medium B than on A and C.

While the specific enzyme activity demonstrated its peak value on day 15 on medium A with 19.7 units, the same was noticed on day 10 on media B and C with 10.6 and 9.1 units respectively. Thereof the activity decayed till day 30. The specific activity was highest on medium A followed by B and C.

(g) F D P A :

The progressive changes of FDPA activity are presented in Tables 26, 27, 28.

Total enzyme activity on medium A recorded its peak value on day 10 with over 30 fold increase; on medium B peak value was observed on day 30 with over 52 fold increase; and on medium C peak value was registered on day 25 with over 65 fold increase. Total FDPA activity on medium B was higher

than on A on any given day. When the activity on medium B was compared with that on medium C, it was found higher on medium B till day 15 and thereafter on medium C. When media A and C were compared, enzyme activity was higher on medium C during the course of culture. While the specific FDPA activity on medium A demonstrated its peak value on day 10 with 18.0 units, on media B and C the peak values were observed on day 5 with 29.1 and 13.8 units respectively. The specific enzyme activity of FDPA was many folds higher on medium B than on A and C. Except on day 25, the specific activity was higher on A than on C.

Expt. 21. Studies with callus tissues of G. hirsutum cultured on 2 mg/l each of IAA, NAA, and KN supplemented with 0.5% glucose + 0.5% fructose, 1% glucose + 1% fructose and 2% glucose + 2% fructose.

The experimental medium was supplied with a mixture of glucose + fructose at 0.5 + 0.5, 1.0 + 1.0 and 2.0 + 2.0% levels and the respective media are referred to A, B and C.

(a) Growth :

Growth expressed as increase in fresh and dry weights is presented in Tables 29, 30, 31.

On fresh weight basis, fold-wise increases were 17.3, 35.2 and 25.7 respectively on media A, B and C. On dry weight

Table : 29. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l kinetin + 0.5% glucose + 0.5% fructose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.20	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	4.00	6.67
5	594.3 (±40.8)	35.6 (±4.2)	3.37	9.47	3.24	9.10	2.47	1.16	4.59	2.15	11.77	5.50	30.50	14.26	35.70	16.69
10	1798.2 (±65.4)	79.0 (±7.2)	11.38	14.40	6.61	8.37	6.81	0.62	17.75	1.62	37.04	3.38	85.70	7.81	134.90	12.30
15	3325.1 (±124.9)	136.5 (±9.6)	17.47	12.80	9.69	7.10	9.77	0.86	19.44	1.72	118.37	10.47	124.10	10.98	36.50	3.24
20	5250.9 (±202.5)	174.5 (±14.4)	17.68	10.13	14.20	8.14	12.09	0.68	27.53	1.54	173.28	9.71	208.30	11.67	157.50	8.82
25	6127.0 (±240.3)	190.4 (±14.9)	9.14	4.80	6.76	3.55	27.66	0.89	17.99	0.68	189.94	6.08	163.40	5.23	79.70	2.55
30	6914.0 (±272.7)	193.3 (±15.8)	8.64	4.47	7.07	3.66	27.45	1.32	4.52	0.22	91.26	4.40	50.70	2.44	117.50	5.67

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

Table : 30. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l kinetin + 1% glucose + 1% fructose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	8.00	6.67
5	938.4 (±43.7)	57.1 (±4.6)	9.98	17.47	5.50	9.63	2.52	0.38	8.41	1.60	4.13	0.99	76.60	14.58	42.40	7.69
10	3427.4 (±139.9)	119.9 (±9.1)	8.96	7.47	3.86	3.22	8.93	0.80	28.03	1.24	134.35	5.94	193.10	8.54	245.60	18.00
15	6009.1 (±240.2)	229.5 (±17.4)	23.25	10.13	17.21	7.50	18.57	1.41	19.47	1.47	206.71	15.64	220.30	16.66	72.10	5.44
20	11812.8 (±470.5)	366.8 (±29.7)	47.43	12.93	22.12	6.03	38.66	1.49	19.84	0.76	408.72	15.73	279.60	11.76	158.60	5.91
25	13316.3 (±524.9)	389.2 (±30.8)	36.86	9.47	29.13	7.50	70.21	1.65	25.44	0.60	420.80	9.88	199.70	4.69	240.20	8.12
30	14096.0 (±561.8)	391.5 (±31.6)	29.25	7.47	21.10	5.39	71.66	1.59	14.64	0.33	310.11	6.88	56.40	1.25	267.80	5.94

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

Table : 31. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l kinetin + 2% glucose + 2% fructose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	4.00	6.67
5	629.5 (±47.2)	46.3 (±5.1)	9.20	19.87	5.02	10.85	1.95	0.55	6.21	1.76	3.40	0.97	39.20	11.12	41.60	11.80
10	1085.4 (±49.9)	78.5 (±6.3)	20.30	25.86	10.72	13.66	3.73	0.52	7.92	1.11	23.23	3.24	64.00	6.93	59.70	8.33
15	2470.4 (±100.6)	182.6 (±14.7)	24.83	13.60	16.62	9.10	10.71	1.06	8.23	0.81	45.46	4.49	63.40	6.26	44.50	4.39
20	4273.2 (±162.5)	244.3 (±20.4)	44.63	18.27	22.23	9.10	19.84	0.68	8.49	0.58	88.03	6.06	126.80	8.73	72.60	5.00
25	8246.2 (±322.8)	420.9 (±33.8)	87.55	20.80	38.30	9.10	32.73	1.13	6.98	0.27	303.46	11.50	123.80	4.69	66.00	2.50
30	10280.0 (±403.4)	534.0 (±45.6)	98.95	18.53	63.65	11.92	48.28	1.16	16.47	0.30	242.61	5.76	212.50	5.04	236.40	5.61

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

basis, fold-wise increases were 12.1, 24.5 and 33.4 respectively. Dry weight of the tissue increased markedly with the increase of carbohydrate level in the medium.

(b) Total and reducing sugar accumulation :

Progressive changes in the accumulation of total and reducing sugars are presented in Tables 29, 30, 31.

Total and reducing sugars on mg% basis accumulated during initial 5 or 10 days on all the three media and thereafter sugar content declined steadily till day 30. Total and reducing sugars both on mg% and on culture basis were the highest on medium C followed by B and A.

(c) Amylase :

The development of amylase activity is presented in Tables 29, 30, 31.

While the tissue on medium A demonstrated its peak value on day 25 with over 10 fold increase, those on media B and C exhibited peak values on day 30 with over 26 and 18 fold increases. The amylase activity on unit culture basis was several folds higher on medium B than on A and C. Except on days 5 and 10, the enzyme activity was higher on C than on A.

The specific amylase activity on all the three media was much reduced and showed lesser values than that of day 0. The

enzyme activity was marginally higher on medium B than on A and C except on day 5. On the whole, the specific amylase activity was higher on A compared to C.

(d) Invertase :

The progressive changes of invertase activity in the tissues grown on media A, B and C are presented in Tables 29, 30, 31.

Total enzyme activity on medium A reached its peak on day 20 with over 162 fold increase, whereas on medium B peak value was observed on day 10 with over 165 fold increase, while on medium C peak value was recorded on day 30 with over 97 fold increase. By and large, total invertase activity was considerably higher on medium B than on A and C. Except on day 30 the activity was higher on medium A than on C.

On all the three media, the specific invertase activity exhibited its peak value on day 5 with 7.4, 5.5 and 6.1 fold increases respectively on media A, B and C. The specific enzyme activity was significantly higher on medium A followed by B and C.

(e) M D H :

The progressive changes in total and specific MDH activity in the cultured tissues are presented in Tables 29, 30, 31.

Substantial increase in the total enzyme activity on all the three media resulted in peak values on day 25 with over 33, 73 and 53 fold increases respectively on media A, B and C. Total MDH activity on medium B was many folds higher than on A and C. When A and C were compared the activity was higher on medium A till day 20 after which it was several folds higher on medium C.

The specific MDH activity on medium A exhibited the peak value on day 15 with 10.5 units, on medium B the peak value was recorded on day 20 with 15.7 units and on medium C the peak value was registered on day 25 with 11.5 units. The specific MDH activity was significantly higher on medium B than on A and C, and out of A and C it was higher on medium A till day 20 and thereafter on medium C.

(f) G-6-PDH :

The progressive changes of G-6-PDH activity are presented in Tables 29, 30, 31.

With a fold-wise increase of over 71 and 95, total G-6-PDH activity exhibited peak values on day 20 on media A and B respectively; while on medium C the peak value was recorded on day 30 with over 72 fold increase. Total enzyme activity was substantially higher on medium B than on A and C. Except on days 5 and 30, the enzyme activity was higher on medium A than on C.

While media A and C exhibited peak specific activity on day 5 with 14.3 and 11.1 units respectively, medium B registered its peak value on day 15 with 16.7 units. The specific enzyme activity was significantly higher on medium B than on A and C; and when A and C were compared it was higher on medium A except on day 30.

(g) F D P A :

The progressive changes of FDPA activity in the cultures are presented in Tables 29, 30, 31.

While peak value of FDPA on unit culture basis was attained on day 20 on medium A with a fold-wise increase of over 39, media B and C exhibited peak values on day 30 with a fold-wise increase of over 67 and 59 respectively. Total enzyme activity was several folds higher on medium B than A and C and between A and C it was higher on medium A than on C except on days 5, 15 and 30.

The FDPA on unit protein basis exhibited peak values on media A and C on day 5 with 16.7 and 11.8 units, whereas medium B registered its peak value on day 10 with 18 units. By and large, the specific FDPA activity was higher on medium B than on A and C. When A and C were compared, it was considerably higher on medium A.

Expt. 22. Studies with callus tissues of G. hirsutum cultured on 2 mg/l each of IAA, NAA and KN supplemented with 1 and 4% sucrose.

The experimental medium was added with sucrose at 1 and 4% levels separately as source of carbon. The media are referred to in the text as A and B respectively. The studies made at 2% sucrose are already described earlier in Experiment 10 in Section C - I.

(a) Growth :

Growth, expressed as increase in fresh and dry weights is presented in Tables 32, 33.

On fresh weight basis, fold-wise increases were 14.3 and 42.1 respectively on media A and B. On dry weight basis, fold-wise increases were 12.8 and 51.9 respectively.

(b) Total and reducing sugar accumulation :

Progressive changes in the accumulation of total and reducing sugars in cotton tissues are presented in the Tables 32, 33.

Total and reducing sugars on mg% basis accumulated rapidly during initial 5 or 10 days in culture and then declined steadily till the termination of culture period. Both total and reducing sugars on mg% and on mg/culture basis were higher on medium B than on A on any given day.

Table : 32. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l kinetin + 1% sucrose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	4.00	6.67
5	758.9 (±44.8)	32.2 (±4.0)	3.69	11.47	2.42	7.50	4.30	1.45	4.82	1.93	22.01	7.44	8.40	2.84	34.20	11.56
10	1372.5 (±45.2)	53.7 (±4.3)	4.01	7.47	1.18	2.19	5.95	1.28	10.29	2.21	35.69	7.65	48.50	10.39	65.90	14.13
15	2914.7 (±118.5)	101.4 (±8.1)	10.27	10.13	2.41	2.38	16.52	1.57	10.91	1.04	59.64	5.68	17.50	1.67	43.70	8.17
20	3570.0 (±142.6)	130.6 (±8.3)	9.76	7.47	2.74	2.10	18.82	2.40	4.21	0.54	137.80	17.56	28.60	3.64	64.30	8.19
25	4676.6 (±183.4)	178.0 (±9.8)	6.41	3.60	4.41	2.48	30.47	2.41	2.76	0.52	211.38	16.74	40.50	3.21	121.60	9.63
30	5717.2 (±224.7)	205.3 (±17.2)	6.71	3.27	4.31	2.10	29.11	1.89	7.92	0.51	221.83	14.37	64.80	4.20	281.60	18.89

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

Table : 33. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l kinetin + 4% sucrose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	4.00	6.67
5	690.6 (±47.5)	50.4 (±4.1)	9.21	18.27	5.31	10.53	2.13	0.61	6.82	1.84	1.24	0.35	56.40	16.00	67.00	19.00
10	2334.3 (±87.9)	150.9 (±12.8)	65.99	43.73	23.15	15.34	6.51	0.61	6.02	0.56	38.75	3.61	154.10	14.40	116.70	10.90
15	5165.8 (±202.4)	327.2 (±25.7)	87.69	26.80	41.69	12.74	10.33	0.41	31.06	0.83	89.88	3.55	297.90	11.80	222.10	8.80
20	7521.4 (±301.8)	440.5 (±36.3)	126.29	28.67	52.51	11.92	23.25	0.67	25.07	0.73	94.77	2.74	348.50	10.10	270.80	7.80
25	10439.4 (±420.7)	633.2 (±52.6)	187.43	29.60	97.13	15.34	43.34	1.15	14.45	0.39	342.41	9.11	160.10	4.30	146.20	3.90
30	16832.3 (±681.5)	829.8 (±67.9)	200.23	24.13	132.77	16.00	76.00	1.23	9.93	0.17	252.48	4.41	168.30	4.90	202.00	3.50

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

(c) Amylase :

The progressive changes of amylase activity are presented in Tables 32, 33.

The total enzyme activity on medium A exhibited its peak value on day 25 with over 11 fold increase; medium B on the other hand, demonstrated its peak value on day 30 with over 28 fold increase. On medium B, total enzyme activity was many folds higher than on medium A.

The specific enzyme activity after initial drop, on medium A, staged a comeback by day 25 with 2.4 units. On the other hand, activity on medium B was always lower than that of day 0. The specific amylase activity was considerably higher on medium A than on B throughout the culture period.

(d) Invertase :

The progressive changes of invertase activity are presented in Tables 32, 33.

On both the media, total enzyme activity attained its peak value on day 15 with over 64 and 182 fold increases respectively on medium A and B. Activity, however, dropped thereof.

The specific invertase activity on medium A and B registered peak values on days 10 and 5 respectively with

2.2 and 1.8 units. Thereof, activity on both the media declined till day 30 in culture. While total activity was several folds higher on medium B, the specific activity showed significantly higher values on medium A than on B.

(e) M D H :

The progressive changes in the total and the specific MDH activity are presented in Tables 32, 33.

The MDH activity on unit per culture basis exhibited peak values with over 38 and 59 fold increases on days 30 and 25 on media A and B respectively.

On unit protein basis, the MDH activity on media A and B demonstrated peak values with 17.6 and 9.1 units on days 20 and 25 respectively. While total activity was higher on medium B, the specific enzyme activity was several folds higher on medium A than on B.

(f) G-6-P D H :

The progressive changes of G-6-PDH activity are presented in Tables 32, 33.

Though total enzyme activity fluctuated much on medium A, peak value was attained on day 30 with over 64 units, whereas on medium B substantial increase in activity resulted in over 348 units to reach the peak value on day 20.

Rapid increase in the specific activity of G-6-PDH on medium A ensued between days 0 and 10 to attain a peak value with 10.4 units; while on medium B peak value was observed on day 5 with 16.0 units. Activity decayed thereof gradually. Both total and the specific activity was higher on medium B than on A on any given day.

(g) F D P A :

The FDPA activity, total and specific, is presented in Tables 32, 33.

Total and the specific FDPA activity on medium A followed an identical pattern of development. The activity though fluctuated on both the accounts exhibited peak values on day 30 with 70.4 and 2.8 fold increases. On medium B, on the other hand, total enzyme activity demonstrated its peak value on day 20 with 67.7 folds rise, while the specific activity registered its peak value on day 5 with 2.9 fold increase. The activity decayed thereafter.

While the total activity was several folds higher on medium B than on A, except on day 30; the specific activity was higher on medium A than on B, except on day 5.

Expt. 23. Studies with callus tissues of G. hirsutum cultured on 2 mg/l each of IAA, NAA and KN supplemented with 1, 2 and 4% maltose.

The experimental medium was supplied separately with maltose at 1, 2 and 4% levels and the media are referred to in the text as A, B and C respectively. Presence of maltase in the tissue was also detected [illegible].

(a) Growth :

Growth, expressed as increase in fresh and dry weights is presented in Tables 34, 35, 36.

On fresh weight basis, total fold-wise increases were over 15, 26 and 23 on media A, B and C respectively. Corresponding increases on dry weight basis were over 10, 17 and 27 folds respectively.

(b) Total and reducing sugar accumulation :

Progressive changes in the accumulation of total and reducing sugars are presented in Tables 34, 35, 36.

Both total and reducing sugars on mg% basis on all the three media increased sharply during the initial 5 days in culture and thereof declined till day 30. Both on mg% and on culture basis total and reducing sugars were higher on medium C followed by those on B and A.

Table : 34. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum.

Medium : MS + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l kinetin + 1% maltose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	4.00	6.67
5	700.4 (±46.1)	32.6 (±4.8)	2.44	7.47	1.81	5.55	3.29	1.57	2.06	0.98	17.79	8.47	26.60	12.67	27.30	12.99
10	1939.9 (±77.8)	75.2 (±6.5)	4.61	6.13	3.62	4.82	5.41	1.87	7.93	1.28	59.75	9.63	66.00	10.63	69.80	11.24
15	3218.4 (±122.5)	102.6 (±8.4)	3.69	3.60	1.77	1.73	13.95	2.71	7.18	1.39	140.32	27.25	51.50	10.00	61.20	11.89
20	4345.8 (±170.2)	112.7 (±8.2)	4.06	3.60	1.84	1.63	20.41	1.88	7.30	0.67	142.54	13.12	60.80	5.60	139.10	12.80
25	5436.3 (±207.4)	156.9 (±12.6)	8.58	5.47	3.73	2.38	34.27	2.34	5.99	0.41	171.79	13.70	68.90	6.69	87.00	8.93
30	6191.0 (±241.2)	171.1 (±13.8)	7.31	4.27	3.11	1.82	27.95	2.05	8.81	0.38	235.26	17.27	144.50	10.61	167.20	12.28

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

Table : 35. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l kinetin + 2% maltose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	4.00	6.67
5	1147.6 (±43.9)	44.5 (±4.7)	6.76	15.20	2.68	6.03	3.29	0.68	11.33	2.41	24.10	5.12	68.90	14.22	66.70	14.18
10	4283.2 (±163.5)	117.4 (±8.1)	6.42	5.47	2.35	2.00	11.94	1.55	11.81	1.53	131.90	17.12	61.40	7.96	111.40	14.45
15	5757.1 (±224.7)	162.6 (±10.4)	6.94	4.27	2.52	1.55	33.84	2.35	7.26	1.50	255.62	17.76	34.50	2.40	69.00	4.79
20	8041.1 (±320.6)	217.2 (±16.5)	7.82	3.60	3.19	1.47	39.23	1.44	27.63	0.91	220.33	8.06	252.00	9.22	201.00	7.35
25	9454.2 (±375.3)	263.9 (±21.2)	12.67	4.80	4.09	1.55	48.13	1.89	10.42	0.41	334.68	13.11	47.30	1.85	189.10	7.41
30	10665.0 (±422.7)	276.5 (±18.8)	9.95	3.60	4.51	1.63	67.22	1.97	6.97	0.20	375.41	11.00	32.00	0.94	304.60	11.56

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

Table : 36. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l kinetin + 4% maltose.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.29	5.75	4.80	2.33	2.44	4.00	6 67
5	764.9 (±62.4)	52.0 (±4.7)	12.06	23.20	5.16	9.92	2.25	0.48	5.75	1.23	13.16	2.82	68.60	14.70	39.00	8.36
10	1035.7 (±69.8)	76.2 (±6.3)	14.53	19.07	7.34	9.63	2.39	0.43	3.77	0.67	17.61	3.15	57.30	10.25	41.40	7.42
15	2152.7 (±81.9)	126.5 (±10.1)	17.20	13.60	8.74	6.91	10.11	1.02	8.80	0.89	40.47	4.09	23.00	2.32	21.50	2.17
20	3623.4 (±100.6)	185.9 (±13.7)	24.04	12.93	16.41	8.83	15.04	1.06	10.64	0.75	105.80	7.49	152.20	10.77	97.80	6.92
25	5713.5 (±219.5)	303.4 (±24.5)	39.23	12.93	25.39	8.37	26.84	0.84	13.26	0.41	80.00	2.50	201.90	6.31	285.70	8.93
30	9521.1 (±395.3)	438.0 (±33.9)	29.78	6.80	26.59	6.07	55.97	1.73	27.10	0.84	365.60	11.29	95.20	2.94	402.20	17.06

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

(c) Amylase :

The progressive changes in amylase activity in cotton callus are presented in Tables 34, 35, 36.

Total enzyme activity on media A, B and C exhibited peak values on days 25, 30 and 30 respectively with over 12, 25 and 21 fold increases. Total activity was higher on medium B than on A and C. Of A and C the enzyme activity was higher on medium A except on day 30.

While media A and B registered peak values in specific activity on day 15, medium C recorded its peak value on day 30, actual values being 2.7, 2.4 and 1.7 units respectively. The specific amylase activity was considerably higher on medium A followed by B and C throughout the culture period.

(d) Invertase :

The progressive changes of invertase activity in cultured tissues are presented in Tables 34, 35, 36.

On unit culture basis while the peak values were attained on day 30 in tissues on media A and C, that on medium B recorded its peak value on day 20, the fold-wise increases being over 52, 162 and 159 respectively. Total enzyme activity was the highest on medium B (except on day 30) and then on C and A in the decreasing order.

While the specific activity of invertase on medium A registered its peak value on day 15 with 1.4 units; media B and C recorded peak values on day 5 with 2.4 and 1.2 units respectively. Activity on all the three media decayed linearly thereof. The specific enzyme activity was higher on medium B than on A and C except on day 30. Of A and C, the activity was higher on medium C except on days 10 and 15.

(e) M D H :

Progressive changes of MDH activity in the callus, total and specific are presented in Tables 34, 35, 36.

On all the three media, total enzyme activity exhibited peak values on day 30 with over 41, 65 and 63 fold increases. On medium B, total enzyme activity was several folds higher than on A and C on any given day. When A and C were compared the enzyme activity was higher on medium A except on day 30.

The specific MDH activity on media A and B attained peak values on day 15, whereas medium C registered the peak value on day 30, actual values being 27.3, 17.8 and 11.3 units respectively. The specific activity of MDH was several folds higher on medium A than on B and C. Of B and C, the enzyme activity was higher on medium B.

(f) G-6-P D H :

Progressive changes of G-6-PDH activity are presented

in Tables 34, 35, 36.

Total enzyme activity on media A, B and C demonstrated peak values on days 30, 20 and 25 respectively with over 49, 86 and 69 fold increases. Total activity was higher on medium A than on B except on days 5 and 20. When B and C were compared, the activity was higher on medium B except on days 25 and 30. Of A and C, the activity was higher on medium C except on day 30 in culture.

The specific G-6-PDH activity attained peak values on day 5 with 5.2, 5.8 and 6.0 fold increases respectively on media A, B and C. Activity on all the three media decayed thereafter by day 30 in culture. The specific activity was the highest on medium A followed by that on C and B.

(g) F D P A :

The FDPA activity, total and specific, is presented in Tables 34, 35, 36.

Peak values in total enzyme activity were recorded on day 30 on all the three media with over 167, 304 and 402 units respectively. By and large, the total activity was higher on medium B than on A and C. Except on days 10 and 15, the enzyme activity was higher on medium C than on A.

FDPA on unit protein basis registered peak values with 1.9, 2.2 and 2.6 fold increases on days 5, 10 and 30 respectively

on media A, B and C. By and large, the activity was higher on medium A followed by that on B and C.

Expt. 24. Studies with callus tissues of G. hirsutum cultured on 2 mg/l each of IAA, NAA and KN supplemented with 1, 2 and 4% starch.

The experimental medium is added with starch at 1,2 and 4% levels as a source of carbon. The media are referred to in the text as A, B and C respectively.

(a) Growth :

Growth, expressed as increase in fresh and dry weights, is presented in Tables 37, 38, 39.

On fresh weight basis, total fold-wise increases were 11.8, 22.6 and 19.6 on media A, B and C respectively. Fold-wise increases on dry weight basis were 8.9, 19.0 and 26.9 respectively on media A, B and C. As in previous experiments, the highest dry weight increase was registered in medium with maximum carbohydrate level.

(b) Total and reducing sugar accumulation :

Progressive changes in the accumulation of total and reducing sugars are presented in Tables 37, 38, 39.

Total and reducing sugars on mg% basis accumulated during

Table : 37. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l kinetin + 1% starch.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	4.00	6.67
5	714.2 (±60.4)	26.2 (±3.4)	1.43	5.47	1.12	4.29	1.75	0.33	3.27	1.17	24.28	10.72	24.50	8.80	22.30	8.91
10	1470.1 (±69.5)	50.7 (±4.4)	2.77	5.47	1.31	2.58	5.37	1.61	4.60	1.16	42.34	10.67	35.30	8.89	41.20	10.38
15	2168.5 (±81.2)	71.9 (±6.0)	4.41	6.13	1.44	2.00	9.40	1.97	5.98	1.25	87.61	18.36	11.70	2.45	19.50	4.09
20	2876.1 (±103.6)	98.1 (±7.5)	5.37	5.47	3.16	3.22	11.94	1.30	15.93	1.73	90.31	19.81	79.60	8.65	80.50	8.75
25	3764.9 (±143.7)	121.4 (±9.3)	2.91	2.40	1.88	1.55	9.81	0.87	9.70	0.86	192.76	17.07	41.40	3.67	143.10	12.67
30	4720.3 (±181.9)	142.6 (±10.5)	5.14	3.60	2.47	1.73	19.60	1.38	5.20	0.37	171.82	12.13	94.40	6.67	213.80	19.34

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

Table : 38. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l kinetin + 2% starch.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.47	0.29	5.75	4.80	2.93	2.44	4.00	6.67
5	656.2 (±53.8)	29.5 (±3.6)	2.40	8.13	1.55	5.25	5.01	3.05	3.95	2.41	24.87	12.72	19.20	8.61	27.60	16.82
10	1560.9 (±61.7)	66.5 (±5.2)	3.19	4.80	2.21	3.33	8.85	1.77	11.71	2.35	45.83	9.38	72.30	14.48	48.40	10.69
15	4107.8 (±160.9)	121.2 (±9.4)	4.36	3.60	2.55	2.10	9.46	1.98	15.37	1.78	154.45	20.88	16.40	2.52	45.20	6.11
20	5183.8 (±202.5)	156.8 (±9.1)	6.70	4.27	4.36	2.78	29.37	1.77	43.73	2.61	121.30	7.31	300.70	18.13	150.30	9.06
25	7345.1 (±280.0)	267.3 (±20.4)	12.83	4.80	7.75	2.90	47.85	2.10	60.08	1.39	177.75	4.10	355.00	8.19	267.90	17.80
30	9052.2 (±350.6)	304.3 (±24.2)	14.00	4.60	8.46	2.78	60.90	1.98	32.03	1.04	343.98	10.18	262.50	8.53	399.90	23.25

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

Table : 39. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDP[illegible] callus cultures of Gossypium hirsutum

Medium : MS + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l kinetin + 4% starch.

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.69	2.24	0.47	0.29	5.75	4.80	2.93	2.44	4.00	6.67
5	590.2 (±40.8)	36.0 (±4.2)	4.42	12.27	2.42	6.72	1.45	0.77	2.35	1.24	19.59	10.37	18.90	10.00	22.70	10.64
10	1325.5 (±49.6)	62.9 (±5.3)	4.70	7.47	3.59	5.71	4.34	0.84	10.84	2.10	32.34	6.26	75.10	14.53	35.60	6.89
15	2373.8 (±83.4)	119.2 (±9.1)	10.49	8.80	8.24	6.91	13.96	1.51	16.37	1.77	56.97	6.15	83.10	8.98	23.70	2.56
20	3889.1 (±150.7)	158.2 (±12.6)	11.82	7.47	3.62	2.29	15.44	1.24	12.56	1.01	133.79	10.75	171.10	13.75	112.80	8.06
25	6856.3 (±260.9)	334.2 (±25.5)	20.49	6.13	13.47	4.03	44.05	2.01	24.26	1.11	268.77	12.25	164.60	7.50	246.80	11.25
30	7820.8 (±305.5)	430.1 (±33.9)	43.60	10.13	32.26	7.50	35.31	1.25	9.73	0.38	303.45	10.78	140.80	5.00	434.00	17.22

Data represents an average of 5 replicates.

Figures in the parenthesis represent standard error.

initial 5 days in culture and declined steadily thereof till the day 30. With increasing concentrations of starch, accumulation of total and reducing sugars both on mg% and on culture basis also increased.

(c) Amylase :

The progressive changes of amylase activity are presented in Tables 37, 38, 39.

While on media A and B total amylase activity exhibited peak values on day 30 with over 7 and 22 fold increases respectively, the peak value was registered on medium C on day 25 with over 16 fold increase. Total enzyme activity was many folds higher on medium B than on A and C; between A and C activity being higher on medium C.

The specific amylase activity on media A, B and C demonstrated peak values on days 15, 5 and 25 respectively, actual values being 1.97, 3.05 and 2.01 units. Throughout the culture period, the specific amylase activity was considerably higher on medium B than on A and C. Except on day 25, the activity was higher on medium A than on C.

Spent medium showed the presence of amylase activity, indicating extracellular digestion of starch.

(d) Invertase :

The progressive changes of invertase activity are presented in Tables 37, 38, 39.

Invertase activity on unit culture basis on medium A attained the peak value on day 20 with over 93 fold increase; while on media B and C peak values were recorded on day 25 with over 353 and 142 fold increases respectively. Total enzyme activity was significantly higher on medium B than on A and C. The activity of invertase on medium C was next higher followed by that on A.

While the specific activity on media A and B registered peak values on day 20 with over 6 and 9 fold increases, medium C attained its peak value on day 10 with over 7 fold increase. Activity was significantly higher on medium B followed by C and A.

(e) M D H :

The progressive changes in total and specific activities of MDH are presented in Tables 37, 38, 39.

The highest total enzyme activity was recorded on medium B (60 fold increase) followed by that on C (53 fold increase) and A (33 fold increase).

The specific MDH activity on media A, B and C registered peak values on days 20, 15 and 25, the actual values being 19.8, 20.9 and 12.3 units respectively. On medium A, the specific activity was higher than on B and C. When the activities on media B and C were compared, it was higher on

medium B till day 15 and thereafter it was higher on medium C.

(f) G-6-P D H :

Progressive changes of G-6-PDH activity are presented in Tables 37, 38, 39.

G-6-PDH activity on unit culture basis on media A, B and C registered peak values on days 30, 25, and 20 with over 32, 121 and 58 fold increases respectively. Total activity was significantly higher on medium B than on A and C. Between A and C it was higher on medium C.

While the specific activity on media A and C recorded peak values on day 10 with 8.9 and 14.5 units respectively; the peak value on medium B was registered on day 20 with 18.1 units. The specific activity was higher on medium B than on A. When B and C were compared, the activity was higher on medium C till day 15 and thereafter it was higher on medium B. The activity was considerably higher on medium C than on A.

(g) F D P A :

The FDPA activity, total and specific, is presented in Tables 37, 38, 39.

Total enzyme activity on all the three media A, B and C exhibited peak values on day 30 with a fold-wise increase of

over 53, 100 and 108 respectively. Total activity was many folds higher on medium B than on A and C. When A and C were compared, it was higher on medium C than on A.

The specific FDPA activity also exhibited peak values on day 30 on all the three media A, B and C with a fold-wise increase of 2.9, 3.5 and 2.6 respectively. Activity was significantly higher on medium B than on A and C. Of A and C, the activity was higher on medium A.

Summary :

With increasing concentrations of glucose, fructose, glucose + fructuse mixture, sucrose, maltose and starch, growth of cotton callus also increased as measured by dry weight. Total and reducing sugar accumulation on mg% basis also increased with the rise in sugar level in the medium.

While the specific activities of the enzymes amylase, invertase, MDH and FDPA in cotton callus were favoured by low glucose (1%), specific G-6-PDH activity was more in the callus grown at higher glucose concentrations (2 and 4%).

On the other hand, fructose at 1% level stimulated higher specific activities of amylase, invertase and G-6-PDH; whereas fructose at 2% level favoured higher specific activities of MDH and FDPA.

By and large, the specific activities of amylase, MDH, G-6-PDH and FDPA were higher on medium containing 1% glucose + 1% fructose; whereas the specific invertase activity was significantly higher in callus tissues grown on medium containing 0.5% glucose +.5% fructose.

While the specific activities of amylase, invertase, MDH and FDPA were promoted by low sucrose (1%) in the medium, higher sucrose (4%) favoured the specific activity of G-6-PDH.

Maltose at 1% level stimulated the specific activities of amylase, MDH, G-6-PDH and FDPA, but at 2% level the specific invertase activity was promoted.

Higher specific activities of the enzymes amylase, invertase, G-6-PDH and FDPA were observed on 2% starch; whereas the specific MDH activity was higher in the tissues grown on 1% starch containing medium.

Section F : Organogenesis in callus cultures of *Nicotiana tabacum* L.

Callus tissues of tobacco were cultured on MS basal medium with varying levels of IAA and sucrose and the results are shown in the Table 40. The cultures were incubated at 26±2°C in continuous light and periodic observations made.

Expt. 25. Influence of IAA and sucrose interaction on organogenesis in callus cultures of tobacco.

Callus tissues cultured on MS basal medium containing 2% sucrose but devoid of phytohormones served as the control. Shoots were differentiated on this medium during the fourth week of culture period. Frequency of response on this medium was 40-50%; 2-10 shoots being formed per callus mass.

On MS medium containing 0.3 mg/l IAA and 1% sucrose, no organogenetic response was observed. However, with the increase of sucrose level to 2% at the same IAA concentration, solitary shoots were differentiated during the fourth week of culture with about 50% frequency. Further increase of sucrose level to 3% in the same medium, resulted in the differentiation of solitary shoots during the second week of culture, frequency of response being 40-50% (Fig. 6, Table 40). Still further increase of sucrose level to 6% in the same medium, however, resulted in differentiation of roots instead of shoots in 12-13 days of culture with about 20-25% frequency.

On MS basal medium containing 1% sucrose and supplemented with 2.0 mg/l IAA no organogenetic response was observed. Roots

Table : 40. Influence of IAA on organogenesis in callus cultures of Nicotiana tabacum

Medium	IAA concentration (mg/l)	Sucrose (%)	Morpho-genetic response	Frequency response (%)	Timé taken for response
MS	-	2.0	Shoot	40 - 50	Many shoots in 4 weeks.
MS	0.3	1.0	-	-	-
MS	0.3	2.0	Shoot	40 - 50	Solitary shoots in 4 weeks.
MS	0.3	3.0	Shoot	40 - 50	Solitary shoot in 12-15 days.
MS	0.3	6.0	Root	20 - 25	Day 12
MS	2.0	1.0	-	-	-
MS	2.0	2.0	Roots	60 - 75	4 weeks.
MS	2.0	3.0	Roots	60 - 75	13-15 days.
MS	2.0	6.0	Roots	20 - 25	Day 12.

Fig. 6. Shoot differentiation in tobacco callus (0.3 mg/l IAA + 3% sucrose).

Fig. 7. Root differentiation in tobacco callus. (2.0 mg/l IAA + 3% sucrose).

were, however, formed in the medium when the sucrose level was increased to 2%. Roots were differentiated during the fourth week of culture with 75% frequency. Further increase of sucrose level to 3% in the medium, roots were differentiated in 13-15 days of culture with about 60-75% frequency (Fig. 7, Table 40). Though further increase of sucrose level to 6% in the medium resulted in root differentiation, the frequency of organogenetic response dropped to a mere 20-25%. Time taken for morphogenetic response on this medium was 12-13 days (Table 40).

Summary :

Organogenesis in callus cultures of tobacco was observed on MS basal medium containing 2% sucrose but without any hormones. Morphogenetic response in this medium was delayed and shoots appeared in culture only in the fourth week. In MS medium containing 0.3 mg/l IAA and 3% sucrose shoots were formed with the same frequency. However, the time taken for organogenetic response was reduced to two weeks. With the increase of sucrose level to 6% on the same medium, there was a morphogenetic shift from shoots to roots. Another notable change was a drop in response frequency to 20-25%. When IAA concentration was raised from 0.3 mg/l to 2.0 mg/l keeping the sucrose level at 3%, roots were differentiated with a high frequency of 75%. However, it dropped down to 20-25% when the sucrose level in the medium was raised to 6%.

Section G - I : Physiological studies with Amylase, Invertase, MDH, G-6-PDH and FDPA and total and reducing sugars and total starch in callus tissues of tobacco and cotton cultured on shoot inducing medium.

From the experiments carried out earlier and described in Section F of Chapter III (Results), it became obvious that callus tissues of N. tabacum exhibited the morphogenetic capability in vitro. It was the exogenous supply of growth hormones, singly or in combinations and also sugar concentration, which determined the organogenetic responses of tobacco callus tissues.

On the other hand, no organogenetic response was noticed in callus tissues of cotton, though the tissues were grown on the shoot forming medium of the tobacco besides many permutations of concentrations and combinations of known hormones.

To examine the physiological changes associated with shoot differentiation in tobacco callus the following parameters were looked into : (a) Growth, (b) Total and reducing sugar accumulation, (c) Total starch, (d) Amylase, (e)Invertase, (f) MDH, (g) G-6-PDH, and (h) FDPA. The said parameters were also examined in non-shoot-forming cotton callus to gain a comparative account.

The shoot differentiating medium used for this study was: MS basal + 0.3 mg/l IAA + 3% sucrose. Shoots were differentiated from callus tissues of tobacco on the above medium in a maximum of 15 days with around 75 per cent frequency.The experiments were terminated as soon as morphogenetic responses were manifested or else were carried on till day 15 of culture in

tobacco. In case of cotton tissues experiments were terminated on day 21. During this period, commencing with day 0, callus tissues were harvested every third day and analysed for the parameters enlisted above.

For the sake of convenience the results obtained are presented under the following heads :

(i) Studies with callus tissues of N. tabacum cultured on shoot inducing medium.

(ii) Studies with callus tissues of G. hirsutum cultured on shoot inducing medium of tobacco.

Expt. 26. Studies with callus tissues of N. tabacum cultured on shoot inducing medium.

Culture flasks inoculated with tobacco tissues (300±30 mg) were harvested on random basis every third day, and analysed for growth, accumulation of sugars, starch and enzymes. On day 15, non-shoot forming portion of the same callus grown on the same medium was analysed separately for all the parameters under study. The results are described below.

(a) Growth :

Growth, measured as increase in fresh and dry weights, is presented in Fig. 8 A and Table 41.

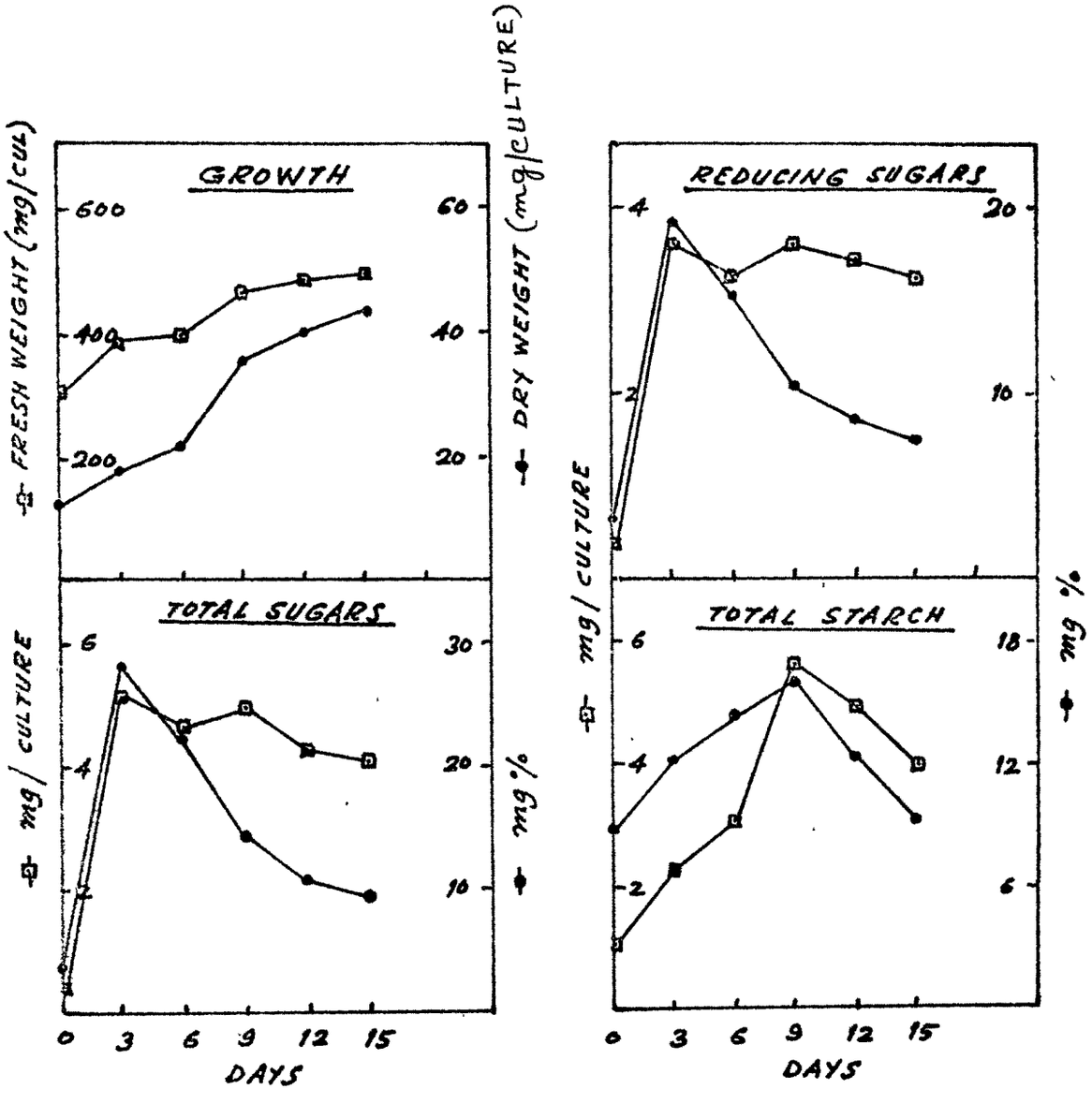

FIG. 8A (REF. TABLE NO. 41)

Table 41 : Growth, accumulation of total and reducing sugars, starch and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Nicotiana tabacum

Medium : MS + 0.3 mg/l IAA + 3% sucrose (Shoot forming medium).

Inoculum : 300±30 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total sugars		Reducing sugars		Total Starch		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult	Unit/mg protein
0	300.0 (±30.0)	12.0 (±3.0)	0.39	3.28	0.35	2.90	1.05	8.75	0.26	0.29	0.31	0.35	1.69	0.88	1.05	1.17	1.50	1.67
3	382.0 (±33.0)	18.6 (±2.3)	5.19	27.93	3.57	19.17	2.26	12.15	0.60	0.38	0.61	0.39	3.57	3.52	9.93	6.34	15.28	9.76
6	392.6 (±24.0)	21.2 (±3.5)	4.69	22.10	3.24	15.26	3.01	14.22	0.76	0.29	0.43	0.17	2.88	2.33	7.85	3.03	16.88	6.51
9	465.0 (±41.0)	35.0 (±4.7)	4.89	13.98	3.57	10.21	5.63	16.09	0.35	0.14	0.42	0.16	1.74	0.99	7.21	2.78	15.12	4.97
12	479.3 (±21.9)	40.3 (±8.9)	4.26	10.57	3.41	8.45	4.91	12.18	1.05	0.39	0.57	0.21	3.12	1.34	5.51	2.05	16.78	6.25
15	493.2 (±22.5)	43.6 (±6.0)	4.05	9.29	3.21	7.36	3.97	9.10	0.66	0.22	0.68	0.23	4.47	1.66	8.88	2.95	16.77	5.57
15	Non-shoot forming portion of callus		4.74	10.87	3.50	8.02	5.52	12.65	0.15	0.07	0.17	0.07	1.25	0.70	1.73	0.76	4.43	2.17

Data represents an average of 5 replicates.

Figures in parenthesis represent standard error.

Growth of tobacco callus on shoot inducing medium increased rapidly during the days 0 and 3 on fresh and dry weight basis. Growth was relatively slow between days 3 and 6. However, growth was again fast between days 6 and 9. Thereon it continued to increase at a slow rate till day 15. During the course of culture for 15 days, fold-wise increases in growth were 1.6 and 3.6 on fresh and dry weight basis respectively.

Shoots differentiated on this medium on day 15 of culture with a frequency of about 75%.

(b) Total and reducing sugar accumulation :

Progressive changes in the accumulation of total and reducing sugars are presented in Fig. 8 A, Table 41.

Total and reducing sugars both on mg% and on mg/culture basis accumulated very rapidly between days 0 to 3. Thereof they went on declining gradually till the termination of culture.

(c) Total starch :

Though total extractable starch accumulated rather rapidly between days 0 and 3, the peak value was attained on day 9 both on mg/culture and mg% basis (Fig. 8 A and Table 41). From day 9, the accumulated starch began to decline. Rapid accumulation was noticed prior to the physiological events leading to organo-

genesis in the shoot forming portion of the callus. On the contrary, in the non-shoot forming portion of callus on day 15, starch content was higher when compared to the organ forming portion of the callus (Table 41).

(d) Amylase :

Progressive changes in the amylase activity in callus cultures of tobacco on the shoot inducing medium are illustrated in Fig. 8 B and Table 41.

Though the total amylase activity declined between days 6 and 9, the peak value attained on day 12. However, the activity declined towards the termination of culture period. The specific amylase activity declined during days 3 to 9 and then increased rapidly till day 12 to attain its peak value. During the days preceding shoot differentiation (i.e. days 12 to 15), the activity declined. Comparatively the non-shoot forming portion of callus exhibited very less activity on day 15 both per culture and also per unit protein.

(e) Invertase :

Changes in the invertase activity in tobacco callus cultured on shoot inducing medium are illustrated in Fig. 8 B and Table 41.

The enzyme activity per culture and per unit protein

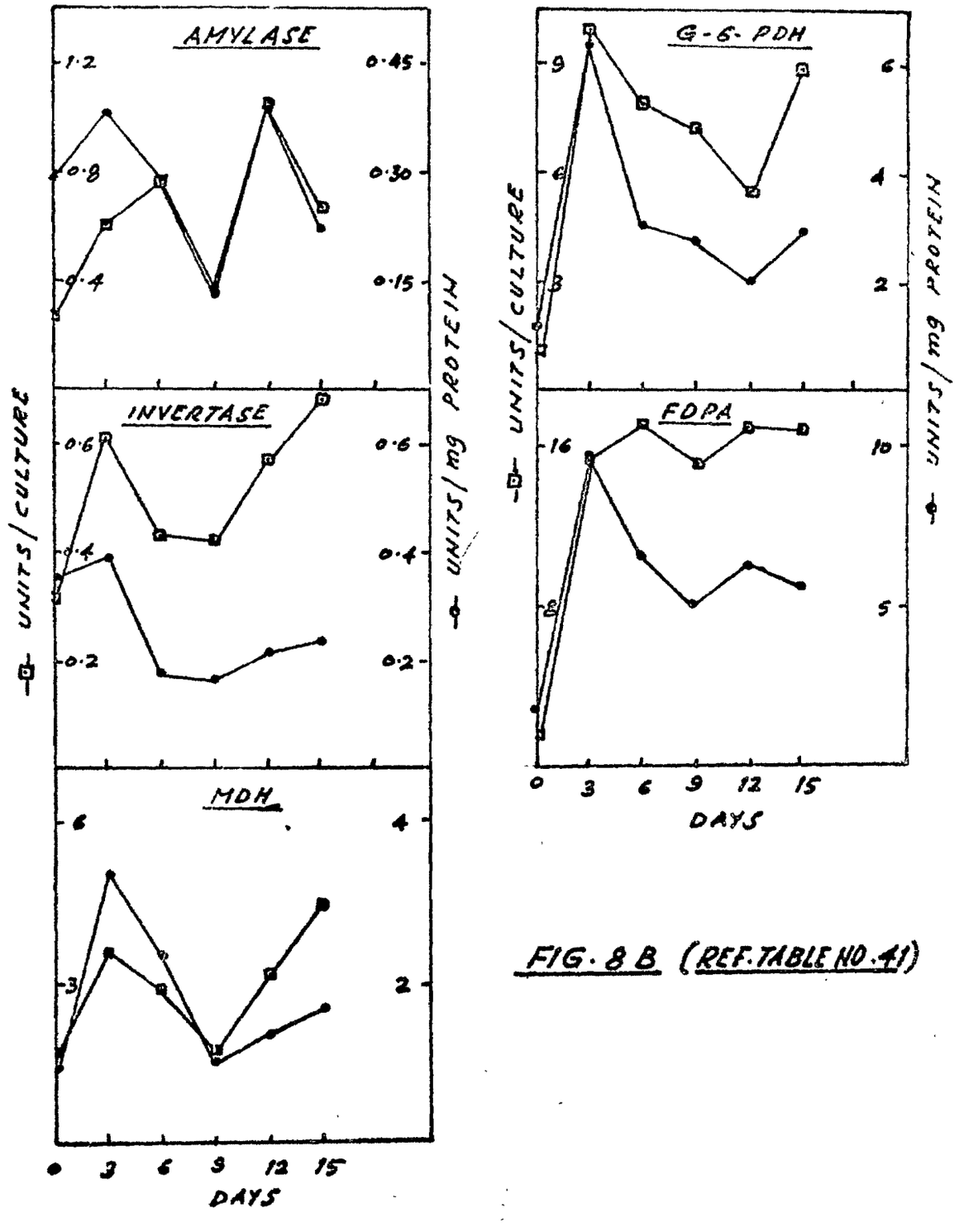

FIG. 8 B (REF. TABLE NO. 41)

followed similar patterns of development. The activity on both the counts increased between days 0 and 3 and then declined till day 9. However, marked (total enzyme) to marginal (specific) increase in the activity was noticed between days 9 and 15. The enzyme activity was on increase during the days immediately preceding shoot differentiation. The invertase activity in the non-shoot forming portion of the callus on the other hand, was comparatively several folds less both on per culture and unit protein basis.

(f) M D H :

The activity of MDH in tobacco callus on shoot differentiating medium is illustrated in Fig. 8 B and Table 41.

The enzyme activity, both on per culture and per unit protein followed an identical pattern of development during the 15 day culture period. The activity on both the counts increased rapidly between days 0 and 3; followed by a decline till day 9. The enzyme activity increased appreciably between days 9 and 15. During the entire culture period two peak values were thus reached, one each on day 3 and 15. The MDH activity on both the counts was on increase during the days preceding shoot formation (days 9 to 15). Non-shoot forming portion of the callus, exhibited very low MDH activity on day 15 when compared to the shoot forming portion of the callus.

(g) G-6-P D H :

Fig. 8 B and Table 41 represent progressive changes of G-6-PDH activity in shoot-forming tobacco callus.

The enzyme activity per culture and per unit protein exhibited similar patterns of development. The activity on both the counts shot up sharply from days 0 to 3 reaching its first peak on day 3. During the period of 3 to 12 days the activity, however, decreased followed by an another increase to attain its second peak value on day 15. The enzyme activity was on increase between days 12 and 15 i.e., the days immediately preceding shoot differentiation.

Non-shoot forming portion of the callus when compared with the shoot forming portion, exhibited lower activities both per culture and per unit protein basis.

(h) F D P A :

Progressive changes in the activity of FDPA are presented in Fig. 8 B and Table 41.

There was a sharp and rapid increase of the enzyme activity both per culture and per unit protein between days 0 to 3. However, the specific activity declined till day 9, followed by marginal increase during the days preceding shoot differentiation.

Non-shoot forming portion of the same callus grown on the same medium, however, exhibited lower FDPA activities when compared to the shoot forming callus of tobacco.

Expt. 27. Studies with callus tissues of G. hirsutum cultured on shoot inducing medium of tobacco.

Culture flasks inoculated with cotton tissues on shoot inducing medium of tobacco (0.3 mg/l IAA + 3% sucrose) were harvested every third day, for measurements of growth, accumulation of sugars and starch, and analysis of enzymes. On day 15, the lower portion of the callus tissue grown on the same medium was also analysed separately for the above parameters to find out the differences in enzyme activities if any. The results are documented below.

(a) Growth :

Growth measured as increase in fresh and dry weights, is illustrated in Fig. 9 A and Table 42.

During days 0 to 15, fold-wise increases in fresh and dry weights were 19.4 and 26.4 respectively. In contrast, in case of tobacco they were barely 1.6 and 3.6 respectively. Clearly, growth of cotton tissue was very rapid compared with that of the tobacco tissue.

In tobacco callus grown on the same medium, differentiation

Table : 42. Growth, accumulation of total and reducing sugars, starch and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 0.3 mg/l IAA + 3% sucrose (Shoot forming medium of tobacco).

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total sugars		Reducing Sugars		Total Starch		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.06	12.90	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	4.00	6.67
3	617.3 (±44.5)	46.1 (±5.9)	16.70	36.23	11.52	24.98	4.91	10.65	2.25	0.94	2.31	0.96	49.38	20.51	12.40	5.15	14.20	5.90
6	1609.4 (±102.8)	80.0 (±7.4)	19.82	24.78	12.79	15.99	10.56	13.20	7.53	2.34	3.07	0.95	7.73	2.40	2.15	0.67	9.70	3.01
9	3675.8 (±126.2)	188.0 (±10.4)	3177	16.98	23.97	12.75	28.41	15.11	12.08	2.53	2.64	0.55	46.32	9.69	12.30	2.57	36.80	7.70
12	6135.1 (±246.7)	353.0 (±18.7)	42.61	12.07	36.01	10.20	50.09	14.19	55.22	2.81	4.40	0.22	380.38	19.38	38.90	1.98	110.40	5.62
15	7743.5 (±318.6)	422.8 (±23.9)	41.73	9.87	32.34	7.65	67.99	16.08	39.55	1.89	4.57	0.22	92.92	4.44	23.20	1.11	69.70	3.33
	Lower portion of callus		44.39	10.50	29.60	7.00	38.18	9.03	2.49	0.20	2.68	0.22	0.00	0.00	0.00	0.00	0.00	0.00
21	8831.9 (±329.5)	441.9 (±26.7)	29.70	6.72	22.49	5.09	44.19	10.00	61.19	4.30	6.91	0.49	220.80	15.63	29.40	2.08	61.80	4.37

Data represents an average of 5 replicates.

Figures in parenthesis represent standard error.

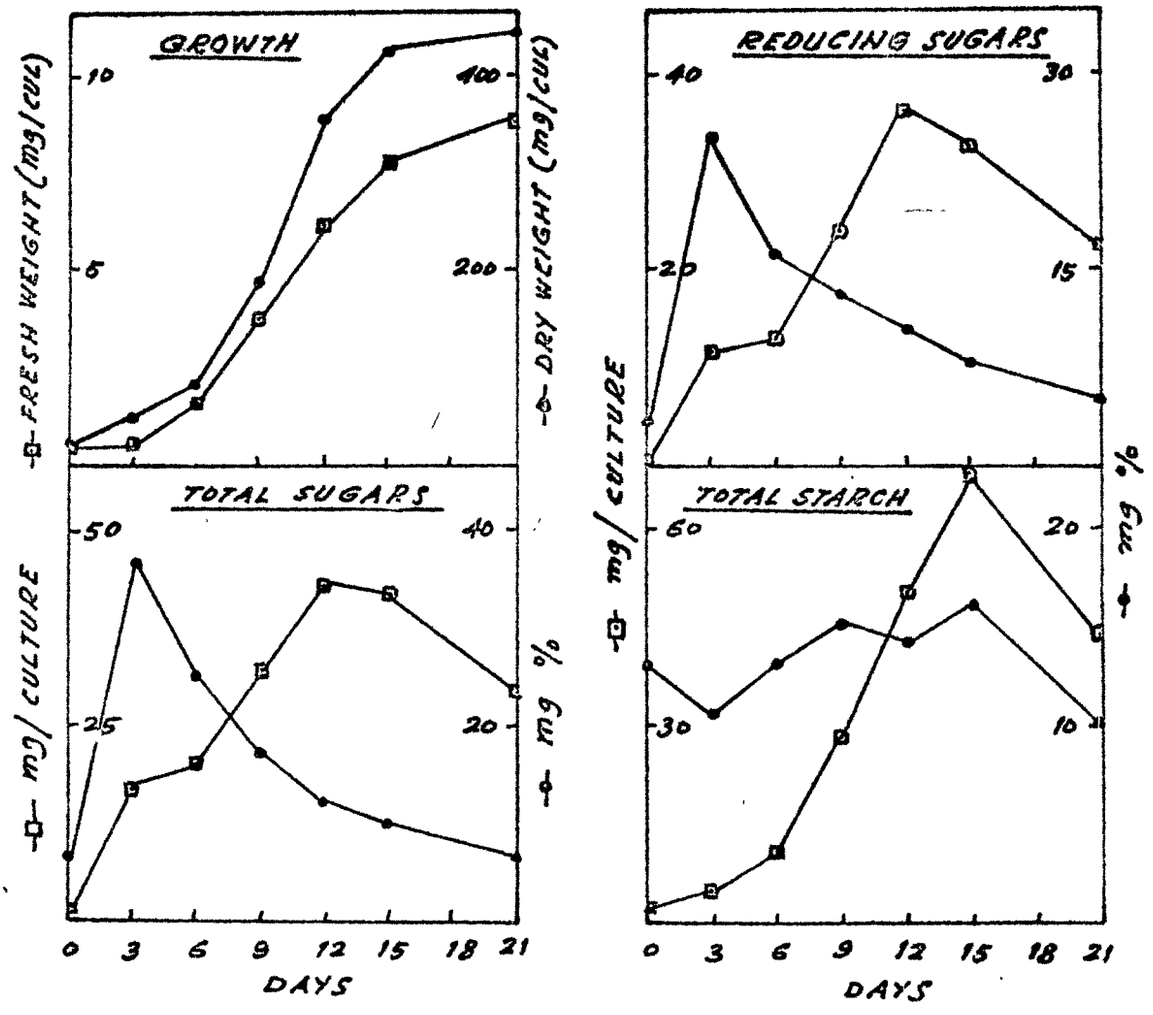

FIG. 9A (REF. TABLE NO. 42)

of shoots occurred on day 15; whereas cotton tissues failed to give any organogenetic response. In case of cotton, tissues were analysed upto the period of 21 days, i.e. 6 days more than in case of tobacco. The cultures were, however, kept under observation till 40 days in case any organogenetic response appeared later.

(b) Total and reducing sugar accumulation :

Accumulation of total and reducing sugars in cotton callus is illustrated in Fig. 9 A and Table 42.

Both total and reducing sugars on mg% basis increased rapidly during the first 3 days in culture. Later on, there was a gradual decline in the sugar content. On culture basis, the total and reducing sugars recorded similar patterns, peak values being registered in both the cases on day 12 followed by gradual decline. Total and reducing sugar content both on mg% and on culture basis on day 15 was more in the lower portion of the callus tissue than that in the upper portion.

(c) Total starch :

Changes in total extractable starch content in cotton callus tissues is illustrated in Fig. 9 A and Table 42.

Total starch on mg% basis decreased by day 3 in culture followed by an accumulation till day 9. After attaining the peak value on day 15, there was considerable depletion of starch content in the callus during days 15 and 21. In lower

portion of the callus total starch was 9.0 mg% against 16.1 mg% in the upper portion.

In tobacco tissues, total starch accumulated till day 12 i.e., prior to shoot formation (Fig. 8 A). During the period of initiation of shoots and their subsequent development, starch content had declined. In contrast, in cotton callus starch accumulated till day 15 to register the peak value. Thereafter it declined, without any sign of organogenesis.

(d) Amylase :

The progressive changes of amylase activity in callus cultures of cotton are illustrated in Fig. 9 B and Table 42.

The total enzyme activity increased rapidly during 9 to 12 days with about 20 folds. After a decline till day 15, the enzyme activity shot up again to show the peak value on day 21 with nearly 23 folds increase. The specific amylase activity increased slowly from day 3 till day 12 followed by a decline. However, the activity increased again between days 15 to 21 to attain the peak value on day 21. In lower portion of the callus on day 15, the activity of amylase on unit/culture and unit/mg protein basis was 2.5 and 0.20 units as against 39.6 and 1.9 units respectively in the upper portion of the callus.

Though there was not much difference in the specific activity of amylase between the cotton and tobacco tissues,

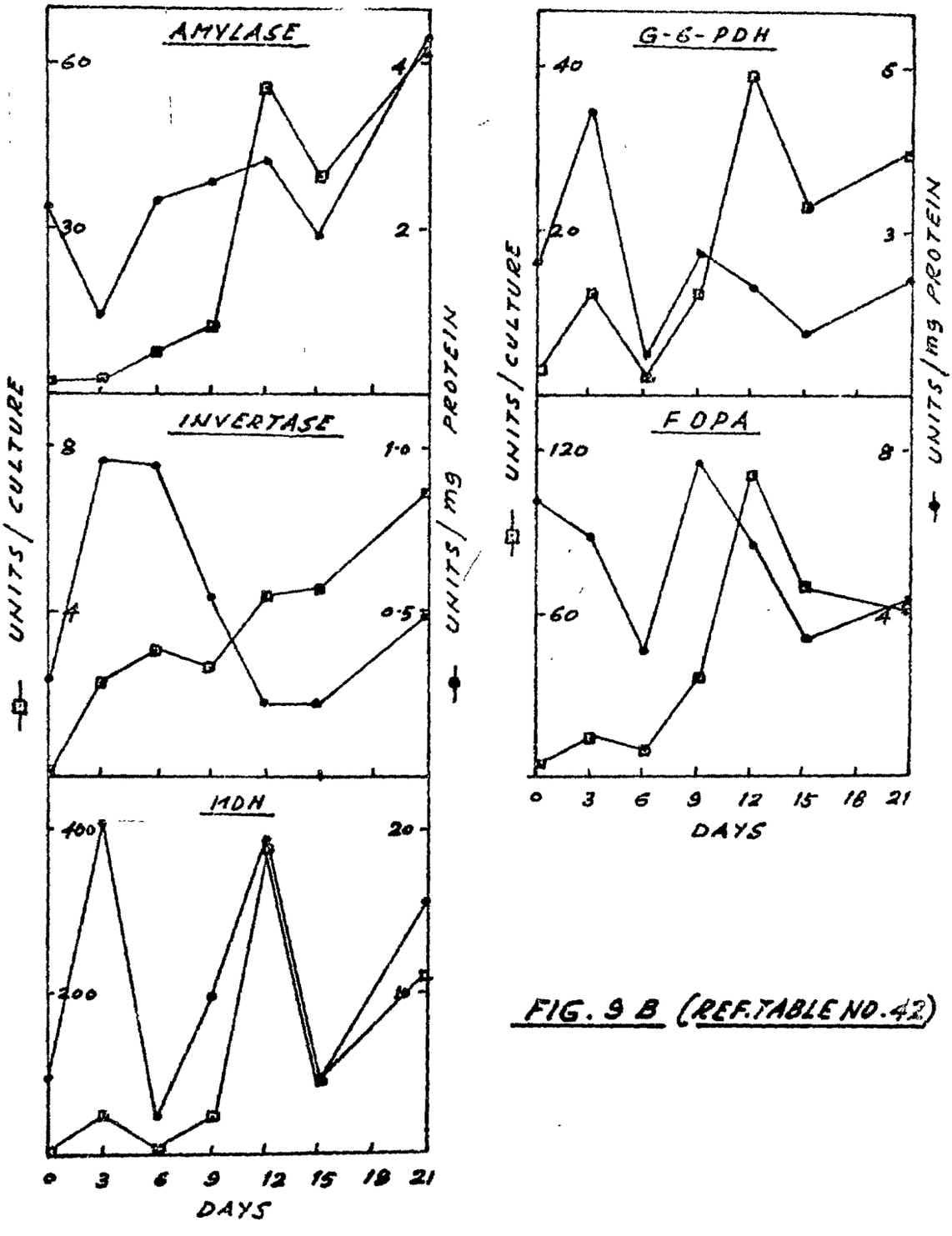

FIG. 9 B (REF. TABLE NO. 42)

the total activity of amylase reached its peak value with about 4 folds increase on day 12 (with pronounced spurt in the activity between days 9 and 12) in tobacco in contrast to 23 folds increase in cotton tissues on day 21 (with marked rise in the activity during 15 to 21 days).

(e) Invertase :

Changes in invertase activity in cotton callus are illustrated in Fig. 9 B and Table 42.

While the total invertase activity increased steadily to attain the peak value on day 21 with over 41 folds increase, the specific enzyme activity exhibited the peak value on day 3 with over 3 folds increase. In lower portion of the callus the total activity was almost half to that in the upper one; but the specific enzyme activity exhibited no significant difference between the upper and the lower portions of the callus.

The enzyme activity increased prior to organogenesis in tobacco callus, whereas in cotton in the corresponding period activity either declined or remained stable.

(f) M D H :

The activity of MDH in cotton callus grown on the shoot inducing medium of tobacco is illustrated in Fig. 9 B and Table 42.

The specific enzyme activity increased sharply during the initial 3 days of culture, attaining its first peak on day 3 with 4.3 folds rise. Both the total and specific activities increased rapidly from day 6 to 12; the total activity registering its peak value on this day (with over 66 folds increase) and the specific activity registering its second peak. In lower portion of the cotton callus the activity of MDH could not be detected at all.

During 12 to 15 days i.e., prior to and during organogenesis, tobacco callus exhibited higher MDH activities on both the counts. In contrast, in cotton callus the enzyme activity declined considerably during that period in culture. Further, the MDH activities, total as well as specific, were several to many folds higher in cotton than in tobacco tissue.

(g) G-6-P D H :

Progressive changes of G-6-PDH activity are presented in Fig. 9 B and Table 42.

The total and specific enzyme activities exhibited two peaks each on day 12 and 3 or 9. In the lower portion of the cotton callus on day 15, the activity of G-6-PDH could not be detected at all.

While the enzyme activity on both the counts was higher during the days preceding shoot differentiation in tobacco,

during the corresponding period in cotton, the activity declined. The specific activity of G-6-PDH in tobacco tissues was higher on any given day than that in cotton tissues.

(h) F D P A :

Progressive changes in the activity of FDPA are presented in Fig. 9 B and Table 42.

While the total enzyme activity registered its peak value on day 12 with a fold-wise increase of 27.6, the specific enzyme activity after an initial decrease demonstrated its peak on day 9 with 7.7 units. The activity decayed, however, between days 9 to 15. In lower portion of the cotton callus, the enzyme activity could not be detected on day 15.

The specific FDPA activity was on increase during the days preceding shoot formation; but the enzyme activity was on decrease in cotton callus during the corresponding period.

Summary :

Growth, measured as increase in fresh and dry weights was several folds higher in cotton when compared with that of the tobacco tissues. While organogenesis was noticed in two weeks in tobacco callus, there was no organogenetic response in cotton callus grown on the shoot inducing medium of tobacco.

In tobacco, starch accumulation was found prior to

organogenesis i.e., till day 9 and then it declined rapidly. On the contrary, starch accumulated till day 15 in cotton callus and declined thereof.

While the peak specific activities of hydrolytic enzymes amylase and invertase were noticed prior to organogenesis in tobacco callus, in cotton tissues, during the corresponding period, the activities either declined or remained stable. In the days preceding shoot formation in tobacco tissues, significant activities of the enzymes MDH, G-6-PDH and FDPA were noticed, whereas in cotton callus the activities of the above enzymes were lower during the corresponding period.

The shoot forming portion of the tobacco callus exhibited higher enzyme activities, while the non-shoot forming portion of the same callus grown on the same medium, when analysed on day 15, exhibited several folds lower activities. In the same way when the lower portion of the cotton callus was analysed on day 15, the activities of the enzymes MDH, G-6-PDH and FDPA could not be detected.

Section G - II : Physiological studies with GOT, ME and PEPC and total and reducing sugars in callus tissues of tobacco and cotton cultured on shoot forming medium in the dark.

From the experiments carried out earlier and described in Section C - II of Chapter III (Results), it became obvious that callus tissues of N. tabacum and G. hirsutum could be grown in dark successfully. Also it was evident that tobacco callus retained its morphogenetic potential when grown in vitro.

In the present study, the role of dark fixation of CO_2 which utilizes phosphoenolpyruvate derived from carbohydrate as a substrate is examined during differentiation of shoots in tobacco callus and in the non-shoot forming callus cultures of cotton grown in the shoot inducing medium. To study the physiological changes associated with shoot differentiation of tobacco callus and the non-shoot forming callus of cotton, the following parameters were examined : (a) Growth, (b) Total and reducing sugar accumulation; and the activities of enzymes : (c) GOT, (d) ME and (e) PEPC.

The shoot differentiating medium used for this study was: MS basal + 0.3 mg/l IAA + 3% sucrose. Shoots were induced on this medium within a maximum of 15 days from callus of tobacco with over 70 per cent frequency; whereas the cotton callus cultures did not differentiate any shoots. The experiments were terminated as soon as morphogenetic responses were manifested or else were carried on till day 15 or 21. During this period the callus tissues were harvested every third day, commencing with day 0 and analysed for the parameters enlisted above.

A comparative account of both the organ forming tobacco and non-organ forming cotton tissues was made.

Results obtained in this section are presented under the following captions :

(i) Studies with callus tissues of N. tabacum cultured on shoot inducing medium in the dark.

(ii) Studies with callus tissues of G. hirsutum cultured on shoot inducing medium of tobacco in the dark.

Expt. 28. Studies with callus tissues of N. tabacum cultured on shoot inducing medium in the dark.

Culture vessels containing 300±30 mg fresh tobacco callus were incubated for a period of 15 days in dark. Every third day, 5 replicate flasks were harvested for determination of growth, accumulation of sugars and analysis of enzymes. On day 15, the non-shoot forming portion of the callus was analysed separately for the above parameters to find out the differences in the enzyme activities if any. The results are described below.

(a) Growth :

Growth measured as increase in fresh and dry weights, of the tobacco callus is illustrated in the Fig. 10 and Table 43.

Table : 43. Growth, accumulation of total and reducing sugars and progressive changes in the activity of GOT, ME and PEPC in callus cultures of Nicotiana tabacum

Medium : MS + 0.3 mg/l IAA + 3% sucrose (shoot forming medium).

Inoculum : 300±30 mg fresh tissue.

Incubation : At 26±2°C in total darkness.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		G O T		M E		P E P C	
			mg/cult.	mg %	mg/cult.	mg %	Unit/cult.	Unit/mg protein	Unit/cult.	Unit/mg protein	Unit/cult.	Unit/mg protein
0	300.0 (±30.0)	12.0 (±3.0)	0.39	3.28	0.35	2.90	8 40	8.24	-	-	-	-
3	390.0 (±32.7)	17.2 (±2.9)	4.92	28.61	3.85	22.36	16.38	10.24	6.24	0.39	3.90	0.24
6	405.0 (±36.4)	19.9 (±3.6)	4.05	20.33	3.05	15.32	12.43	7.22	12.15	1.11	6.48	0.59
9	438.9 (±37.6)	26.8 (±4.0)	5.07	18.91	3.29	12.28	11.32	5.61	4.39	0.22	7.90	0.88
12	468.1 (±35.0)	34.0 (±3.9)	4.38	12.87	3.10	9.13	14.64	7.92	4.75	0.29	14.81	1.46
15	489.9 (±36.9)	39.1 (±4.1)	3.13	8.01	2.35	6.00	22.98	15.39	9.86	0.69	29.89	2.97
15	Non-shoot forming portion of callus		3.86	9.86	2.75	7.03	3.09	1.47	1.06	0.10	2.94	0.14

Data represents an average of 5 replicates.

Figures in parenthesis represent standard error.

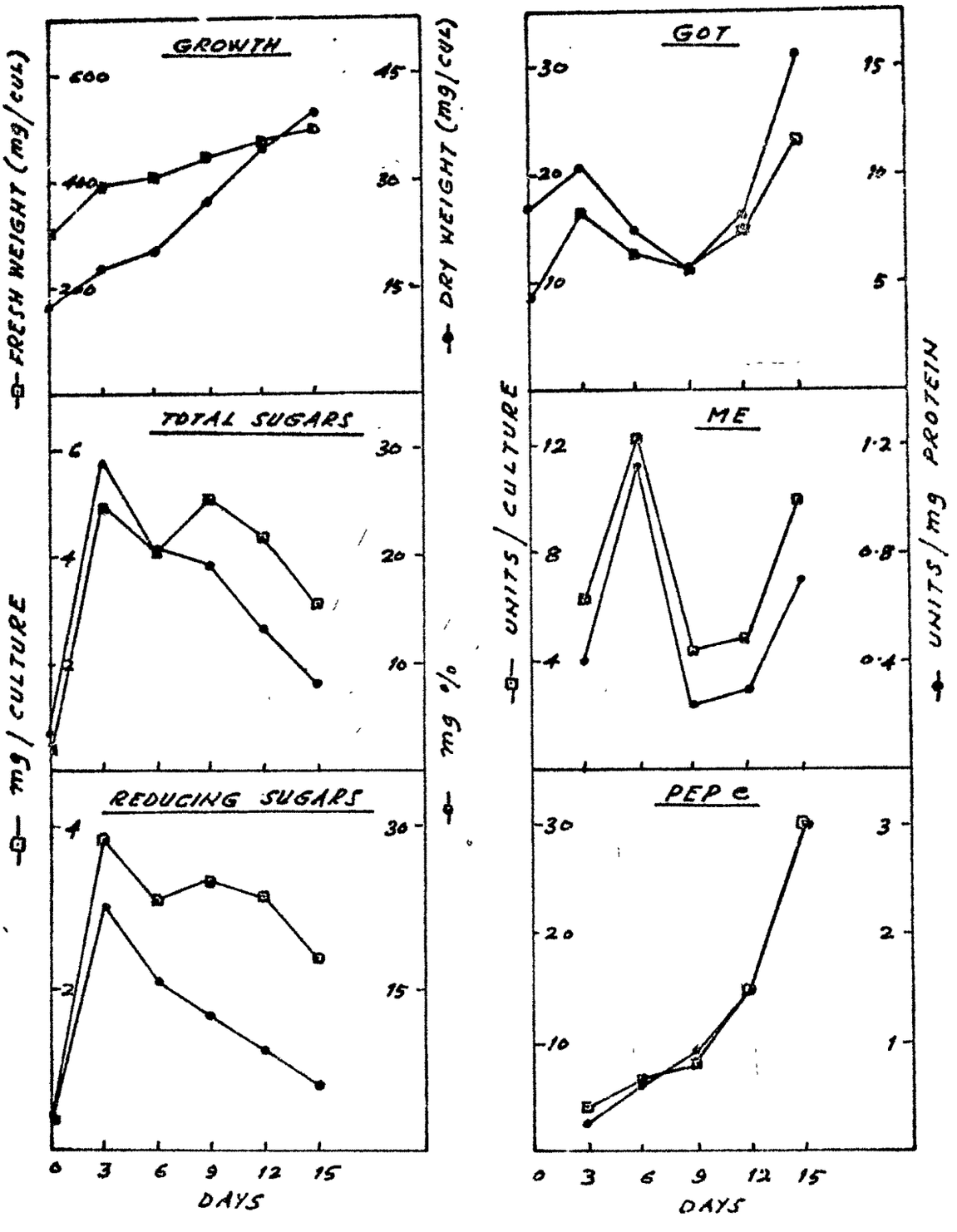

FIG. 10 (REF. TABLE NO. 43)

On fresh and dry weight basis, growth of tobacco callus was little less in dark than when grown in the light (Experiment 26). Total fold-wise increases of fresh and dry weights during the entire 15 day culture period were 1.6 and 3.3 respectively.

Shoot formation was noticed in callus cultures of tobacco on day 15 of culture with over 70 per cent frequency.

(b) Total and reducing sugar accumulation :

The total and reducing sugar accumulation during growth of the tobacco tissues in dark on shoot inducing medium is presented in Fig. 10 and Table 43.

There was a rapid accumulation of total and reducing sugars on mg% basis during the initial 3 days in culture. Thereafter sugar content gradually decreased till day 15. In the non-shoot forming portion of the callus, total and reducing sugar content on mg% was slightly higher (on day 15) when compared to the shoot forming portion of the callus.

(c) G O T :

Progressive changes in the activity of GOT are illustrated in Fig. 10 and Table 43.

Both total and specific enzyme activities followed a similar developmental pattern. The total activity doubled

during the initial 3 days in culture. Following gradual decline in the subsequent 6 days (i.e. days 3 to 9), rapid increase in enzyme activity was observed to attain the peak value on day 15. While the total activity in the non-shoot forming portion of the callus (on day 15) was 7.4 folds less, the specific GOT activity was 10.5 folds less than that in the shoot forming part of the callus.

(d) M E :

The activity of ME in tobacco callus cultured on shoot inducing medium in the dark is presented in Fig. 10 and Table 43.

The total and the specific activities of ME could not be detected on day 0 in culture. However, total and the specific enzyme activities followed an identical pattern of development. The enzyme activity on both the counts increased significantly from day 3 to 6 to attain the peak value. The total enzyme activity declined considerably from 12.2 units to 4.4 during the days 6 to 9, followed by another increase till day 15 (with 9.86 units). In other words, the enzyme activity increased during the days preceding shoot formation (from day 9 to 15). In the non-shoot forming portion of the callus on day 15, total and the specific enzyme activities were 9.3 and 6.9 folds less respectively than that in the shoot forming portion of the callus.

(e) P E P C :

Fig. 10 and Table 43 represent progressive changes of PEPC activity in tobacco callus cultured on the shoot inducing medium in the dark.

The PEPC activity could not be detected on day 0. However, the enzyme activity followed a similar developmental pattern both on culture and unit protein basis. Though the enzyme activity on both the counts increased rapidly from day 3 till day 9, thereafter the increase was more pronounced reaching the peak value on day 15. The PEPC activity increased sharply between days 9 and 15; i.e. the days preceding shoot formation. In the non-shoot forming portion of the callus on day 15, the enzyme per culture and per unit protein basis exhibited 10.2 and 21.2 folds less activity respectively.

Expt. 29. Studies with callus tissues of G. hirsutum cultured on shoot inducing medium of tobacco in the dark.

Cotton callus weighing 400±40 mg fresh weight was inoculated into the culture flasks containing shoot inducing medium of tobacco (MS + 0.3 mg/l IAA + 3% sucrose) and incubated in dark for a period of 21 days. Every third day, five replicate flasks were harvested and analysed for growth, total and reducing sugar accumulation and enzymes. The results

are documented below.

(a) Growth :

Growth, measured as increase in fresh and dry weights of the tissue is illustrated in the Fig. 11 and Table 44.

Growth of the cotton callus on fresh and dry weight basis increased 17.6 and 25.5 folds by day 15 and 20 and 26.3 folds by day 21 in culture. Tobacco tissues, in contrast, exhibited only 1.6 and 3.3 fold increases over the initial inoculum during the 15 day culture period.

Differentiation of shoots occurred in tobacco tissues on the above medium on day 15, while cotton tissues failed to give any organogenetic response on that medium. Cotton tissues were analysed upto 21 days and kept under observation till 40 days with the view that organogenesis might occur late.

(b) Total and reducing sugar accumulation :

Changes in the accumulation of sugars are presented in Fig. 11 and Table 44.

Total and reducing sugars on mg% basis increased rapidly during the initial 3 days in culture and declined thereof gradually till day 21. However, on culture basis the peak value was attained on day 12. No significant difference in accumulation of sugars was seen between tobacco and cotton callus tissues.

Table : 44. Growth, accumulation of total and reducing sugars and progressive changes in the activity of GOT, ME and PEPC in callus cultures of Gossypium hirsutum

Medium : MS + 0.3 mg/l IAA + 3% sucrose (shoot forming medium of tobacco).

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in total darkness.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		G O T		M E		P E P C	
			mg/cult.	mg %	mg/cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	3.61	4.00	-	-	-	-
3	817.4 (±76.3)	47.0 (±6.2)	12.11	25.76	9.29	19.77	39.24	7.87	26.16	0.53	17.98	0.36
6	1735.3 (±150.7)	91.4 (±12.0)	18.56	20.31	15.63	17.10	17.35	5.56	52.06	1.07	20.82	0.67
9	4612.8 (±214.9)	226.8 (±25.7)	29.35	12.94	19.73	8.70	12.51	1.45	28.88	0.33	24.06	0.28
12	6520.9 (±296.5)	346.2 (±24.4)	35.42	10.23	21.85	6.31	19.56	1.20	39.13	0.24	13.04	0.08
15	7038.2 (±299.4)	408.7 (±26.8)	30.16	7.38	21.29	5.21	15.08	1.11	-	-	-	-
21	8008.8 (±306.5)	420.6 (±27.5)	21.20	5.04	16.36	3.89	-	-	-	-	-	-

Data represents an average of 5 replicates.

Figures in parenthesis represent standard error.

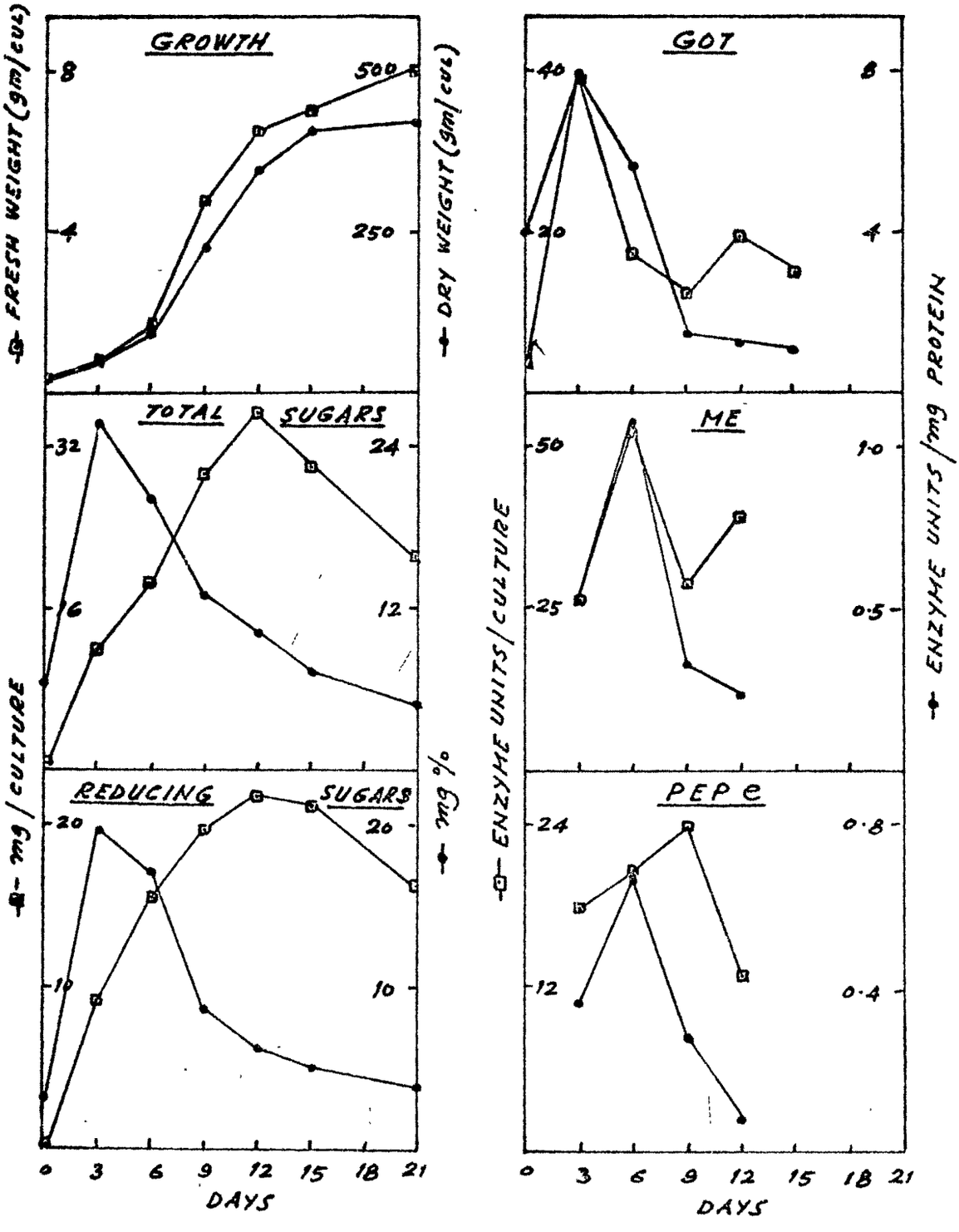

FIG. 11 (REF. TABLE NO. 44)

(c) G O T :

Progressive changes in the activity of GOT are illustrated in Fig. 11 and Table 44.

The total enzyme activity increased nearly 11 folds by day 3 to register the peak value. Thereafter the enzyme activity declined till day 15 and could not be detected beyond days 15 in culture. The specific activity of GOT followed the similar pattern of development. While the enzyme activity in tobacco tissues exhibited sharp rise during the days preceding shoot differentiation (days 9 to 15), cotton callus registered the peak enzyme activity on day 3 and declined rapidly thereafter till day 15.

(d) M E :

The activity of ME in callus tissues of cotton cultured on shoot inducing medium of tobacco in dark is presented in Fig. 11 and Table 44.

Activity of the ME could not be detected on day 0. Total enzyme activity doubled between days 3 to 6 to register the peak value followed by a decay in the activity till day 9 in culture. However, the enzyme activity partially revived back by day 12 and could not be detected thereafter. The specific ME activity also doubled during the period of 3 to 6 days to attain the peak value and declined rapidly thereof till day 12. In tobacco

tissues, the specific activity of ME exhibited its peak value on day 6 like in cotton, but the activity again increased during days 9 to 15, preceding organogenetic development. In contrast, in cotton callus, the specific ME activity declined from day 6 till day 12 and thereof it could not be detected in the tissue.

(e) P E P C :

Fig. 11 and Table 44 represent progressive changes of PEPC activity in callus cultures of cotton grown on shoot inducing medium of tobacco.

Total and the specific activities of PEPC could not be detected on day 0 in culture. The total enzyme activity increased from 18.00 units (on day 3) to 24.1 units by day 9 to attain the peak value and declined considerably by day 12. Activity, however, could not be detected beyond day 12 in culture. The specific enzyme activity demonstrated its peak value on day 6 (0.67 units) and declined sharply till day 12 (0.08 units). The enzyme activity in tobacco callus was on increase prior to shoot formation, while in cotton the enzyme activity declined significantly between days 6 and 12 and could not be detected on day 15.

Summary :

Growth of cotton callus, both on fresh and dry weight basis was superior to that of tobacco (17.6 and 25.5 folds higher respectively). Shoot formation was noticed only in tobacco, while cotton tissues exhibited no organogenetic development when grown on shoot inducing medium of tobacco.

In tobacco tissues, the activities of the enzymes GOT, ME and PEPC increased between days 9 to 15, i.e. during the days preceding shoot formation. In contrast, in cotton callus, all the above mentioned enzyme activities declined considerably between days 6 to 12 and could not be detected at all on day 15 in culture.

From the experiments carried out earlier and described in Sections F and G of Chapter III (Results), it was clear that callus tissues of N. tabacum retained their morphogenetic potential in vitro. The organogenetic responses of these callus cultures rested largely on the exogenous supply of phytohormones, singly or in combinations, and also the sugar concentration.

To examine the physiological changes associated with root differentiation of tobacco callus and the non-root differentiating callus of cotton the following parameters were studied : (a) Growth, (b) Total and reducing sugar accumulation, (c) Total starch, and the activities of enzymes: (d) Amylase, (e) Invertase, (f) MDH, (g) G-6-PDH and (h) FDPA.

The root differentiating medium used for this study was : MS basal + 2.0 mg/l IAA + 3% sucrose. Roots were induced on this medium any time between days 13 and 15 from callus cultures of tobacco with over 70 per cent frequency. The experiments were terminated as soon as morphogenetic responses manifested or else were carried on till day 15 or 21 and analysed for the parameters enlisted above. A comparative account of both tobacco and cotton tissues was made.

Results obtained in this section are presented under following heads :

Table : 45. Growth, accumulation of total and reducing sugars, starch and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Nicotiana tabacum

Medium : MS + 2 mg/l IAA + 3% sucrose.

Inoculum : 300±30 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Total Starch		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	300.0 (±30.0)	12.0 (±3.0)	0.39	3.28	0.35	2.90	1.05	8.75	0.26	0.29	0.31	0.35	1.69	0.88	1.05	1.17	1.50	1.67
3	309.6 (±22.0)	15.2 (±2.6)	4.87	32.03	3.02	19.87	1.53	10.08	0.53	0.31	0.54	0.31	0.98	0.66	6.20	4.88	5.60	4.41
6	438.5 (±41.2)	38.4 (±6.2)	10.28	26.77	7.68	20.01	4.54	11.83	0.85	0.29	0.55	0.19	2.11	0.99	9.20	3.75	14.50	5.91
9	460.9 (±36.)	40.6 (±5.0)	8.22	20.25	6.39	15.74	5.12	12.62	0.83	0.35	0.54	0.23	0.76	0.32	9.70	3.76	35.00	13.56
12	499.8 (±32.4)	50.3 (±4.3)	7.47	14.86	6.22	12.36	4.54	9.03	0.96	0.34	0.49	0.18	0.53	0.26	3.30	1.18	12.50	4.47
15	557.9 (±51.2)	61.4 (±7.7)	7.04	11.47	5.65	9.21	4.91	8.00	0.54	0.14	0.69	0.24	10.73	2.66	5.90	1.04	54.10	11.87
15	Non-root forming portion of callus		6.20	10.09	4.52	7.36	6.58	10.72	0.22	0.05	0.27	0.06	1.80	0.30	6.45	1.20	61.90	12.67

Data represents an average of 5 replicates.

Figures in parenthesis represent standard error.

(i) Studies with callus tissues of N. tabacum cultured on root inducing medium.

(ii) Studies with callus tissues of G. hirsutum cultured on root inducing medium of tobacco.

Expt. 30. Studies with callus tissues of N. tabacum cultured on root inducing medium.

Tobacco callus weighing 300±30 mg fresh weight was inoculated into the culture flasks containing root inducing medium (2.0 mg/l IAA + 3% sucrose) and incubated for a period of 15 days in light. Every third day, five replicate flasks were harvested and analysed for growth, sugar accumulation and enzymes. On day 15, non-root forming portions of the same callus grown on the same medium were analysed separately to find out the differences in the enzyme activities if any. The results are described below.

(a) Growth :

Growth measured as increase in fresh and dry weights of the tissues is illustrated in Fig. 12 A and Table 45.

Growth on both fresh and dry weight basis exhibited typical double sigmoid curve. Initial lag phase of 3 days was followed by 3 days of rapid growth. During this period fresh weight increased approximately 1.5 folds and dry weight by

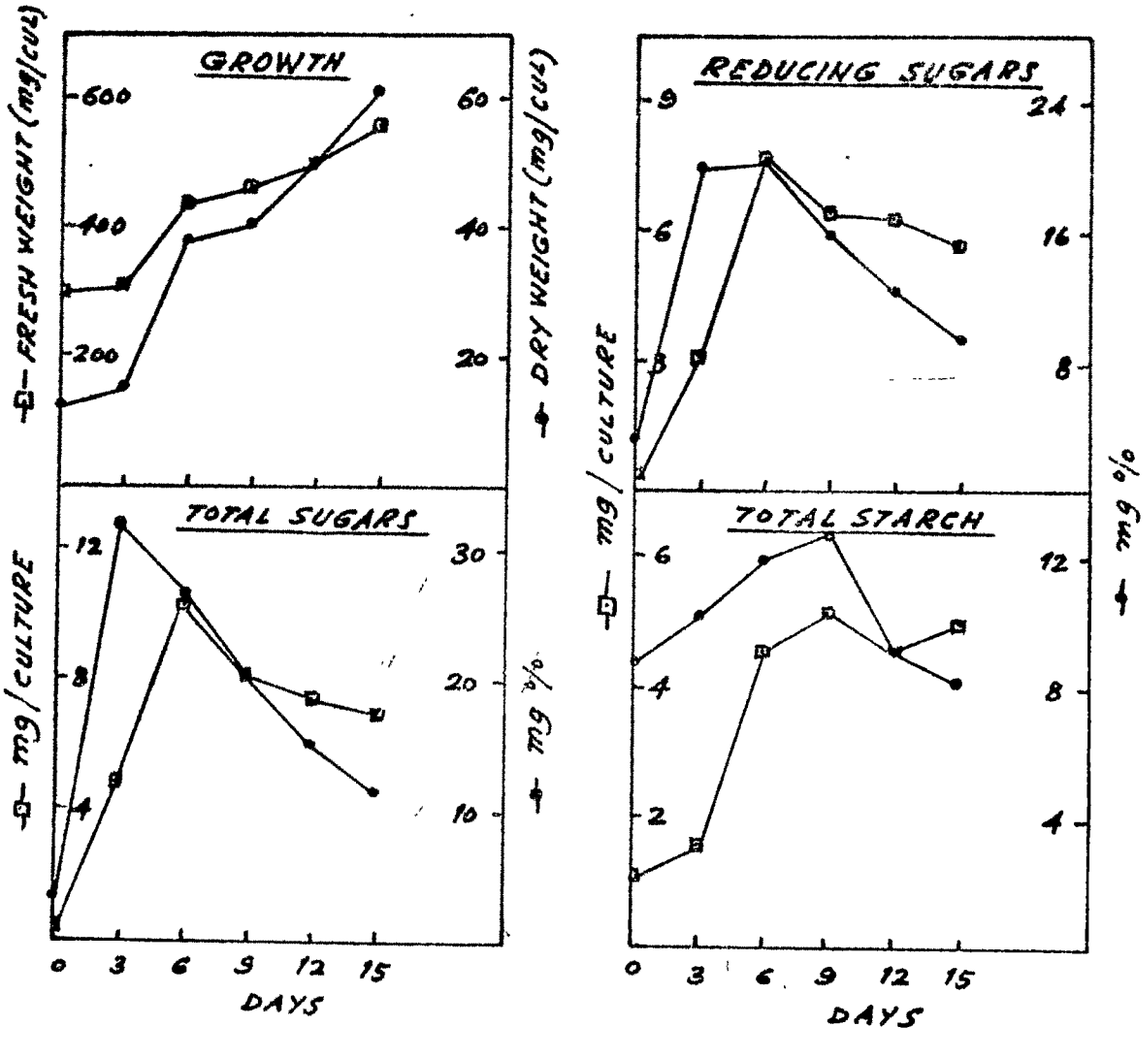

FIG. 12 A (REF. TABLE NO. 45)

2.5 folds. After an another slow period of growth from days 6 to 9, growth increased linearly till day 15. During the entire culture period fresh weight increased 1.9 folds and dry weight by 5.1 folds.

From the cultured callus masses, 4-6 roots were formed between day 13 to 15 with a frequency response around 70%.

(b) Total and reducing sugar accumulation :

Progressive changes in the accumulation of total and reducing sugars in tobacco callus grown on root inducing medium are illustrated in Fig. 12 A and Table 45.

Total and reducing sugars on mg% basis accumulated rapidly during the initial 3 days in culture and declined thereof gradually till day 15. In the non-root forming portion of the callus both total and reducing sugar content was less when compared to the root forming part of tissue.

(c) Total starch :

Variation in total extractable starch content in tobacco callus cultured on root inducing medium are illustrated in Fig. 12 A and Table 45.

Both on mg/culture and on mg% basis, total starch content increased upto day 9 to attain the peak value, followed by a decline till day 15. Thus starch accumulated continuously

prior to root formation and began to decline from day 9 onwards. In the non-root forming portion of the callus, total starch content on mg/culture and on mg% basis was more when compared to the root forming portion of the callus.

(d) Amylase :

The development of amylase activity is represented in Fig. 12 B and Table 45.

The total enzyme activity increased from day 0 till day 12 to register the peak value and dropped rapidly by day 15 in culture. On the other hand, the specific amylase activity exhibited no significant changes till day 12. However, after attaining the peak value on day 9, the activity declined sharply after day 12. In the non-root forming portion of the callus, the enzyme activity on day 15 was 3 folds less than in the root-forming portion of the callus.

(e) Invertase :

The progressive changes in invertase activity are illustrated in Fig. 12 B and Table 45.

The total enzyme activity after an initial rapid rise, remained more or less stable in culture till day 12. The peak value was observed on day 15. The specific invertase activity decayed nearly 1.8 folds upto day 6 and then fluctuated.

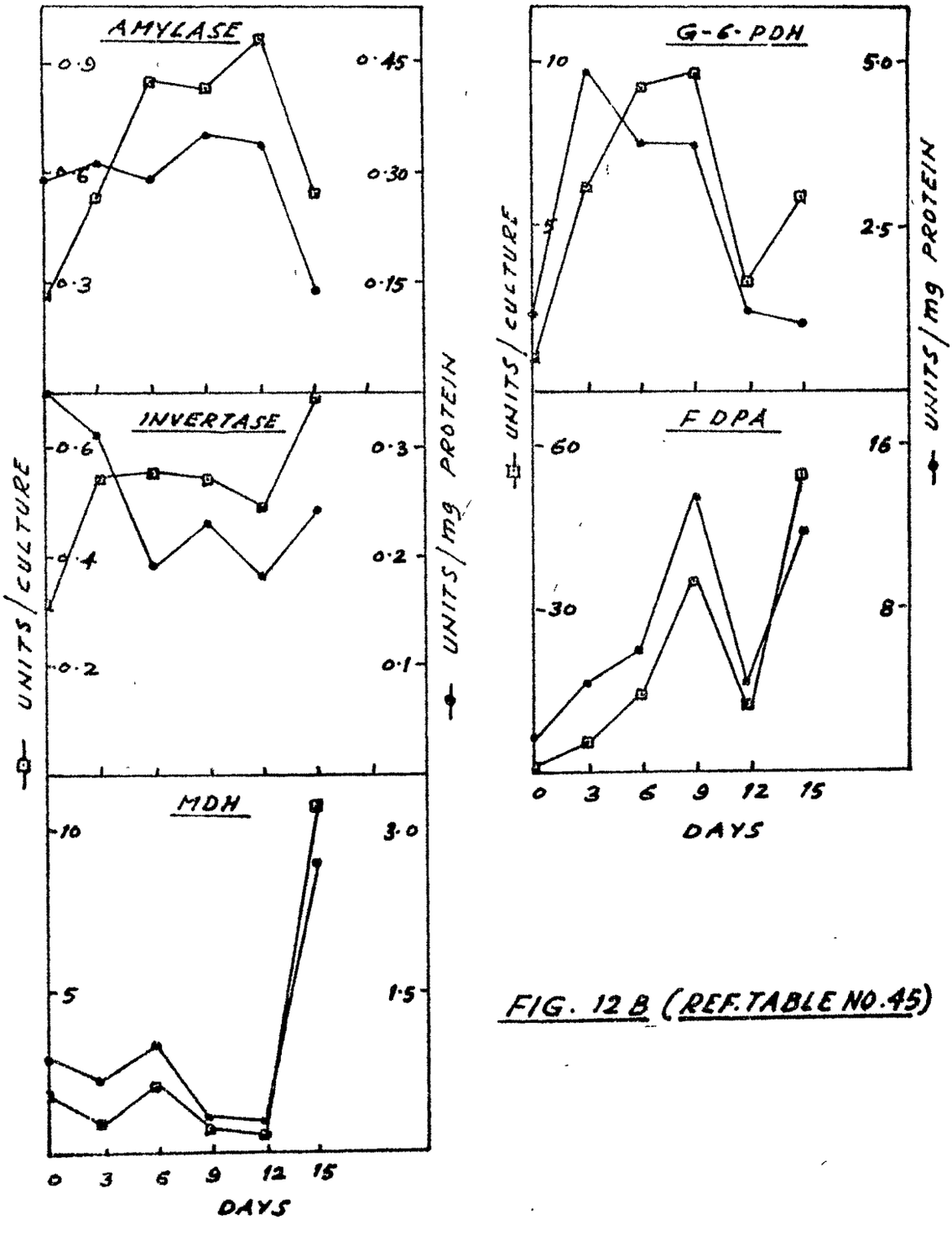

FIG. 12 B (REF. TABLE NO. 45)

However, the activity was on increase during days 12 to 15. Both total and the specific invertase activities were on increase just before the appearance of roots in culture (between days 12 to 15). In the non-root forming portion of the callus, the specific invertase activity on day 15 was 4 folds less than in the root forming portion of the tissue.

(f) M D H :

The progressive changes of enzyme MDH activity in tobacco callus are illustrated in Fig. 12 B and Table 45.

The total activity of MDH declined slightly between days 0 and 3. After an increase in next 3 days (3 to 6 days), it dropped again till day 12. But by day 15, the enzyme activity increased dramatically (nearly 20 folds). The specific enzyme activity followed essentially identical developmental pattern. The appearance of roots from the callus tissues on day 15 was preceded by a sharp increase in the total and the specific activities of MDH. The specific activity of MDH on day 15 in the non-root forming portion of the callus was almost 9 folds less when compared to that in the root forming portion of the callus.

(g) G-6-P D H :

Activity of G-6-PDH in tobacco callus, cultured on the

root inducing medium is illustrated in Fig. 12 B and Table 45.

Total enzyme activity increased sharply till day 9 to attain the peak value. The activity, however, declined by day 12 followed by another increase (from 3.3 units to 5.9 units) by day 15. Though the specific enzyme activity increased 4 folds during the initial 3 days in culture, it declined significantly thereof till the termination of culture period. While the total activity exhibited its peak value on day 9, the peak in specific activity was attained on day 3, much earlier to the formation of roots. Though the non-root forming portion of the callus exhibited slightly higher specific activity on day 15, it was not significant.

(h) F D P A :

Progressive changes in the activity of FDPA are illustrated in Fig. 12 B and Table 45.

Total and the specific activities of FDPA exhibited very similar developmental patterns. There was a rapid rise in the enzyme activity till day 9 to attain the first peak (more than 23 folds in the total and 8 folds in the specific activities). After rapid decline by day 12, the enzyme activity again jumped up by day 15 to register the second peak value. The FDPA activity on both the counts registered sharp rise during the period immediately preceding root formation. In the non-root forming portion of the callus on

day 15, the specific activity was slightly higher when compared to that in the root forming portion of the callus.

Expt. 31. Studies with callus tissues of G. hirsutum cultured on root inducing medium of tobacco.

Culture flasks inoculated with cotton tissues on root inducing medium of tobacco (2.0 mg/l IAA + 3% sucrose) were harvested every third day, for measurements of growth, accumulation of sugars and starch, and analysis of enzymes. On day 15, the lower portion of the callus tissue grown on the same medium was also analysed separately for the above parameters to find out the differences in enzyme activities if any. The results are documented below.

(a) Growth :

Growth measured as increase in fresh and dry weights is illustrated in Fig. 13 A and Table 46.

On fresh and dry weight basis growth increased by 17.4 and 21.3 folds by day 15 and still further increase was observed by day 21 in culture. This increase in growth of cotton callus was certainly many folds higher than that of tobacco tissue (1.9 and 5.1 folds increase on fresh and dry weight basis respectively).

In tobacco callus grown on the same medium, differentiation

Table : 46. Growth, accumulation of total and reducing sugars, starch and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Gossypium hirsutum

Medium : MS + 2 mg/l IAA + 3% sucrose (Root forming medium of tobacco).

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Total Starch		Amylase		Invertase		M D H		G-6-P D H		FDPA	
			mg/ cult.	mg %	mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	2.06	12.90	2.69	2.24	0.17	0.29	5.75	4.80	2.93	2.44	4.00	6.67
3	824.7 (±64.6)	48.7 (±6.1)	14.63	30.04	11.04	22.67	6.34	13.01	3.86	1.06	3.99	1.10	49.40	13 64	15.10	4.16	10.70	2.95
6	1668.1 (±115.9)	78.8 (±7.4)	18.16	23.05	13.07	16.59	11.63	14.76	9.23	0.91	4.45	0.44	30.69	3.02	28.90	2.84	33.40	3.28
9	4419.7 (±174.5)	202.5 (±18.9)	26.67	13.17	21.55	10.64	28.03	13.84	27.47	1.52	5.50	0.30	114.91	6.34	150.30	8.29	198.90	10.98
12	5282.2 (±195.4)	309.5 (±19.6)	31.01	10.02	26.43	8.54	35.96	11.62	35.28	2.47	3.12	0.22	113.04	7.93	140.10	2.09	95.10	6.67
15	6949.3 (±209.9)	340.6 (±21.3)	27.25	8.00	23.54	6.91	33.24	9.76	74.70	2.99	4.54	0.18	350.25	14.00	122.80	4.91	132.00	5.28
	Lower portion of callus		23.94	7.03	20.27	5.95	31.44	9.23	3.97	.21	1.60	0.09	—	—	—	—	—	—
21	8215.4 (±302.2)	440.2 (±27.8)	26.54	6.03	21.83	4.96	42.26	9.60	49.29	2.73	5.37	0.30	279.32	15.45	11.00	0.61	115.00	6.36

Data represents an average of 5 replicates.

Figures in parenthesis represent standard error.

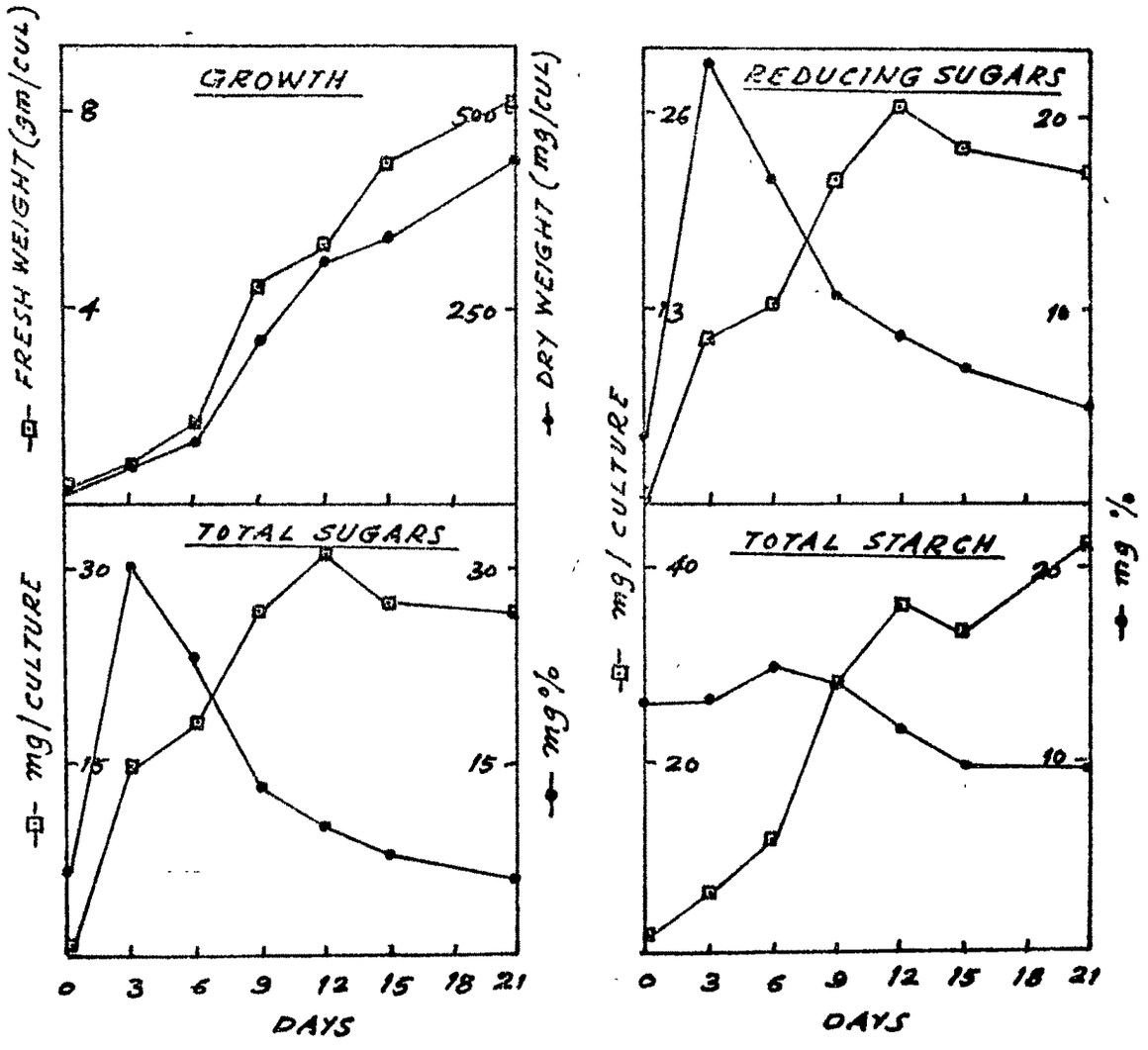

FIG. 13A (REF. TABLE NO. 46)

of roots occurred any time between days 13 to 15; whereas cotton tissues failed to give any organogenetic response. In case of cotton, tissues were analysed upto the period of 21 days, i.e. 6 days more than in case of tobacco. The cultures were, however, kept under observation till 40 days in case any organogenetic response appeared later.

(b) Total and reducing sugar accumulation :

Accumulation of total and reducing sugars in callus cultures of cotton grown in the root inducing medium of tobacco are illustrated in Fig. 13 A and Table 46.

Both total and reducing sugars on mg% basis increased rapidly during the initial 3 days in culture and declined thereof gradually till day 21. On mg/culture basis also peak values were observed on day 12 in both the cases. In the lower portion of the callus total and reducing sugar content was less when compared with the upper portion of the callus.

(c) Total starch :

Changes in total extractable starch content are illustrated in Fig. 13 A and Table 46.

Total starch on mg% basis accumulated from 12.9 to 14.8 mg% by day 6 to attain the peak value and declined thereof till day 21 in culture. But on mg/culture basis the peak value was attained on day 21. In lower portion of the cotton

callus on day 15 total starch content was slightly less than that in the upper part. Starch content on mg% basis was much higher in cotton tissues than that in the tobacco callus. While starch accumulated till day 6 in cotton tissues and declined thereof, in case of tobacco starch accumulated till day 9 and then declined (till day 15).

(d) Amylase :

The development of amylase activity is represented in Fig. 13 B and Table 46.

The total enzyme activity increased 28 folds to reach the peak value on day 15 followed by a decline thereof. The specific amylase activity on the other hand, declined from 2.2 units to 0.9 units by day 6 and increased thereafter till day 15 to attain the peak value. However, the activity declined by day 21 in culture. In the lower portion of the callus, the specific amylase activity exhibited 0.2 units in contrast to 3.0 units (14 folds increase) in the upper part of the callus on day 15.

While the specific amylase activity in tobacco increased prior to root initiation (till day 9) and declined during the period of root formation (days 9 to 15), cotton callus exhibited increased specific activity from day 6 to 15 in culture.

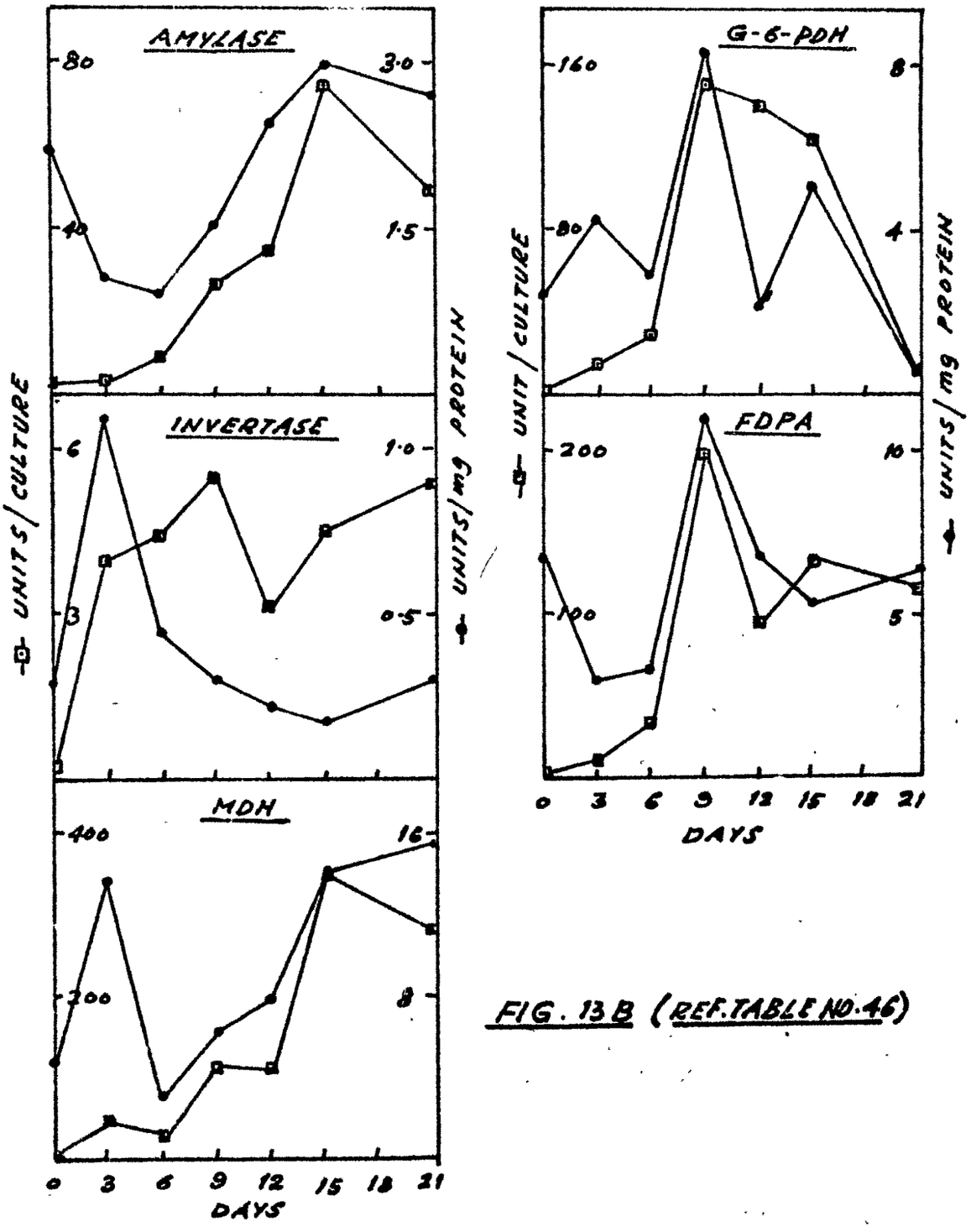

FIG. 13 B (REF. TABLE NO. 46)

(e) Invertase :

The development of invertase activity is represented in Fig. 13 B and Table 46.

Total enzyme activity showed double peak developmental pattern. The activity increased more than 32 folds till day 9 to register the first peak value. The drop in activity in subsequent 3 days (9 to 12) was followed by another rise to register the second peak value on day 21.

The specific invertase activity on the other hand, increased sharply (nearly 4 folds) by day 3 to register the peak value and declined significantly thereof. In the lower portion of callus on day 15, both the total and specific activities were considerably less when compared with that in the upper part of the cotton callus.

Both total and the specific invertase activity was considerably higher in cotton on any given day than that in the tobacco tissue.

(f) M D H :

The progressive changes in the enzyme activity of MDH are illustrated in Fig. 13 B and Table 46.

The total enzyme activity after initial fluctuations, demonstrated the peak value on day 15 with nearly 61 folds

increase. On the other hand, the specific MDH activity exhibited double peak developmental pattern, one each on day 3 (13.6 units) and 21 (15.5 units). In lower portion of the cotton callus on day 15, the enzyme activity could not be detected.

In tobacco tissues, the MDH activity on both the counts increased sharply between days 12 to 15 (prior to organogenesis). Such a sharp increase in the activity of MDH was also noticed between days 9 to 15 in cotton callus, but it was not followed by any organogenetic response till as late as 40 days.

(g) G-6-P D H :

The progressive changes in the enzyme activity of G-6-PDH are illustrated in Fig. 13 B and Table 46.

The total enzyme activity developed sharply till day 9 (51.3 folds increase) to demonstrate the peak value, followed by a rapid decline till day 21 in culture. On the other hand, the specific G-6-PDH activity after declining on day 6, increased considerably to register a pronounced peak on day 9 (3.4 fold increase). Following decline in activity by day 12, it enhanced again to attain the second peak value on day 15. In lower part of the callus, the enzyme activity on day 15 could not be detected.

In tobacco tissues, the peak specific activity was

observed on day 3 (the activity declined thereof), while in cotton callus two peaks were observed one each on days 9 and 15.

(h) F D P A :

Total and the specific FDPA activity in cotton callus tissues grown in the root inducing medium of tobacco is illustrated in Fig. 13 B and Table 46.

Total enzyme activity increased rapidly till day 9 to attain the peak value (50 fold increase) followed by a decay till day 12. However, the activity increased marginally thereafter. On the other hand, the specific enzyme activity dropped during the initial 3 days in culture; but increased thereafter to register the peak value on day 9 (1.7 fold increase). A decline in the enzyme activity from 11.0 units to 5.3 units ensued thereafter till day 15. In lower portion of the callus, the enzyme activity could not be detected on day 15.

Contrary to the results with cotton, the total FDPA activity in tobacco showed the peak value on day 15 (in cotton, peak was attained on day 9). While the specific FDPA activity demonstrated double peaks one each on day 9 and 15 in tobacco callus, only one peak was observed in cotton callus tissues on day 9. Moreover, the specific FDPA activity was several folds higher in tobacco on any given day than that in cotton tissue.

Summary :

Growth of cotton callus as measured by increase in fresh and dry weights was several folds higher than that of tobacco on the root inducing medium. While roots were formed in tobacco tissues, no such organogenetic response was noticed in cotton callus. Starch accumulated till day 6 in cotton, contrary to day 9 in tobacco tissues. Starch content on mg% basis was higher in cotton callus than that in tobacco callus.

The specific amylase activity in tobacco dropped between days 12 to 15 (prior to organogenesis); whereas in cotton callus the specific amylase was on increase from day 6 till day 15.

While the specific invertase activity in tobacco exhibited always lower values than that of day 0, in cotton the enzyme activity was higher between days 0 to 9 and declined thereof.

Appearance of roots from callus tissues of tobacco on day 15 was preceded by a sharp increase in the activity of MDH. In cotton also such a development of enzyme activity was noticed between days 12 to 15 but no organogenetic response was observed.

The specific activity of G-6-PDH in tobacco and cotton followed almost similar developmental pattern. The FDPA activity on unit protein basis increased several folds prior to root formation in tobacco, but the specific enzyme activity in cotton declined during the days 12 to 15 in culture.

Section H - II : Physiological studies with GOT, ME and PEPC and total and reducing sugars in callus tissues of tobacco and cotton cultured on root differentiating medium in the dark.

From the experiments carried out earlier and described in Sections C - II and G - II of Chapter III (Results), it was clear that callus tissues of N. tabacum and G. hirsutum could be grown in dark successfully. It was also evident that tobacco callus retained its morphogenetic capability when grown in vitro.

In the present study, the role of dark fixation of CO_2 which utilizes phosphoenolpyruvate derived from carbohydrate as a substrate is examined during differentiation of roots in tobacco callus and in the non-root forming callus cultures of cotton when grown on the root inducing medium of tobacco.

To examine the physiological changes associated with root differentiation of tobacco callus and the non-root forming callus of cotton the following parameters were studied: (a) Growth, (b) Total and reducing sugar accumulation, and the activities of enzymes: (c) GOT, (d) ME and (e) PEPC.

The root differentiating medium used for this study was: MS basal + 2.0 mg/l IAA + 3% sucrose. Roots were induced in tobacco callus on this medium within a maximum of 15 days with over 70 per cent frequency. Cottom callus cultures, on the other hand, did not differentiate any roots. The experiments were terminated as soon as morphogenetic responses were observed or else were carried out till day 15 or 21. During this period the callus tissues were harvested every third day,

and analysed for the parameters enlisted above. A comparative study of both the organ forming tobacco and the non-organ forming cotton tissues was made.

Results obtained in this section are presented under the following heads :

(i) Studies with callus tissues of N. tabacum cultured on root inducing medium in the dark.

(ii) Studies with callus tissues of G. hirsutum cultured on root inducing medium of tobacco in the dark.

Expt. 32. Studies with callus tissues of N. tabacum cultured on root inducing medium in the dark.

Culture vessels containing 300±30 mg fresh tobacco callus were incubated for a period of 15 days in the dark. Every third day, 5 replicate flasks were harvested for determination of growth, accumulation of sugars and analysis of enzymes. On day 15, root forming and non-root forming portions of the callus grown on the same medium were analysed separately and the results are described below.

(a) Growth :

Growth measured as increase in fresh and dry weights of the tobacco callus is illustrated in Fig. 14 and Table 47.

On fresh and dry weight basis, growth of tobacco callus

Table : 47. Growth, accumulation of total and reducing sugars and progressive changes in the activity of GOT, ME and PEPC in callus cultures of Nicotiana tabacum

Medium : MS + 2 mg/l IAA + 3% sucrose (Root forming medium)

Inoculum : 300±30 mg fresh tissue.

Incubation : At 26±2°C in total darkness.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		G O T		M E		P E P C	
			mg/cult.	mg %	mg/cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	300.0 (±30.0)	12.0 (±3.0)	0.39	3.28	0.35	2.90	8.40	8.24	-	-	-	-
3	328.0 (±28.4)	16.8 (±2.7)	4.94	29.38	4.45	26.49	11.15	7.39	5.90	0.39	13.12	0.87
6	417.6 (±33.5)	34.5 (±4.0)	7.58	21.97	6.25	18.12	11.67	7.88	8.35	0.91	5.01	0.55
9	486.2 (±35.7)	40.3 (±4.1)	7.04	17.46	5.86	14.53	15.27	6.16	4.89	0.36	7.78	0.39
12	506.9 (±38.2)	50.0 (±4.8)	6.94	13.88	5.37	10.74	14.19	7.99	4.06	0.45	8.03	0.56
15	543.1 (±39.0)	58.9 (±4.7)	6.01	10.20	4.43	7.52	25.81	17.56	6.92	0.72	21.72	1.68
15	Non-root forming portion of callus		5.89	10.00	3.59	6.10	4.84	3.03	0.97	0.10	4.03	0.27

Data represents an average of 5 replicates.

Figures in parenthesis represent standard error.

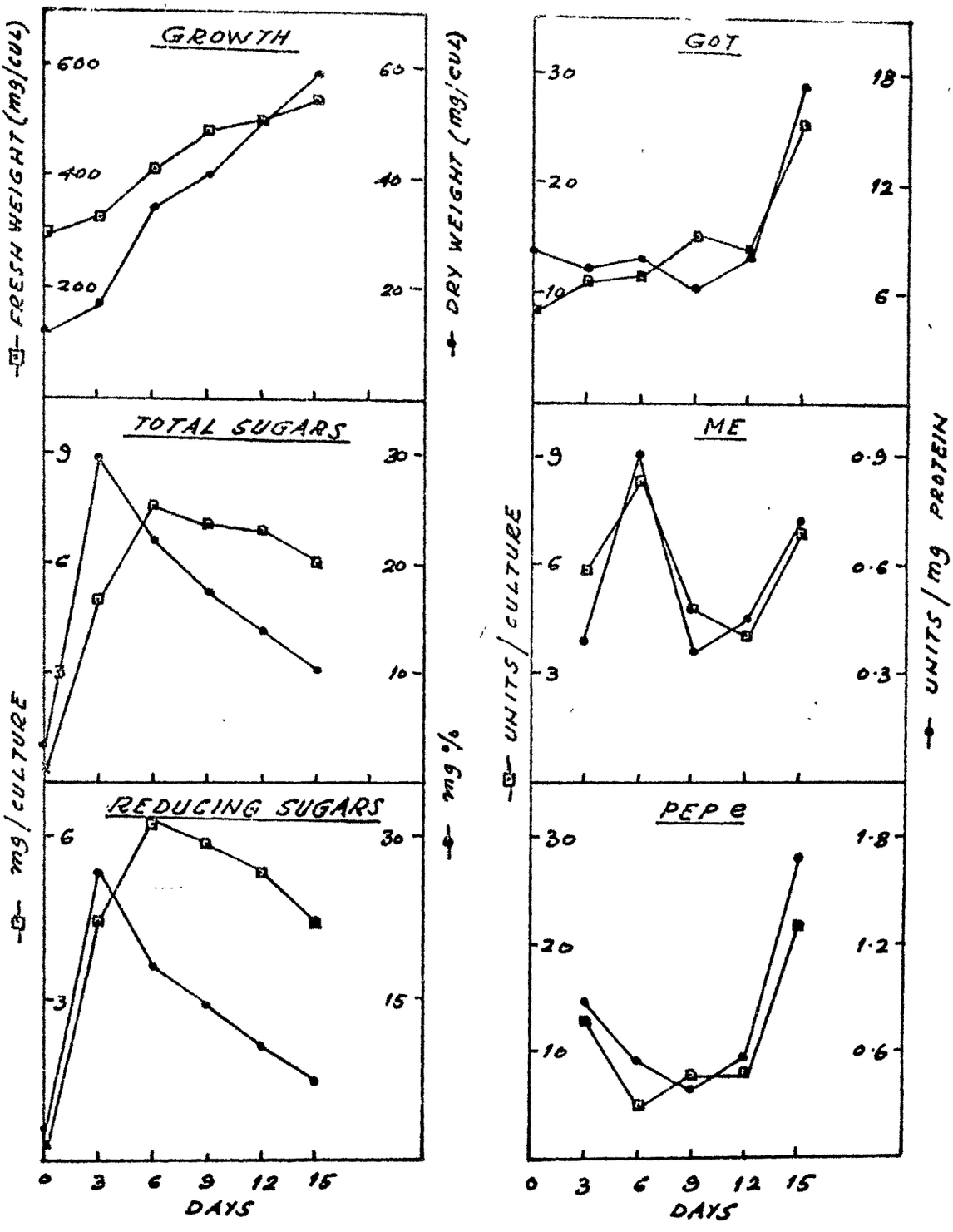

FIG. 14 (REF. TABLE NO. 47)

was comparatively less in dark than when grown in the light (Experiment 30). Total fold-wise increases of fresh and dry weights during the entire culture period of 15 days were 1.8 and 4.9 respectively.

Root formation was noticed in callus cultures of tobacco within a maximum of 15 days with a frequency response of over 70%, 4-5 roots being formed per callus mass.

(b) Total and reducing sugar accumulation :

Total and reducing sugar contents in tobacco callus cultured on root inducing medium in the dark are illustrated in Fig. 14 and Table 47.

There was a dramatic rise in total (29.4%) as well as reducing sugars (26.5%) during the initial 3 days in culture on mg% basis. Thereafter, the sugar content declined gradually till day 15. In the non-root forming portion of the callus on day 15, total and reducing sugar content was less when compared with that in the root forming portion of the callus.

(c) G O T :

The development of GOT activity is represented in Fig. 14 and Table 47.

After a slow rise till day 9, the total enzyme activity increased rapidly during days 12 to 15 to attain the peak

value. On the other hand, the specific enzyme activity fluctuated till day 9 and then increased sharply till day 15 to attain the peak value. Both total and the specific GOT activities were on the increase prior to the formation of roots (from days 9 to 15). In the non-root forming portion of the callus on day 15, the enzyme activity on both the counts was 5.3 and 5.8 folds less (total and the specific) when compared with that in the root forming portion of callus.

(d) M E :

The progressive changes in ME activity are represented in Fig. 14 and Table 47.

Activity of the ME could not be detected on day 0. Total and the specific enzyme activity followed almost the similar developmental pattern. The enzyme activity exhibited double peaks one each on day 6 and 15. Just before the differentiation of roots, the enzyme activity on both the counts increased. In the non-root forming portion of the callus on day 15, the activities of ME were 7.1 and 7.2 folds less (total and the specific activities respectively) when compared with that in the root forming part of the callus.

(e) P E P C :

The progressive changes in the enzyme activity of PEPC during differentiation of roots in tobacco callus are illustrated in Fig. 14 and Table 47.

Like malic enzyme, the PEPC activity also could not be detected on day 0. The developmental patterns of the total and specific enzyme activities were further quite similar, both showing steep rise from day 12 till day 15. The appearance of roots from callus tissues of tobacco on day 15 was thus preceded by a sharp rise in the activity of PEPC. In the non-root forming portion of the callus on day 15, total and the specific activities of PEPC were 5.4 and 6.2 folds less respectively when compared with that in the root forming part of the callus.

Expt. 33. Studies with callus tissues of G. hirsutum cultured on root inducing medium of tobacco in the dark.

Cotton callus weighing 400±40 mg fresh weight was inoculated into the culture flasks containing root inducing medium of tobacco (MS + 2.0 mg/l IAA + 3% sucrose) and incubated in dark for a period of 21 days. Every third day, five replicate flasks were harvested and analysed for growth, total and reducing sugar accumulation and enzymes. The results are documented below.

(a) Growth :

Growth measured as increase in fresh and dry weights of the cotton tissue is illustrated in the Fig. 15 and Table 48.

Table : 48. Growth, accumulation of total and reducing sugars and progressive changes in the activity of GOT, ME and PEPC in callus cultures of Gossypium hirsutum

Medium : MS + 2 mg/l IAA + 3% sucrose (Root forming medium of tobacco).

Inoculum : 400±40 mg fresh tissue.

Incubation : At 26±2°C in total darkness.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		G O T		M E		P E P C	
			mg/cult.	mg %	mg/cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	400.0 (±40.0)	16.0 (±4.0)	1.04	6.50	0.52	3.28	3.61	4.00	-	-	-	-
3	890.7 (±50.4)	47.1 (±5.1)	13.16	27.94	8.45	17.94	48.10	12.27	23.16	0.59	8.91	0.23
6	1928.5 (±79.8)	100.7 (±8.0)	23.42	23.26	20.23	20.09	32.40	5.25	30.86	0.50	30.86	0.50
9	3455.9 (±140.6)	178.1 (±16.3)	24.26	13.62	14.16	7.95	6.91	1.11	20.74	0.33	13.82	0.28
12	5300.0 (±205.5)	326.1 (±24.2)	36.03	11.05	23.12	7.09	8.48	1.23	21.20	0.31	10.86	0.15
15	7657.8 (±251.6)	419.7 (±33.4)	37.77	9.00	25.39	6.05	3.83	0.39	-	-	-	-
21	8368.8 (±300.2)	420.1 (±35.6)	26.97	6.42	18.36	4.37	-	-	-	-	-	-

Data represents an average of 5 replicates.

Figures in parenthesis represent standard error.

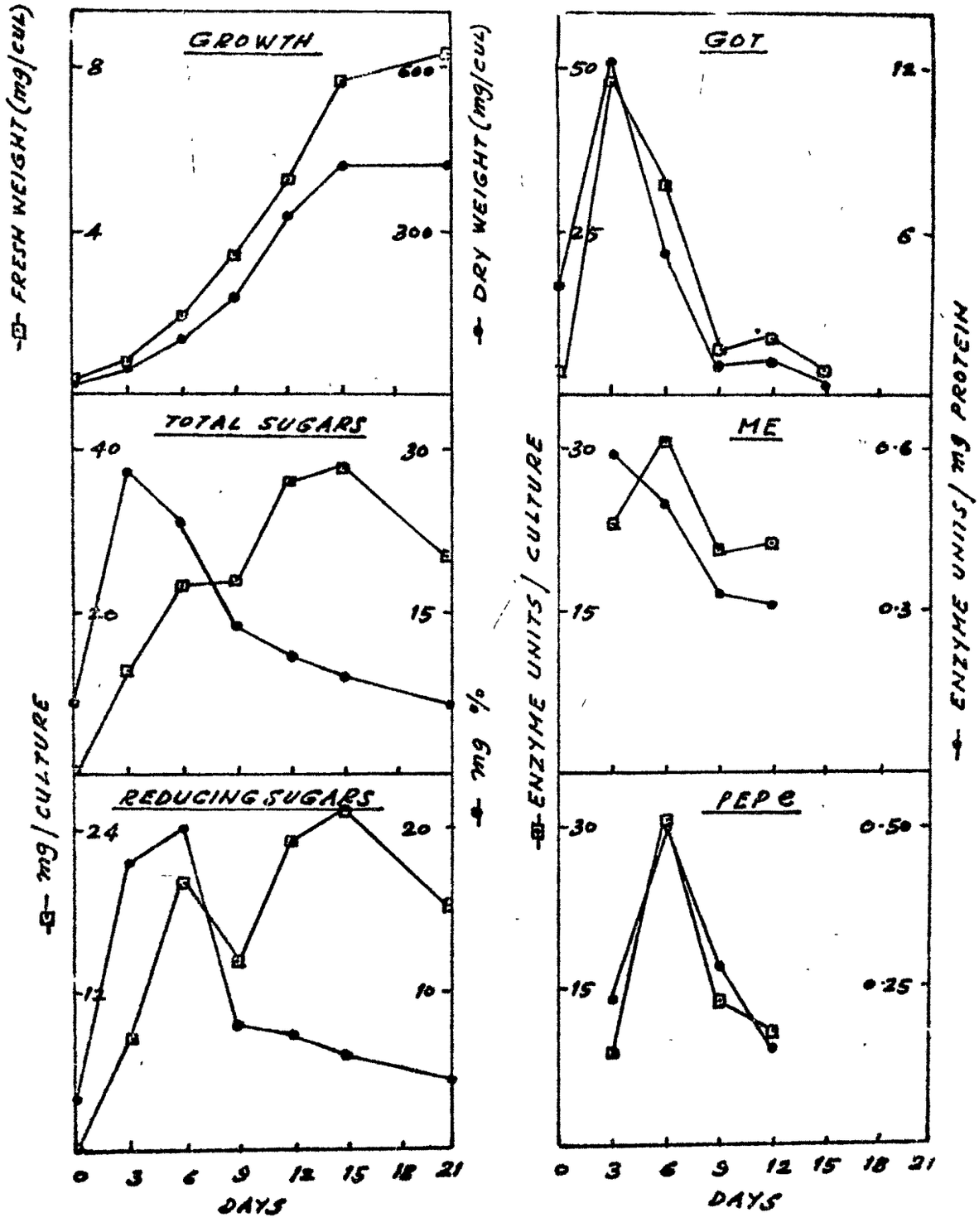

FIG. 15 (REF. TABLE NO. 48)

Growth of the cotton callus on fresh and dry weight basis increased 19.1 and 26.2 folds respectively by day 15 and thereafter there was no significant increase in growth till day 21. Growth is several folds higher in cotton when compared with that of tobacco tissues.

(b) Total and reducing sugar accumulation :

Accumulation of total and reducing sugars in the callus tissues are illustrated in Fig. 15 and Table 48.

Total and reducing sugars on mg% basis accumulated rapidly during the initial 3 days and declined gradually thereof till day 21 in culture. In both the cases, peak values on mg/culture basis was observed on day 15 in culture.

(c) G O T :

The development of GOT activity in callus cultures of cotton grown in root inducing medium of tobacco in the dark is represented in Fig. 15 and Table 48.

The enzyme activity both on culture and on mg protein basis (total and specific) increased very rapidly during the first 3 days to attain the peak values and declined thereof till day 15. In contrast, in tobacco callus, the specific GOT activity had increased sharply between days 12 to 15, i.e. prior to organogenetic response.

(d) M E :

The progressive changes in ME activity are presented in Fig. 15 and Table 48.

Activity of ME could not be detected on day 0 as in the case of tobacco. The total enzyme activity progressed from 23.2 units (on day 3) to 30.9 units (on day 6) to attain the peak value followed by a decline till day 12 after which it could not be detected. The specific activity of ME dropped steadily from day 3 to day 12. Both on culture and unit protein basis, the activity of ME increased between days 12 to 15 (prior to organogenesis) in tobacco, whereas in cotton the ME activity, total as well as specific, was on decline from day 6/3 onwards and could not be detected after day 12.

(e) P E P C :

The progressive development in enzyme activity of PEPC is illustrated in Fig. 15 and Table 48.

Like malic enzyme, the activity of PEPC could not be detected on day 0, as was observed with tobacco. Further, the enzyme activity both on culture and on unit protein basis, followed similar developmental pattern. Activity on both the counts increased from day 3 to 6 to attain the peak values

and declined thereof till day 12 in culture. In tobacco tissues, on the other hand, the PEPC activity on both the counts had increased significantly between days 9 to 15.

Summary :

Growth of cotton callus, cultured on root inducing medium of tobacco was several folds higher when compared to that of tobacco tissues.

The specific activities of the enzymes GOT, ME and PEPC increased considerably in tobacco tissues during the days preceding root differentiation; whereas in cotton callus, the peak activities were recorded either on day 3 or 6 and declined thereof sharply till day 12 and could not be detected at all on day 15 in culture.

Section I : Osmotic requirement for shoot and root formation in callus cultures of tobacco.

The developmental sequence leading to shoot and root formation in tobacco callus cultures has been described in earlier Sections G and H of this Chapter III. From these studies it was clear that exogenously supplied IAA and sucrose are playing an important role in organogenesis. Both the shoot and root formation have been correlated with a number of biochemical parameters such as starch content, total and reducing sugar accumulation and their utilization, including some enzymes of respiration and glucose oxidation.

For the normal development of shoot and root a continuous supply of carbohydrate in the medium is necessary. Probably absorbed free sugars and accumulated starch are utilised during the meristemoid initiation, organ primordia formation and subsequent development of shoots and roots. This utilization pattern during organogenesis reflects the high energy requirement for the organogenetic process. Besides being a readily available source of energy, an additional possible role for the degradation products of starch and the exogenously supplied carbohydrate in tobacco callus may well be osmotic.

To investigate any possible osmotic involvement during shoot and root formation in tobacco callus cultures the present experiments were conducted. Also from the Experiment 25 (Chapter III), it became obvious that enhancement of sucrose level in the medium from 3 to 6% caused a shift in morphogenetic response from shoot to root formation. Further, one

would question if 6% sucrose is really needed for organogenesis in tobacco callus cultures.

In an attempt to investigate the osmotic requirement for shoot and root formation in tobacco callus cultures, the medium was supplemented with the osmotic agent mannitol, a sugar alcohol. Mannitol has been selected because though it does enter the tobacco callus tissue (Klenovska, 1971), it has been shown not to support the growth (Hildebrandt and Riker, 1949), nor does it get metabolised (Trip et al., 1964) by the tobacco callus.

Expt. 34. The effect of mannitol and the osmotic requirement for shoot and root formation in callus cultures of Nicotiana tabacum L.

Callus tissues of tobacco weighing 300±30 mg were inoculated on 40 ml MS basal medium supplemented with different concentrations of IAA and sucrose or mannitol to give the molar sucrose equivalent to 2, 3 or 6% sucrose. The culture vessels were incubated in continuous light at 26±2°C. The various treatments provided and the results obtained are presented in the Table 49.

Auxin (0.3 or 2.0 mg/l IAA) singly or in presence of low (1%) sucrose level in the medium could not invoke any organogenetic response. When supplied singly mannitol also did not

induce any morphogenetic differentiation. Ultimately tissues died on this medium. However, sucrose (2%) alone in absence of auxin did induce shoot formation. In presence of low auxin (0.3 mg/l IAA), sucrose at 1% along with mannitol to give the molar equivalent of 2% sucrose induced shoot formation within 4 weeks. However, these shoots were dwarf and slender (Fig. 16). Further enhancement in mannitol level to give molar equivalent of 3% sucrose decreased the number of shoots produced per callus piece though the response frequency remained constant. Shoot formation was, however, completely suppressed when the mannitol level was increased still further in the (1% sucrose containing) medium to give molar equivalent of 6% sucrose (Table 49).

When mannitol was added in 2% sucrose (low auxin) medium to give molar equivalent of 3% sucrose, not only the number of shoots produced per callus and the response frequency increased but also the time taken for organogenesis was reduced by a week (Fig. 17). Further increase in mannitol level in the above medium to give molar equivalent of 6% sucrose resulted in complete suppression of organogenesis (Table 49).

In presence of high auxin (2.0 mg/l IAA), addition of mannitol in 1% sucrose containing medium to give molar equivalents of 2, 3 and 6% sucrose failed to invoke any

Table : 49. The effect of mannitol and the osmotic requirement for shoot and root formation in callus cultures of Nicotiana tabacum

Medium	IAA Concentration (mg/l)	Sucrose (%)	Mannitol	Morphogenetic response	Frequency response (%)	Time taken for response	Number of shoots or roots per callus mass
MS	-	-	-	-	-	-	-
MS	-	2.0	-	Shoots	40 - 50	4 weeks	Many
MS	-	-	To give the molar sucrose equivalent to 2%	-	-	-	-
MS	0.3	1.0	-	-	-	-	-
MS	0.3	1.0	To give the molar sucrose equivalent to 2%	Dwarf shoots	35 - 45	20-25 days	2 to 5
MS	0.3	1.0	To give the molar sucrose equivalent to 3%	Shoots	35 - 45	20-25 days	Solitary
MS	0.3	1.0	To give the molar sucrose equivalent to 6%	-	-	-	-
MS	0.3	2.0	-	Shoot	40 - 50	4 weeks	Solitary
MS	0.3	2.0	To give the molar sucrose equivalent to 3%	Shoots	55 - 65	3 weeks	7 to 9
MS	0.3	2.0	To give the molar sucrose equivalent to 6%	-	-	-	-
MS	2.0	1.0	-	-	-	-	-
MS	2.0	1.0	To give the molar sucrose equivalent to 2%	-	-	-	-
MS	2.0	1.0	To give the molar sucrose equivalent to 3%	-	-	-	-
MS	2.0	1.0	To give the molar sucrose equivalent to 6%	-	-	-	-
MS	2.0	2.0	-	Roots	60 - 75	4 weeks	1 to 2
MS	2.0	2.0	To give the molar sucrose equivalent to 3%	Roots	45 - 55	3 weeks	3 to 4
MS	2.0	2.0	To give the molar sucrose equivalent to 6%.	Roots	45 - 55	3 weeks	2 to 10

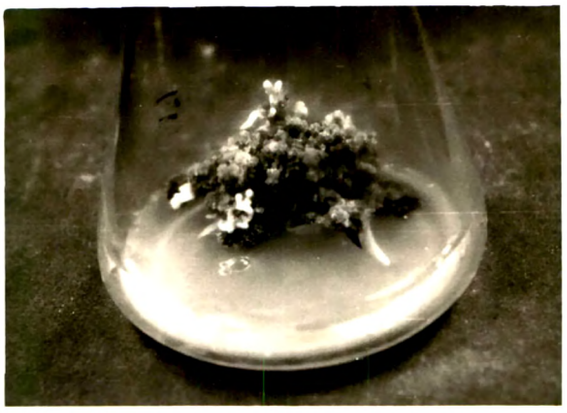

Fig. 16. Shoot differentiation.
(1% sucrose + Mannitol to give the molar equivalent of 2% sucrose).

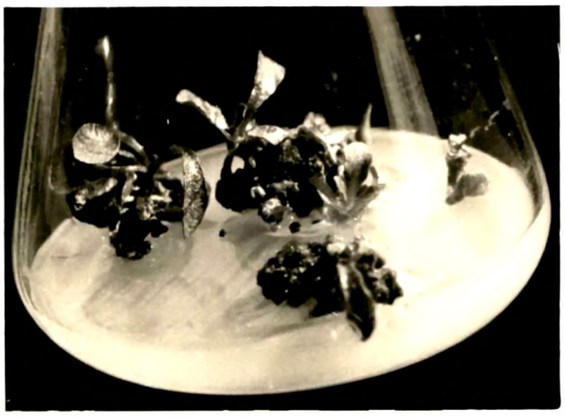

Fig. 17. Shoot differentiation
(2% sucrose + Mannitol to give the molar equivalent of 3% sucrose).

Fig. 18. Root differentiation
(2% sucrose + Mannitol to give the molar sucrose equivalent to 6%).

organogenetic response. On the other hand, in presence of high auxin (2.0 mg/l IAA), 2% sucrose alone could induce root formation. The root formation, however was improved and expediated (though the frequency of response was reduced) when mannitol was incorporated into this medium to give molar equivalents of 3% and 6% sucrose (Fig. 18).

The above observations indicate that not only auxin and sucrose levels affect organogenetic responses of the cultured tobacco tissues but also osmoticum of the medium has an important role to play in the processes leading to organ differentiation, as osmotic-hormonal interactions are clearly indicated.

Section - J : Effect of an inhibitor (Rifamycin) on organogenesis and carbohydrate metabolizing enzymes in callus cultures of tobacco.

Rifamycin is an antibiotic and claimed to be a specific RNA polymerase inhibitor. In the bacterium E. coli a concentration of only 3 X 10^{-8}M of rifamycin caused a 50 per cent inhibition of the enzyme. It is commonly held opinion that the antibiotic is specific to bacteria since mouse RNA polymerase was not affected. However, in plants, evidence exists that at least in certain families it has activity.

An attempt is made in the present study to examine the effect of rifamycin on organogenesis and physiological changes associated therewith in callus cultures of tobacco. The following parameters were investigated : (a) Growth, (b) Total and Reducing sugar accumulation, (c) Amylase, (d) Invertase, (e) M D H, (f) G-6-P D H and (g) F D P A.

The experiments were terminated by day 15 and the tissues analysed for the parameters enlisted above. Results obtained in this section are presented under the following heads :

(i) Studies with callus tissues of N. tabacum cultured on standard medium supplemented with 0.01 mg/l and 0.1 mg/l rifamycin.

(ii) Studies with callus tissues of N. tabacum cultured on shoot inducing medium supplemented with 0.01 mg/l and 0.1 mg/l rifamycin.

(iii) Studies with callus tissues of N. tabacum cultured on

root inducing medium supplemented with 0.01 mg/l and 0.1 mg/l rifamycin.

Expt. 35. Studies with callus tissues of N. tabacum cultured on standard medium supplemented with 0.01 mg/l and 0.1 mg/l rifamycin.

Callus masses of tobacco weighing 300±30 mg by fresh weight were cultured on MS basal medium supplemented with 2.0 mg/l and of IAA, NAA and KN and 2% sucrose and the levels of rifamycin incorporated were 0.01 mg/l and 0.1 mg/l. These are referred to in the text as medium A and B respectively. Medium was autoclaved with all the supplements except rifamycin. Rifamycin was filter sterilized and syringed out into each flask after autoclaving the medium. The culture vessels were incubated at 26±2°C in continuous light for a period of 15 days. Every three days, 5 replicate flasks were harvested and analysed for growth, total and reducing sugar content, and the enzymes. The results are described below.

(a) Growth :

Growth measured as increase in fresh and dry weight is presented in Tables 50, 51.

The growth of tobacco callus on medium A (i.e. 0.01 mg/l rifamycin containing medium) was better than on medium B (i.e. 0.1 mg/l rifamycin containing medium). On the former, fresh

Table : 50. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Nicotiana tabacum

Medium : MS + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l kinetin + 0.01 mg/l Rifamycin + 2% sucrose.

Inoculum : 300±30 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry Weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	300.0 (±30.0)	12.0 (±3.0)	0.39	3.28	0.35	2.90	0.26	0.29	0.31	0.35	1.69	0.88	1.05	1.17	1.50	1.67
3	389.1 (±32.4)	21.7 (±2.7)	4.14	19.07	1.77	8.14	0.61	0.10	0.71	0.19	5.65	0.96	3.80	0.98	5.60	1.86
6	598.3 (±36.9)	34.7 (±3.2)	5.00	14.40	1.92	5.52	0.80	0.15	0.58	0.21	10.74	1.59	6.90	1.31	8.40	1.23
9	797.9 (±37.4)	51.6 (±4.0)	5.23	10.13	1.54	2.99	1.10	0.21	0.99	0.16	26.17	1.81	8.80	1.45	10.37	1.72
12	967.5 (±39.5)	54.1 (±4.6)	4.76	8.80	2.25	4.16	0.97	0.15	0.88	0.24	10.26	0.65	5.80	1.67	17.52	3.62
15	1444.1 (±42.7)	80.6 (±6.3)	2.90	3.60	1.61	2.00	1.25	0.15	0.80	0.13	28.83	0.97	12.00	1.09	33.20	3.24

Data represents an average of 5 replicates.

Figures in parenthesis represent standard error.

Table : 51. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Nicotiana tabacum.

Medium : MS + 2 mg/l IAA + 2 mg/l NAA + 2 mg/l KN + 0.1 mg/l Rifamycin + 2% sucrose.

Inoculum : 300±30 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	300.0 (±30.0)	12.0 (±3.0)	0.39	3.28	0.35	2.90	0.26	0.29	0.31	0.35	1.69	0.88	1.05	1.17	1.50	1.67
3	413.4 (±29.7)	21.7 (±3.7)	3.79	17.47	1.67	7.71	0.55	0.10	0.40	0.20	2.57	0.91	2.50	0.78	5.30	1.05
6	626.4 (±32.4)	38.1 (±3.9)	6.05	15.87	1.54	4.03	0.86	0.15	0.74	0.22	8.80	1.07	5.13	0.96	8.10	1.20
9	742.1 (±40.3)	49.6 (±5.1)	5.02	10.13	2.32	4.67	0.53	0.12	0.87	0.14	19.89	1.42	6.30	0.82	10.80	1.15
12	1034.9 (±60.6)	66.6 (±6.2)	4.98	7.47	1.65	2.48	0.80	0.17	0.81	0.15	9.94	0.68	5.20	0.47	14.80	1.80
15	1349.0 (±63.8)	73.0 (±6.9)	3.99	5.47	1.53	2.10	0.97	0.14	1.73	0.19	21.15	0.86	2.70	0.24	17.21	1.07

Data represents an average of 5 replicates.

Figures in parenthesis represent standard error.

weight increased 4.8 folds and dry weight by 6.7 folds (Table 50). On the latter, fresh and dry weight increases were 4.5 and 6.1 folds respectively (Table 51). Higher concentrations of rifamycin inhibited growth of tobacco callus. When compared with the standard medium (i.e., without rifamycin) growth on rifamycin containing media was much less. Increases in fresh and dry weights on the standard (control) medium were 14.0 and 14.5 folds respectively (Table 9).

(b) Total and reducing sugar accumulation :

Changes in total and reducing sugar accumulation in callus tissues of tobacco during the course of culture for 15 days are presented in Tables 50 and 51.

Total sugars increased during the first 3 days of culture in both the media A and B. On mg% basis it was 19.1 on medium A and 17.5 on medium B. By day 15 it had come down to 3.6 and 5.5 mg% on media A and B. Reducing sugars also followed the same pattern. There was a dramatic accumulation of reducing sugars on both the media during the first 3 days of culture and thereafter it started decreasing till day 15. There was no marked difference in the trend of accumulation of total and reducing sugars on the two media.

(c) Amylase :

The development of amylase activity is presented in Tables 50, 51.

On both the media A and B, total enzyme activity demonstrated the peak values on day 15. The specific amylase activity also revealed no significant difference between the two media. On medium A, both total and the specific amylase activity was slightly higher than that on medium B. In the standard medium (without rifamycin) the amylase activity was higher, more so the total than the specific (Table 9).

(d) Invertase :

Progressive changes in invertase activity are presented in Tables 50, 51.

While medium A registered the peak total invertase activity on day 9 (1.0 unit), medium B demonstrated the peak on day 15 (1.7 units). The specific invertase activity on media A and B followed similar pattern with marginal fluctuations. There was no marked difference in enzyme activity between the two media, but when compared with the standard medium (Table 9), activity was suppressed in ribamycin containing media.

(e) M D H :

Changes in MDH activity are presented in Tables 50, 51.

Total enzyme activity on both the media increased rapidly till day 9 followed by a decline. However, the peak values were registered on day 15 in both the media (28.8 and

21.2 units respectively on media A and B). The specific MDH activity also followed similar developmental pattern on both the media without any significant differences. Activity of MDH on both the counts was lower on medium B on any given day when compared with that on medium A.

On standard medium (Table 9) the activity of MDH on both the counts was several folds higher than that on media A and B on any given day.

(f) G-6- P D H :

The progressive changes in enzyme activity of G-6-PDH in tobacco callus are presented in Tables 50, 51.

While on medium A, the peak total activity of G-6-PDH was registered on day 15 (12.0 units) medium B demonstrated the peak value on day 9 (6.3 units). The specific enzyme activity on medium A recorded the peak on day 12 (1.7 units), whereas in case of B, the activity was always less than that of day 0. Activity of G-6-PDH on both the counts was adversely affected at higher concentration of the antibiotic.

On standard medium (Table 9) G-6-PDH exhibited considerably higher activities when compared with that on medium A and B.

(g) F D P A :

The progressive changes of enzyme activity of FDPA are presented in Tables 50, 51.

Total enzyme activity on both the media followed similar developmental pattern. In both the cases, total enzyme activity increased gradually from day 0 till day 15 to attain the peak values with 22.1 and 11.5 folds rise respectively on media A and B. While the specific activity on medium A exhibited the peak value on day 12 (with 2 fold increase), marginal fluctuations in enzyme activity were noticed on medium B. Activity of FDPA on both the counts was considerably higher on medium A when compared with that on medium B.

On standard medium, the activity of FDPA on both the counts was many folds higher than that on the media A and B (Table 9).

Expt. 36. Studies with callus tissues of N. tabacum cultured on shoot inducing medium supplemented with 0.01 mg/l and 0.1 mg/l rifamycin.

Tobacco callus weighing 300±30 mg fresh tissue was inoculated into MS basal medium containing 0.3 mg/l IAA + 3% sucrose and the levels of rifamycin incorporated were 0.01 and 0.1 mg/l separately. These two media are referred to in the text as A and B respectively. Every third day, five replicate flasks were harvested and analysed for growth, total and reducing sugar content and the enzymes upto the period of 15 days. The results are described below.

(a) Growth :

Growth measured as increase in fresh and dry weights, is presented in Tables 52, 53.

The growth of tobacco callus was better on medium A than on medium B. The respective fold-wise increases in fresh weight were 1.6 and 1.5, whereas dry weight were 3.4 and 3.1. While shoots were differentiated in normal shoot inducing medium of tobacco (Expt. 26) within a period of 2 weeks, the organogenetic response was delayed on media A and B. On medium A shoots appeared during the 4th week of culture period with 50-60% frequency (Fig. 19), while on medium B the shoots were differentiated after 30 days of culture with only 5-10% frequency (Fig. 20).

(b) Total and reducing sugar accumulation :

Variations in total and reducing sugar content in tobacco tissues cultured on shoot inducing medium containing 0.01 mg/l and 0.1 mg/l rifamycin are presented in Tables 52, 53.

Both total and reducing sugars on mg% basis accumulated during the initial days of culture and declined gradually thereof. This is in contrast to the rapid utilization of sugars in the shoot forming medium (Table 41).

(c) Amylase :

The progressive changes in total and the specific activities

Table : 52. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Nicotiana tabacum.

Medium : MS + 0.3 mg/l IAA + 0.01 mg/l Rifamycin + 3% sucrose.

Inoculum : 300±30 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	300.0 (±30.0)	12.0 (±3.0)	0.39	3.28	0.35	2.90	0.26	0.29	0.31	0.35	1.69	0.88	1.05	1.17	1.50	1.67
3	339.9 (±31.6)	15.1 (±2.8)	5.07	33.60	1.69	11.18	0.47	0.34	0.35	0.25	3.17	1.78	0.20	0.14	2.30	1.61
6	370.8 (±36.3)	18.7 (±3.4)	5.54	29.60	1.37	7.30	0.19	0.15	0.29	0.13	2.60	1.27	3.90	2.02	6.29	2.83
9	440.7 (±37.5)	27.9 (±3.6)	7.40	26.53	1.82	6.54	0.22	0.14	0.25	0.16	1.29	0.56	4.40	1.50	8.34	3.11
12	456.2 (±42.7)	35.8 (±5.5)	8.84	24.70	3.16	8.83	0.84	0.25	0.38	0.15	2.17	0.85	5.10	1.72	11.50	2.16
15	478.1 (±47.4)	40.5 (±6.1)	9.29	22.93	1.84	4.54	1.13	0.20	0.54	0.18	2.28	0.87	6.70	1.91	12.38	2.04

Data represents an average of 5 replicates.

Figures in parenthesis represent standard error.

Table : 53. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of Nicotiana tabacum

Medium : MS + 0.3 mg/l IAA + 0.1 mg/l Rifamycin + 3% sucrose.

Inoculum : 300±30 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars mg/cult.	Total Sugars mg %	Reducing Sugars mg/cult.	Reducing Sugars mg %	Amylase Unit/cult.	Amylase Unit/mg protein	Invertase Unit/cult.	Invertase Unit/mg protein	M D H Unit/cult.	M D H Unit/mg protein	G-6-P D H Unit/cult.	G-6-P D H Unit/mg protein	F D P A Unit/cult.	F D P A Unit/mg protein
0	300.0 (±30.0)	12.0 (±3.0)	0.39	3.28	0.35	2.90	0.26	0.29	0.31	0.35	1.69	0.88	1.05	1.17	1.50	1.67
3	329.5 (±32.2)	13.9 (±2.9)	4.82	34.67	1.55	11.18	0.48	0.33	0.22	0.14	3.15	1.47	1.30	0.63	2.50	1.40
6	359.1 (±31.5)	19.1 (±3.2)	4.76	24.93	1.60	8.37	0.19	0.13	0.18	0.09	2.51	1.17	2.65	1.42	6.48	2.08
9	407.6 (±40.2)	27.1 (±3.7)	6.24	23.03	2.09	7.71	0.24	0.13	0.21	0.06	1.46	0.59	3.30	0.94	7.60	2.00
12	421.9 (±42.3)	32.0 (±4.1)	6.93	21.67	1.93	6.03	0.70	0.19	0.35	0.09	2.02	0.68	4.30	0.86	8.70	2.05
15	452.4 (±56.0)	37.7 (±5.0)	7.58	20.10	2.33	6.19	0.55	0.09	0.30	0.05	2.05	0.71	6.05	1.04	8.40	1.23

Data represents an average of 5 replicates.

Figures in parenthesis represent standard error.

Fig. 19. Shoot differentiation.
(0.3 mg/l IAA + 3% sucrose +
0.01 mg/l rifamycin).

Fig. 20. Shoot differentiation.
(0.3 mg/l IAA + 3% sucrose +
0.1 mg/l rifamycin).

of amylase are presented in Tables 52, 53.

No marked difference in the total and the specific amylase activity was observed (except on day 15) between the two media. In comparison with shoot inducing medium of tobacco (without rifamycin, Table 41), amylase activity on both the counts on media A and B was less considerably.

(d) Invertase :

The changes in the activity of invertase are presented in Tables 52, 53.

The enzyme activity both on culture and unit protein basis was much suppressed in both the media when compared with the shoot forming medium (without rifamycin, Table 41). The specific enzyme activity in both the media was less than that of day 0. Higher rifamycin containing medium (B) always showed less activity than that in presence of low concentration of rifamycin (medium A).

(e) M D H :

The progressive changes in total and specific activities of MDH are presented in Tables 52, 53.

Both total and the specific enzyme activities on media A and B exhibited peak values on day 3 followed by a decline thereof. However, no significant difference in enzyme development

pattern between the two media was noticed, only the suppression of activity was more in presence of higher concentration of the antibiotic. In comparison with normal shoot inducing medium of tobacco (Table 41). The MDH activity was considerably low on media A and B.

(f) G-6-P D H :

The progressive changes in total and the specific activities of G-6-PDH are presented in Tables 52, 53.

Total enzyme activity on both the media exhibited peak values on day 15 with about 6 folds increase. On the other hand, the specific G-6-PDH activity recorded the peak value on day 6 with 2.0 and 1.4 units respectively. However, the enzyme activity on medium B was less than that on medium A (except on day 3). In normal shoot forming medium of tobacco, G-6-PDH activity on both the counts was several folds higher than in case of media A and B.

(g) F D P A :

Total and the specific activities of FDPA during the culture of tobacco callus on rifamycin containing media are presented in Tables 52, 53.

While medium A recorded the peak value on day 15 with over 8 folds increase, medium B exhibited peak value on day 12

with nearly 6 folds increase. On the other hand, medium B demonstrated marginal increase in the specific enzyme activity on day 6 (2.1 units), whereas medium A recorded the peak value on day 9 with about 2 fold increase. The specific enzyme activity on medium A was slightly higher on all the days than that on medium B.

Activity of FDPA on both the counts was higher on the normal shoot inducing medium (Table 41) than those on media A and B.

Expt. 37. Studies with callus tissues of N. tabacum cultured on root inducing medium supplemented with 0.01 mg/l and 0.1 mg/l rifamycin.

Tobacco callus weighing 300±30 mg (fresh weight) was inoculated into MS basal medium containing 2.0 mg/l IAA + 3% sucrose. In the experimental media 0.01 and 0.1 mg/l rifamycin was incorporated separately. The media are referred to in the text as A and B respectively. Every third day, 5 replicate flasks were harvested and analysed for growth, total and reducing sugar content and the enzymes for a period upto 15 days. The results are described below.

(a) Growth :

Growth measured as increase in fresh and dry weights is presented in Tables 54, 55.

Table : 54. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA in callus cultures of *Nicotiana tabacum*.

Medium : MS + 2.0 mg/l IAA + 0.01 mg/l Rifamycin + 3% sucrose.

Inoculum : 300±30 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	300.0 (±30.0)	12.0 (±3.0)	0.39	3.28	0.35	2.90	0.26	0.29	0.31	0.35	1.69	0.88	1.05	1.17	1.50	1.67
3	355.9 (±34.1)	18.1 (±3.2)	6.28	34.67	2.47	13.66	0.55	0.31	0.48	0.23	0.69	0.53	4.80	2.30	3.70	1.77
6	416.5 (±37.4)	31.2 (±3.4)	8.65	27.73	2.47	7.90	0.47	0.21	0.24	0.11	1.76	0.80	7.51	2.23	10.30	2.92
9	459.1 (±40.0)	40.3 (±3.7)	10.32	25.60	3.37	8.37	0.75	0.25	0.52	0.12	0.74	0.27	6.22	1.22	11.50	2.27
12	488.6 (±40.9)	48.8 (±4.1)	11.52	23.60	3.56	7.30	0.68	0.25	0.50	0.16	0.46	0.19	2.10	0.61	12.10	2.61
15	533.6 (±43.8)	56.9 (±4.7)	12.44	21.87	4.63	8.14	0.61	0.21	0.61	0.13	1.08	0.71	2.19	0.65	20.50	3.63

Data represents an average of 5 replicates.

Figures in parenthesis represent standard error.

Table : 55. Growth, accumulation of total and reducing sugars and progressive changes in the activity of Amylase, Invertase, MDH, G-6-PDH and FDPA and in callus cultures of Nicotiana tabacum.

Medium : MS + 2.0 mg/l IAA + 0.1 mg/l Rifamycin + 3% sucrose.

Inoculum : 300±30 mg fresh tissue.

Incubation : At 26±2°C in continuous light.

Day	Fresh weight mg/cult.	Dry weight mg/cult.	Total Sugars		Reducing Sugars		Amylase		Invertase		M D H		G-6-P D H		F D P A	
			mg/ cult.	mg %	mg/ cult.	mg %	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein	Unit/ cult.	Unit/mg protein
0	300.0 (±30.0)	12.0 (±3.0)	0.39	3.28	0.35	2.90	0.26	0.29	0.31	0.35	1.69	0.88	1.05	1.17	1.50	1.67
3	348.8 (±32.3)	17.8 (±3.2)	6.74	37.67	1.99	11.18	0.70	0.30	0.47	0.20	0.62	0.52	2.20	1.84	2.60	1.12
6	410.6 (±37.7)	32.9 (±3.5)	9.43	28.67	2.91	8.83	0.42	0.17	0.19	0.05	1.02	0.69	6.40	2.04	5.50	2.04
9	463.6 (±39.5)	42.8 (±3.9)	11.47	26.80	2.88	6.72	0.42	0.07	0.28	0.05	0.47	0.20	5.40	0.15	8.49	1.34
12	477.8 (±40.0)	44.5 (±3.9)	11.09	24.93	3.62	8.14	0.45	0.14	0.32	0.10	0.40	0.18	1.70	0.37	10.21	1.40
15	510.3 (±42.6)	51.6 (±4.5)	11.54	22.37	4.32	8.37	0.50	0.16	0.40	0.05	0.51	0.25	1.80	0.42	15.42	1.37

Data represents an average of 5 replicates.

Figures in parenthesis represent standard error.

On medium A fresh and dry weight increases during 15 day culture period were 1.8 and 4.7 folds; whereas on medium B they were 1.7 and 4.3 folds respectively. There was no pronounced difference in growth of the tissues on media A and B on one hand, and between these and the control, on the other (Table 45). While roots were formed in the normal root inducing medium (Experiment 30), the tissues on rifamycin containing media did not show any organogenetic response even at the end of 4 weeks of culture period.

(b) Total and reducing sugar accumulation :

Variations in the accumulation of total and reducing sugar content is presented in Tables 54, 55.

Total and reducing sugars on mg% basis increased rapidly during the first 3 days in culture and declined gradually thereof. On mg/culture basis peak values were recorded on day 15 in both the media.

Total and reducing sugars depleted fast in the normal root forming medium prior to and during organogenesis (Table 45); whereas in rifamycin containing media A and B the total and reducing sugars were not utilised fast between days 6 to 15 in culture.

(c) Amylase :

The development of amylase activity is presented in

Tables 54, 55.

Total as well as the specific enzyme activity in both the media followed similar patterns with marginal fluctuations. In comparison with normal root forming medium of tobacco, amylase activity on both the counts on any given day was less in rifamycin containing media. There was a sharp drop in the specific activity with corresponding decrease in starch contents prior to organ formation, which was not the case on media A and B.

(d) Progressive changes in invertase activity are presented in Tables 54, 55.

Both total and the specific enzyme activities were much suppressed on media A and B. The specific enzyme activities were always lower than that of day 0 in both the media. In comparison with the normal root forming medium, the specific invertase activity was several folds lower in case of media A and B.

(e) M D H :

Changes in MDH activity in tobacco callus during the culture period of 15 days are presented in Tables 54, 55.

Total as well as the specific MDH activity in both the media was always less than that of day 0 (except the total activity on medium A). However, the enzyme activity was

marginally higher on medium A than that on medium B. Activity of MDH was appreciably higher during rhizogenesis in the normal medium (Table 45), indicating rifamycin suppression of the activity on media A and B.

(f) G-6-P D H :

The progressive changes of the enzyme G-6-PDH activity are presented in Tables 54, 55.

Total enzyme activity in both the media increased by 6 to 7 folds from day 0 till day 6 to attain the peak values, followed by a decline. The specific enzyme activity on medium A doubled by day 3 to register the peak value, whereas the activity on medium B almost doubled by day 6 and declined thereafter. Enzyme activity was more suppressed in presence of higher level of rifamycin. In the normal root forming medium of tobacco, the activity of G-6-PDH was several folds higher than in tissues on rifamycin containing media.

(g) F D P A :

The progressive changes in enzyme activity of FDPA are presented in Tables 54, 55.

In media A and B, total enzyme activity increased gradually from day 0 till day 15 to attain the peak values with 14 and 10 folds rise respectively. While medium A exhibited the peak specific activity on day 15 with 2 folds increase, medium B

recorded the peak on day 6 with 1.2 folds increase. Both total and the specific activities of FDPA on medium A were higher than that on medium B. On media A and B, enzyme activity was many folds less than that on normal root inducing medium (Table 45). On root inducing medium, the specific activity dropped sharply during the initiation of organogenesis (days 9 to 12), but it enhanced with the development of organ (root) primordia (days 12 to 15). On the other hand, in rifamycin containing media the activity either marginally increased or remained stable (days 9 to 15).

Summary :

Low rifamycin (0.01 mg/l) containing medium always exhibited higher growth and all enzyme activities than that of high rifamycin (0.1 mg/l) containing medium. Growth as well as the enzyme activities in the standard medium (without rifamycin) were higher than that on the rifamycin (0.01 and 0.1 mg/l) containing media.

Rifamycin delayed not only morphogenetic response in the shoot inducing medium of tobacco but also suppressed the enzyme activities of amylase, invertase, MDH, G-6-PDH and FDPA (Expt. 36).

In case of root inducing medium of tobacco, rifamycin both at low and high concentrations suppressed the root formation completely. Also, growth as well as the enzyme activities were many folds low in rifamycin containing media than that on normal root inducing medium of tobacco.