PROLIFERATIVE GROWTHS AND ORGANOGENESIS IN COCONUT EMBRYO AND TISSUE CULTURES[1]

E.V. de Guzman, A.G. del Rosario and E.M. Ubalde*

INTRODUCTION

In a basal medium including vitamins, excised mature coconut embryos could develop into complete seedlings. The addition of auxin-like substances elicited varied morphological responses. When naphthalene acetic acid (NAA) was added to the solid medium the embryos developed a profusion of thin adventitious roots (Sajise and de Guzman, 1972). As a supplement to the liquid or solid medium 2,4 dichlorophenoxyacetic acid (2,4-D) induced tissue proliferations that disrupted the structural integrity of the embryo (Balaga *et al.*, 1973).

Cellular proliferations leading to surface callus formation were obtained by Eeuwens (1976) in stem, leaf and inflorescence explants from mature coconut palms especially when cultured in a specially developed mineral medium containing several organic constituents including gibberellic acid, 2,4-D and kinetin. From the same laboratory with stem explants from mature palms Apavatjrut and Blake (1977) observed more extensive growth with cell division occurring throughout the explants. At the surface pro-embryo-like group of cells seemingly arising from internal division of a single large cell could be identified.

METHODS AND TREATMENTS

Embryos were initially cultured following a liquid solid sequence (de Guzman *et al.*, 1971). The solid medium was sometimes modified as to sugar or Ca^{++} and/or K^{+} levels. Since no distinct effect of treatment was obtained after a period of eight weeks in the solid medium, the embryos were resubjected to the same treatments. The treatments used were: (a) 8% sugar; (b) minus calcium; (c) twice the normal amount of potassium, (d) four times the normal amount of potassium, and (e) low calcium but high potassium. The solid media were supplemented with 0.1 ppm 2,4-D and sometimes also with kinetin and para-chlorophenoxyacetic acid (pCPA).

Sections of the proliferated tissues were subcultured in callus-inducing media. In some experiments the medium contained 1% activated charcoal.

DISCUSSIONS

Morphological observations. Development of the seedling was inhibited or was abnormal in the presence of 2,4-D. The shoot might emerge and retain the usual form but its development was very much reduced. The root was even more adversely affected; it might not emerge or its tip showed callus-like growth. The cotyledon proper usually remained undeveloped and left intact. Other types of abnormalities were observed in both root and shoot. Characteristic effects, although variable in frequency, of 2,4-D in the media were the proliferations occuring at the cotyledonary sheath (CS) region, at the proximal end of embryo at the base of the root and near the midsection at the junction of the root, shoot and haustorium.

The CS is an upward extension of the haustorium which ensheaths the plumule. Normally it develops as a thin-non-fleshy sheath but in the presence of 2,4-D it may undergo hyper-development. The resulting growth might be a thickened collar of tissue at the base of the scale leaf (Fig. 1) or a mass of nodular proliferations (Fig. 2). Growth derived from the CS have the characteristics of shoot tissues. The surface texture resembled that of the leaf and might turn green. Surface callusing appeared in the proximal region starting at the base of the root on the side away from the shoot but it could extend to the area between the shoot and the root. Lower into the junction region the proliferations might be more extensive (Fig. 3). The intactness of the embryo was disrupted revealing creamy white tissues that appeared like hard callus. Some root-like proliferations with callus-like surface appeared. Despite the extensiveness of the disorganization the lower portion of the haustorium and the root and the shoot remained intact.

In liquid culture with basal medium the response was chiefly embryonic growth resulting in enlargment of the embryos. Supplementing the liquid medium with 0.1 ppm 2,4-D produced slight growth inhibition but no morphological abnormalities. Although appearing normal the 2,4-D treated embryos have undergone marked histological change in the procambial region (Fig. 4). These embryos, when transferred to solid medium without exogenous auxin, could undergo extensive internal cellular and tissue proliferation leading to disorganization of the embryo.

[1] Part of research supported by grants from the National Research Council of the Philippines (NRCP) and the International Atomic Energy Agency.

* Professor, Instructor and former Research Assistant, respectively, Department of Horticulture, University of the Philippines at Los Baños, College, Laguna, Philippines.

Effect of treatments. Increase of the sugar level to 8% markedly increased the degree of proliferations. This response is similar to the reported effect of high sugar in the production of adventitious roots.

As shown in Table 1 there is no consistent effect of treatments on the frequency of proliferations.

Subculture of the proliferations. The CS proliferations were subcultured to continue proliferative growth and to induce the new growths to differentiate into shoots. The subculture of the root-like bodies aimed to promote more proliferations as well as to induce a break-up of their structural integrity and thereby give real callus growth.

Explants of both types continued to grow in subculture. In the case of the CS proliferations the surface in contact with the medium became discolored. A few weeks after transfer, new growth became very evident. However the older growth lost their viability; they became discolored. Therefore the net effect was a little accumulation of viable tissue mass. However, in medium in which carbon was incorporated, sustained growth became possible (Figs. 5 and 6, Table 2).

Continued growth of the root-like proliferations gave rise to enlarged bodies. The original nodule or root-like bodies sometimes transformed into irregularly shaped forms. Some developed rootlets. The resulting growths mostly retained the intact surface. Rupturing was observed in some cultures but did not result in much callus growth.

Morpho-anatomical observations. In freshly excised embryos, procambial strands radiate peripherally from the junction zone down the haustorium as well as occur randomly in the inner zones of the haustorium. After 8 weeks of culture in basal medium xylem elements with annular thickenings (Fig. 7) differentiated from the procambium. In the presence of 2,4-D the procambium strand, instead of differentiating into xylem, became converted into broad longitudinal bands of meristematic cells (Fig. 4). It is the further growth activity in these meristematic regions during the solid culture that gave rise to proliferations of tissue masses that resulted in the break up of the embryonic structure.

On further passage and subculture of the CS proliferations, two morphological types of growth emerged. One was of a nodular type (Fig. 5) which developed green color in the presence of light. Sometimes the nodules were very tiny, giving the growing surface a coarse granular appearance. The second type (Fig. 6) consisted of a few lumps or a single lump of tissues with a relatively smooth surface and a spongy texture. In light they acquired a yellow-green color with some areas on certain cultures remaining white. A culture could be classified strictly as belonging to either type but there were cultures where both types occurred. In the first type discrete bodies made up of small, prominently nucleated, closely arranged cells such as those found in apical meristems were observed (Fig. 8). Forms suggestive of a tendency to organize shoot primordia and embryoid-like structures could be identified (Fig. 8 and 9). In the second type, the bulk of the tissue consists of lightly staining, large parenchymatous cells (Fig. 10). Delineating the large cells into lumps was an outline of small cells.

The nodules may actually be units of organization similar to the protocorm in orchids. A strong proof for this function is the differentiation of scale leaves in one of the cultures (Fig. 11). The spongy tissue, through further manipulations, may be directed into a real callus type of growth from which plantlets can be obtained through subsequent organogenesis. Thus in coconut, as in the orchid, two pathways of plantlet regeneration may be possible: direct plantlet regeneration involving "protocorm" formation and an indirect regeneration through a callus phase.

LITERATURE CITED

Apavatjrut, P. and J. Blake. 1977. Tissue culture of stem explants of coconut (*Cocos nucifera* L.). Oléagineux *32*(6): 267-270.

Balaga, H.Y., A.G. Del Rosario and E.V. De Guzman. 1973. Morphological responses of the makapuno embryo to 2,4-D in vitro. NRCP Research Bulletin *28*(1): 1-5.

De Guzman, E.V., A.G. Del Rosario and E.C. Eusebio. 1971. The growth and development of coconut makapuno embryo in vitro. III. Resumption of root growth in high sugar media. The Phil. Agric. *53*(10): 566-579.

Eeuwens, C.J. 1976. Mineral requirements of cultured coconut tissue. Physiol. Plant *36*:23-28.

Sajise, J.U. and E.V. De Guzman. 1972. Formation of adventitious roots in coconut macapuno seedlings grown in medium supplemented with naphthaleneacetic acid. Kalikasan, Phil. Jour. Biol. *1*: 197-206.

Table 1. The effect of sugar, calcium and potassium content of modified Murashige and Skoog's medium (Mo) supplemented with 0.1 ppm 2,4-D on the occurrence of various types of responses[1].

Treatment	Trial	Type of Response (%)			
		Proliferation in shoot area	Proliferation in root and junction area	Normal development	Ungerminated and dead
Mo4D	a	25.0(4)	43.7(7)	18.7(3)	12.5(2)
	b	0	66.6(6)	11.1(1)	22.2(2)
	c	7.1(1)	50.0(7)	0	50.0(7)
	Ave	10.7	53.4	9.9	28.2
Mo8D	a	46.1(6)	46.1(6)	7.6(1)	15.4(2)
	b	9.1(1)	72.7(8)	0	18.2(2)
	c	0	73.3(11)	0	26.6(4)
	Ave	18.4	64.0	2.5	20.0
Mo8D -Ca	a	28.5(4)	64.2(9)	7.1(1)	7.1(1)
	b	27.2(3)	27.2(3)	18.2(2)	27.2(3)
	c	6.6(1)	73.2(11)	0	20.0(3)
	Ave	20.7	54.9	8.4	18.1
Mo8D + 3K	a	50.0(7)	42.8(6)	0	28.5(4)
	b	0	18.2(2)	0	8.2(9)
	c	0	60.0(9)	6.6(1)	33.3(5)
	Ave	16.6	40.3	2.2	23.3
Mo8D 1/2 Ca + 3K	a	50.0(8)	50.0(8)	6.2(1)	18.7(3)
	b	0	20.0(2)	30.0(3)	50.0(5)
	c	6.6(1)	66.6(10)	6.6(1)	20.0(3)
	Ave	18.8	45.5	14.2	44.3
Mo8D + 1K	a	53.3(8)	53.3(8)	0	13.3(2)
	b	0	33.3(3)	11.1(1)	55.5(5)
	c	0	60.0(9)	6.6(1)	33.3(5)
	Ave	17.7	48.8	5.9	34.0

[1] Figures in parentheses indicate the number of cultures showing the types of response, 4D-4% dextrose; 8D-8% dextrose.

Table 2. Growth of subcultured cotyledonary sheath proliferations in various media with and without charcoal.

Treatments [1]	Average Growth Rating [2]	% cultures with profuse growth	% cultures with very profuse growth
1. MS + 0.1 ppm 2,4-D + 1.0 ppm Ki	1.4	0	0
2. MS + 0.1 ppm 2,4-D + 1.0 ppm Ki + 0.1 ppm pCPA	1.7	0	0
3. SH + 0.1 ppm 2,4-D + 1.0 ppm Ki	2.1	0	0
4. as in treatment 1 + 1% charcoal	3.1	0	31
5. as in treatment 2 + 1% charcoal	2.3	7	0
6. as in treatment 3 + 1% charcoal	2.4	18	0

[1] MS – modified Murashige and Skoog's medium
SH – Schenk and Hildebrandt's medium

[2] 0 – no growth
1 – very slight growth
2 – slight growth
3 – moderate growth
4 – profuse growth
5 – very profuse growth

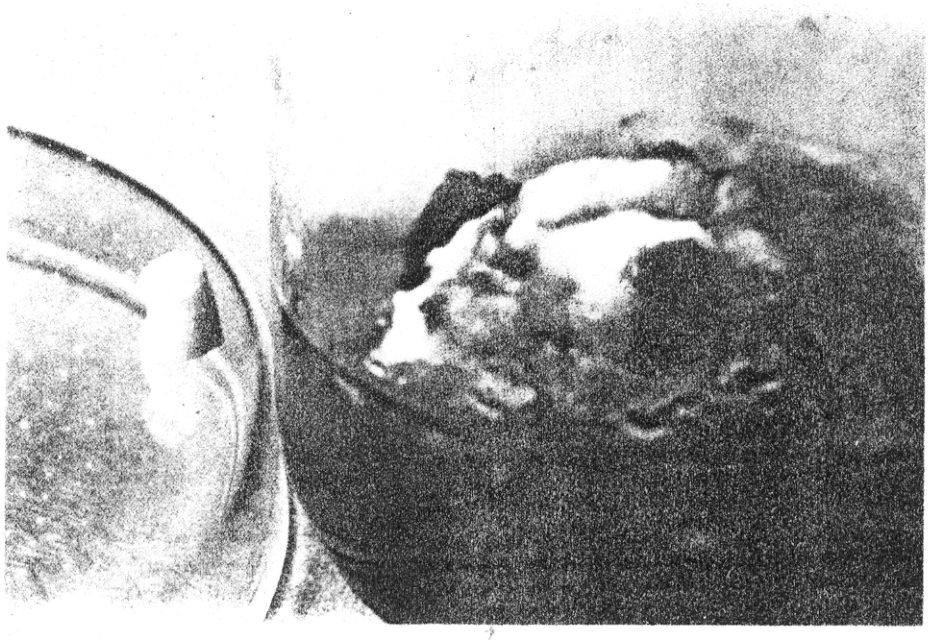


Fig. 1. Embryo showing a thickened cotyledonary sheath around the base of the first scale leaf.

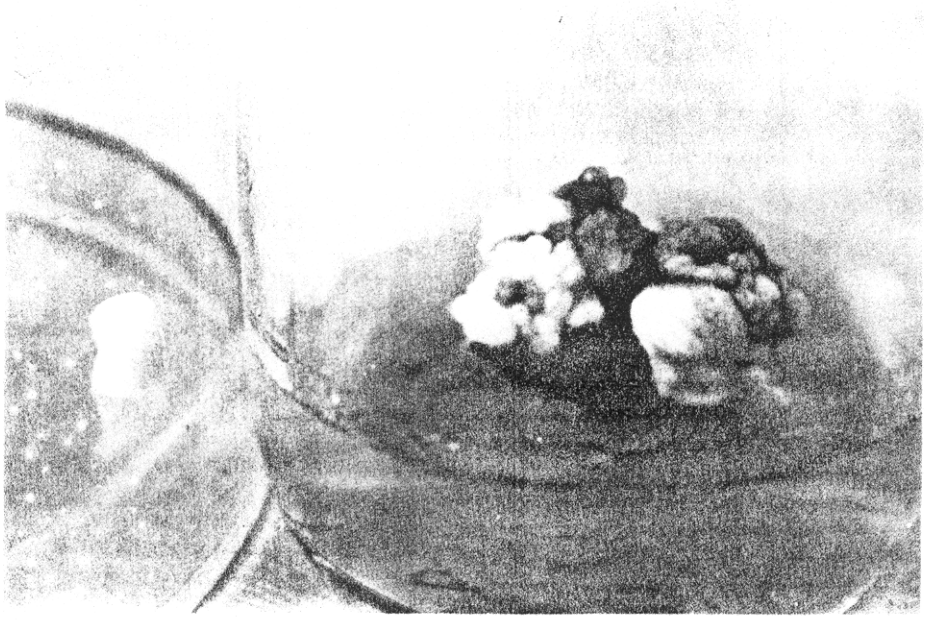


Fig. 2. Embryo showing nodular proliferations arising from the cotyledonary sheath.

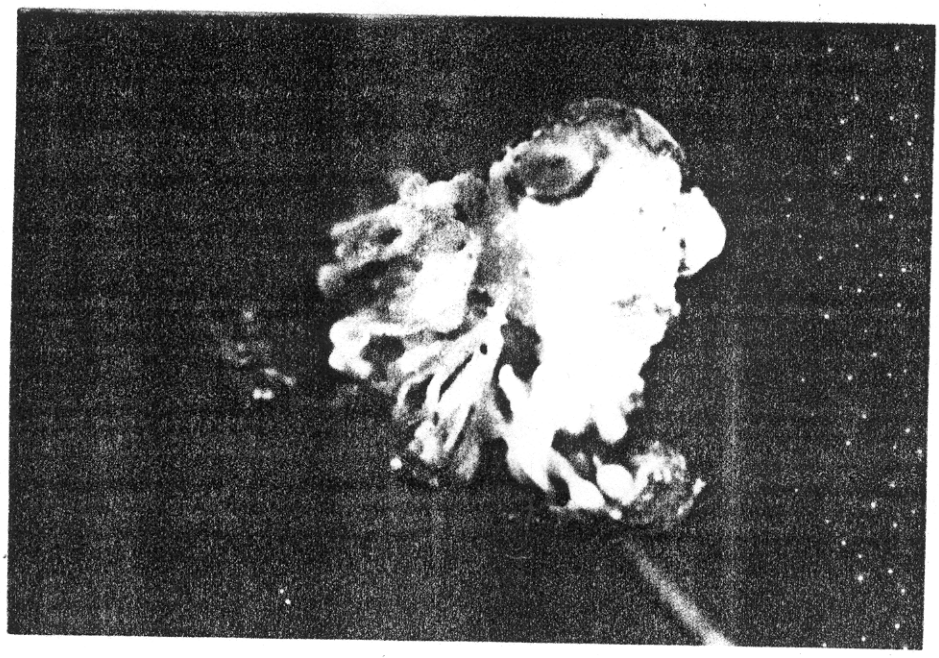


Fig. 3. Extensively disorganized embryo.

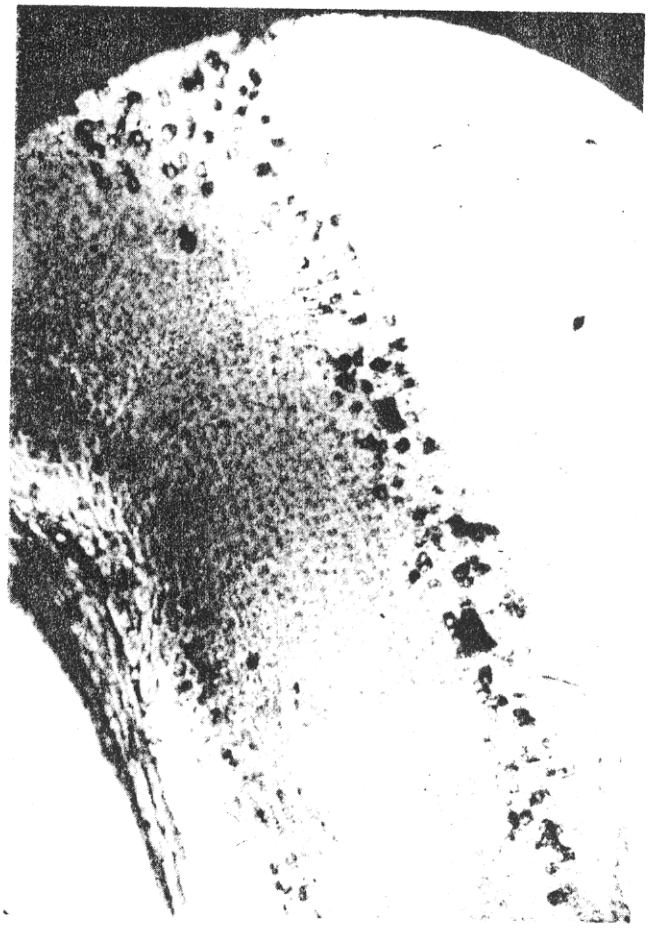


Fig. 4. Section showing conversion of the procambium into a band of actively dividing cells.

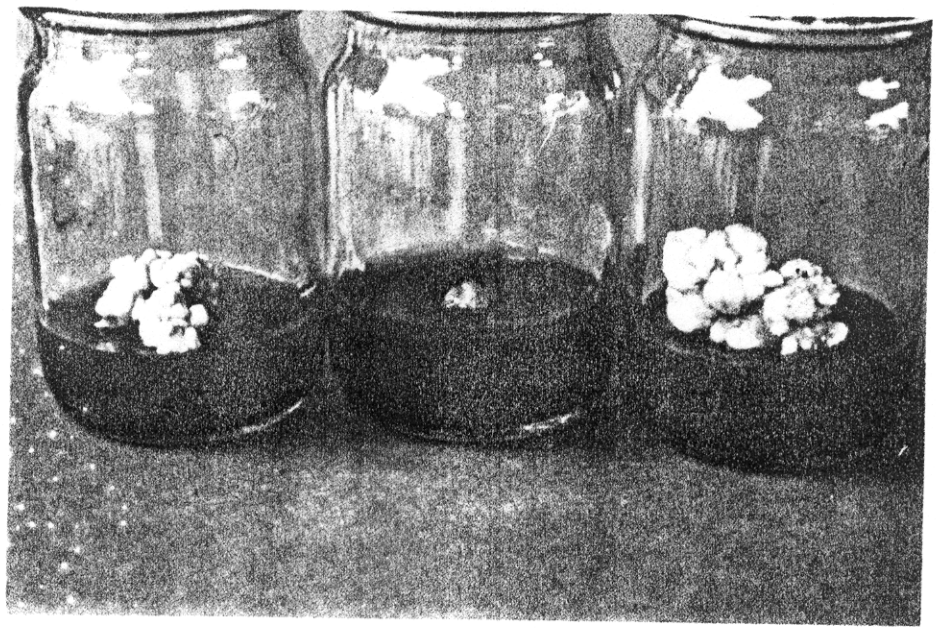


Fig. 5. Culture showing a nodular type of growth in a sub-culture of the cotyledonary sheath proliferation.

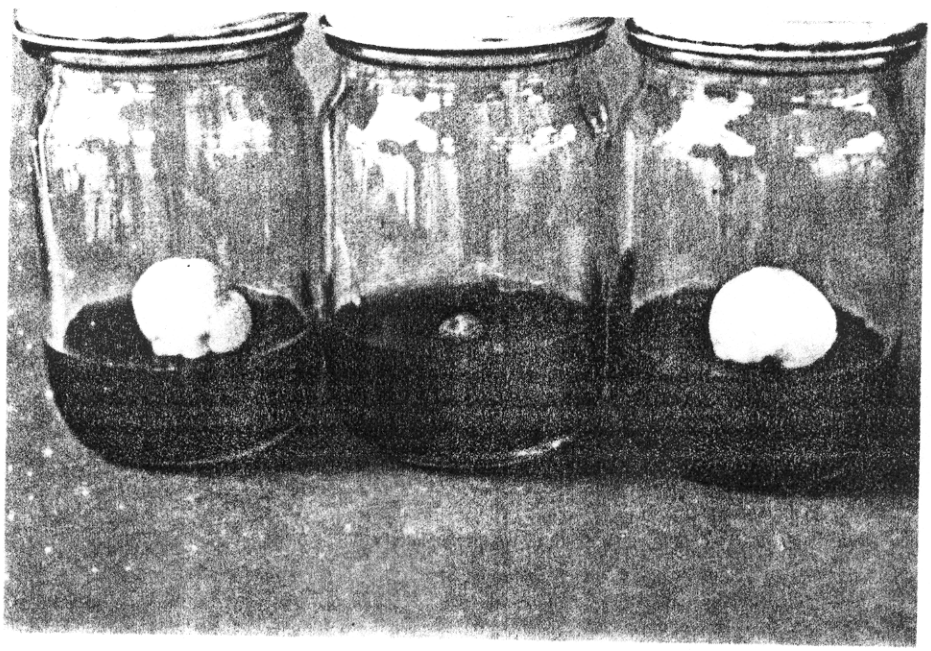
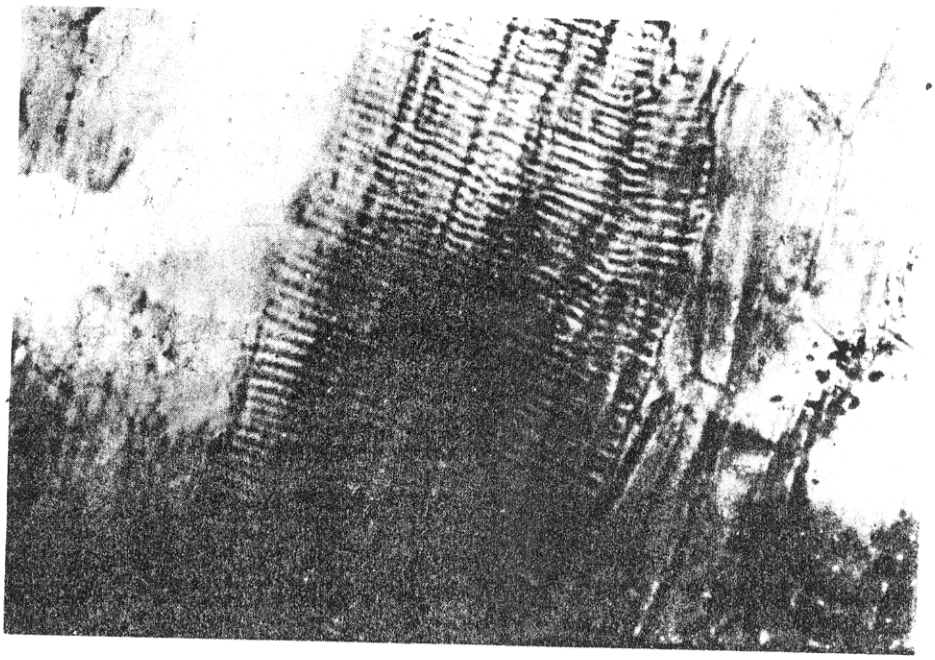
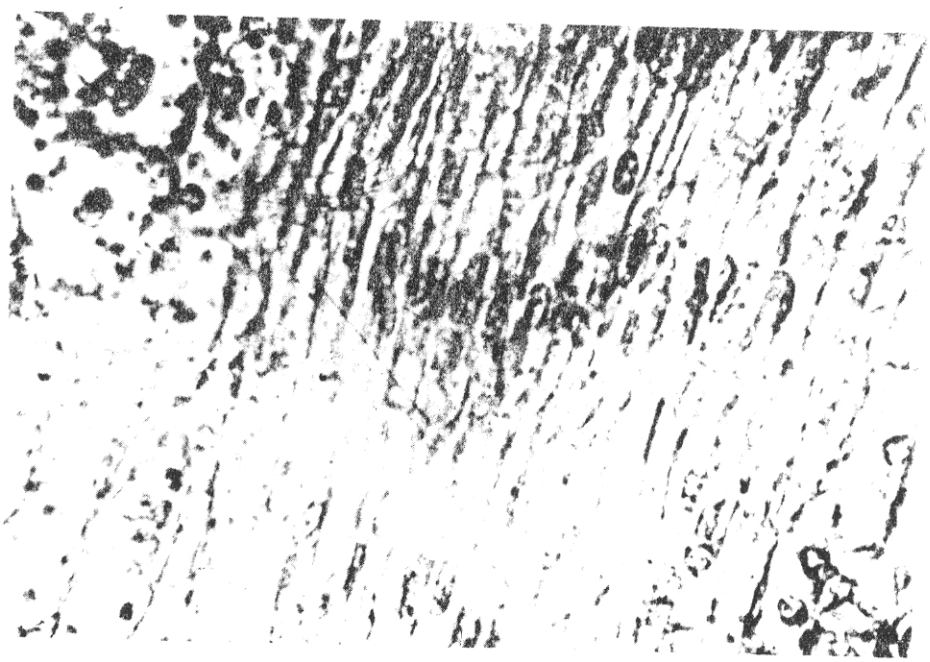


Fig. 6. Culture showing the spongy type of growth in a sub-culture of the cotyledonary sheath proliferation.



(a)



(b)

Fig. 7. Section of embryo showing xylem elements differentiated from the procambium (a) and procambium in freshly excised embryo (b).

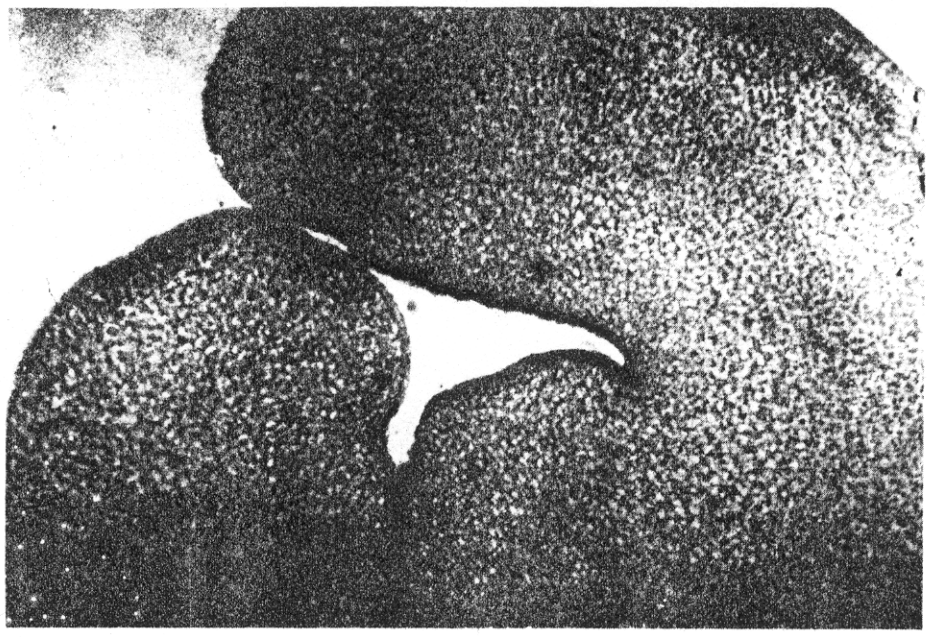


Fig. 8. Close-up of a nodular proliferation showing conversion of cells to the primary meristematic state.

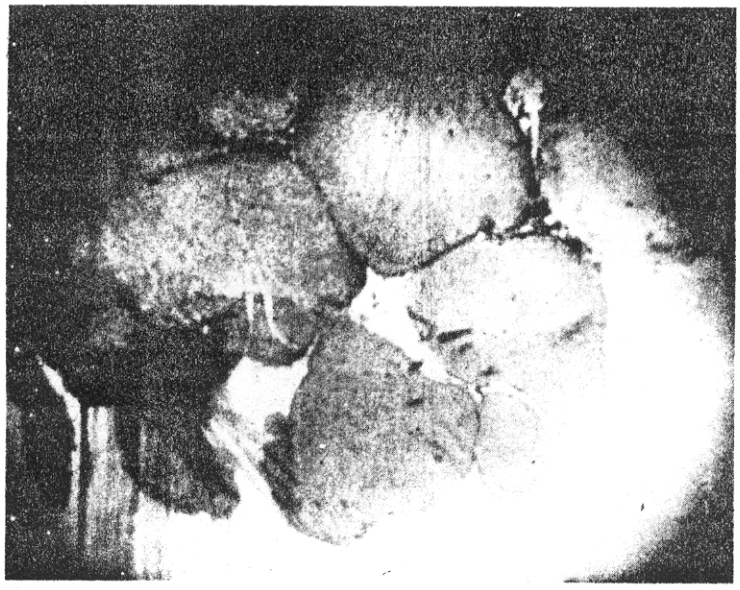


Fig. 9. Section of nodular proliferations showing discrete bodies.

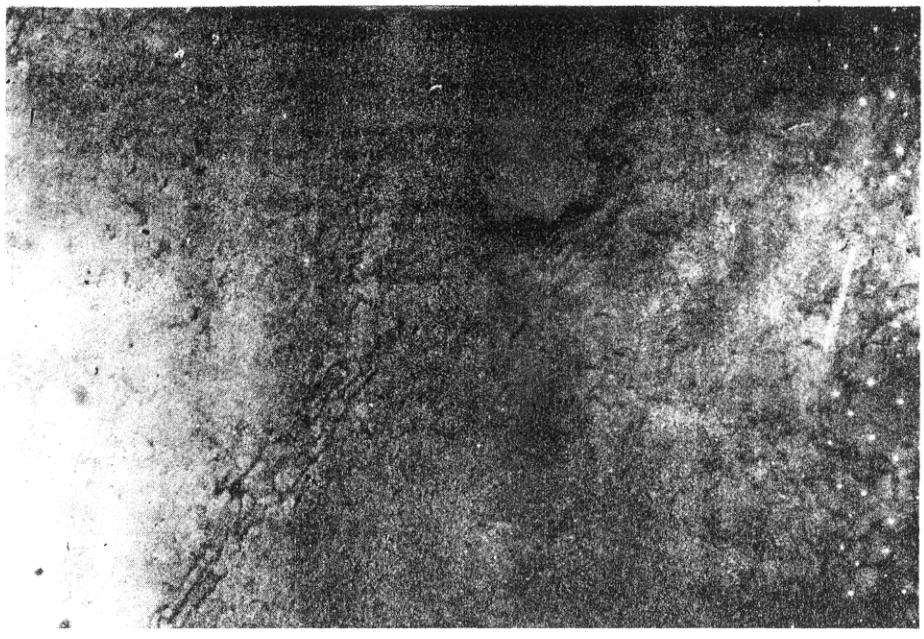


Fig. 10. Section of spongy type of growth showing large, lightly staining cells.

Fig. 11. Culture showing differentiation of shoots from nodular type of proliferation.