PLANTLET REGENERATION FROM MERISTEMS OF PHILIPPINE SWEETPOTATO VARIETIES

Villaluz Z. Acedo

Instructor, Philippine Root Crop Research and Training Center, Visayas State College of Agriculture, Baybay, Leyte, Philippines.

ABSTRACT

The solidified Murashige and Skoog (MS) medium added with gibberellic acid (GA_3) induced direct organogenesis without any callus formation of the meristems of sweetpotato, varieties VSP-1, VSP-2 and VSP-3. The optimum GA_3 concentration varied with variety. On the other hand, benzylaminopurine as medium supplement caused profuse callus growth on the basal portions of the meristem before shoots developed.

Shoot growth of the meristems was much faster in liquid than in solid MS medium supplemented with GA_3. Rapid shoot and root development was induced when the cultures were transferred to the solid MS medium containing high sucrose level 1.5 months after.

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KEY WORDS: Sweetpotato. *Ipomoea batatas* L. Meristem culture. Organogenesis. Murashige and Skoog. BAP. GA_3.

INTRODUCTION

The sweetpotato, *Ipomoea batatas* (L) Lam., is a dicotyledonous plant belonging to the family Convolvulaceae. It is vegetatively propagated by stem cuttings obtained directly from growing plants or from plants of the previous cropping season. This system of propagation renders possible the carry-over and build-up of diseases, especially those caused by viruses, in the succeeding cropping, thus, likely to cause great reduction in root yield.

Apical meristem culture has been reported to successfully eliminate a number of sweetpotato viruses (Alconero *et al.*, 1975; Frison and Ng, 1981). It has also been proven to be effective in producing virus-free clones of many vegetatively propagated plants (Quak, 1977; Hu and Wang, 1984).

Meristems may be regenerated into plantlets more quickly than tissues from other plant parts if cultured on suitable medium. The regenerated plants retain the genetic characteristics of the parent plant because the diploid nature of the meristematic cells is more uniform (Murashige, 1974).

This study determined the culture medium formulation that would effectively induce direct organogenesis of meristems in selected Philippine sweetpotato varieties.

MATERIALS AND METHODS

Preparation of meristems

Freshly harvested roots of sweetpotato, varieties VSP - 1, VSP - 2 and VSP - 3 were washed with tap water to remove adhering soil and dirt. These were then surface-sterilized using fungicide and insecticide solution, planted in clay pots containing sterilized soil and sand (1:1) mixture, and maintained under greenhouse condition. When sprouts were produced, 15 mm long shoot tips were taken. The expanded leaves were removed before washing the whole shoot tips with soapy water.

In a laminar flow cabinet, the tissues were sterilized with 2% sodium hypochlorite solution for 5 minutes and rinsed with sterile distilled water three times. Meristems of about 0.2 - 0.4 mm consisting of the apical dome with one leaf primordium were dissected under a stereomicroscope and inoculated into the culture medium.

Medium preparation

The Murashige and Skoog (MS) nutrients added with 2% sucrose was used as the basal medium. In the first experiment, 0.7% agar was added to gel the medium. Benzylaminopurine (BAP) and gibberellic acid (GA_3) were added singly at 0.5 mg/l and 0.25 mg/l, respectively. The second experiment utilized the liquid MS medium. A filter paper bridge was used to support the meristems. Different levels of GA_3 as medium supplement were tried.

After 1.5 months of culture in liquid medium, the meristems were transferred to solid MS medium supplemented with higher sucrose concentration of 5%. The MS medium alone served as the control. The pH of the medium was adjusted to 5.8. Ten ml of the medium formulation was dispensed into each culture vessel and sterilized by autoclaving for 15 minutes. The medium was allowed to cool down before use. Twenty culture vessels were used for each treatment. Three sweetpotato varieties, VSP - 1, VSP - 2 and VSP - 3, were used in the two experiments.

Culture incubation and maintenance

The cultures were maintained in an air-conditioned room with a temperature range of 25 - 28°C. They were exposed to an illumination of about 1,000 lux for the first few days of culture and then transferred to higher illumination condition of about 2,000 lux until the termination of the experiment.

Observations were made on the magnitude of callus, shoot and root formations.

RESULTS AND DISCUSSION

Influence of BAP and GA_3 on meristem growth

Meristems of VSPs 1, 2 and 3 cultured on BAP-added solid MS medium showed profuse callus formation after 4 months (Table 1 and Fig.1). Callus growth started at the base of the slow-growing meristem. This preceded shoot development of the meristems. No meristem formed roots (Table 1). In GA_3-added medium, the meristems exhibited direct organogenesis without forming callus (Table 1 and Fig.1). All meristems developed shoots which were about 3 - 5 mm in length after 4 months in culture. However, only 20 - 40% of the meristems produced roots (Table 1). This was observed only in VSP - 1 and VSP - 3 varieties. VSP - 2 meristems failed to form roots. Meristems cultured on MS medium alone did not show any sign of growth (Table 1). They rapidly turned brown during the first week of incubation.

The promotive effect of GA_3 on direct organogenesis of sweetpotato meristems follows that observed in potato, *Dahlia* and *Chrysanthemum* (Morel, 1975). From the result of the study, it appeared that meristem

Table 1. Response of meristems of different sweetpotato varieties to different culture medium formulations after 4 months in culture.

		Callus Formation		Shoot Formation		Root Formation	
Med.	Var.	%	degree[1]	%	length[2] (mm)	%	length[2] (mm)
MS	VSP - 1	0	0	0	-	0	-
	VSP - 2	0	0	0	-	0	-
	VSP - 3	0	0	0	-	0	-
MS + BAP 0.5 mg/l	VSP - 1	100	3	100	2.3$\pm$0.5	0	-
	VSP - 2	100	3	80	8.0$\pm$5.0	0	-
	VSP - 3	100	3	100	3.7$\pm$1.7	0	-
MS + GA_3 0.25 mg/l	VSP - 1	0	0	100	4.0$\pm$1.8	40	3.5$\pm$0.5
	VSP - 2	0	0	100	3.1$\pm$1.1	0	-
	VSP - 3	0	0	100	4.4$\pm$2.6	20	16.3$\pm$0

[1]Degree of Callusing:
0 - no callus
1 - slight callusing (2 mm in diameter and below)
2 - moderate callusing (2.1 - 6.0 mm in diameter)
3 - profuse callusing (6.1 mm and above)
[2]Means $\pm$ standard error (SE)

growth and development, particularly root formation differed with variety of sweetpotato (Table 1). In BAP-added medium, the complete inhibition of root development was probably due to the presence of callus which could have physically blocked the development of root initials or competed for available nutrients. Root growth inhibitors associated with excessive callus growth may have also been produced as has been observed in *Pistacia vera* (Barghchi and Alderson, 1983).

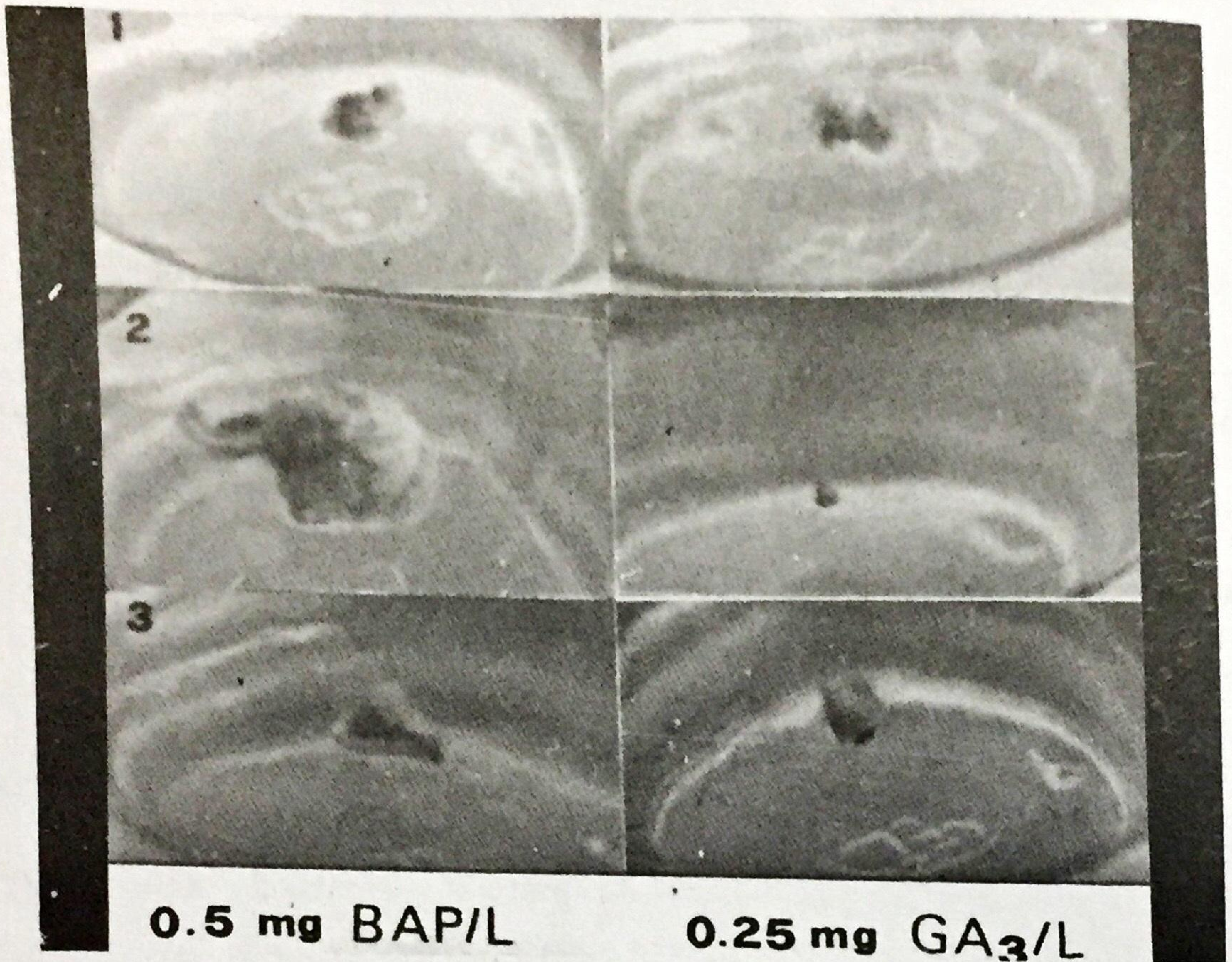

Figure 1. Meristems of (1) VSP - 1, (2) VSP - 2, and (3) VSP - 3 after 2 months of culture in solid MS medium supplemented either with 0.5 mg BAP/l or 0.25 mg GA_3/l.

Meristem growth in liquid MS medium with GA_3

In this experiment, only GA_3 as medium supplement was used since it enhanced both shoot growth and root formation and did not induce callusing as observed in the preceding experiment. Different GA_3 levels (0 - 0.75 mg/l) were tried to determine the optimum concentration for rapid shoot development. The growing meristems were then transferred to the sucrose-enriched solid MS medium to induce root development.

Table 2 shows the promotive effect of GA_3 on shoot growth of meristems. The three sweetpotato varieties differed in GA_3 requirement for shoot development. For higher percentage formation and faster growth of shoots, the meristems of VSP - 1, VSP - 2 and VSP - 3 required 0.25, 0.5, and 0.75 mg GA_3/l, respectively. Figures 2a and 2b further illustrate the growth of meristems after only 1 month of culture. On MS medium alone, the meristems did not grow and eventually turned brown.

Table 2. Effects of different levels of GA_3 supplemented to the liquid Murashige and Skoog basal medium on shoot and root growth of sweetpotato meristems after 1.5 months of incubation.

	GA_3 concentration (mg/l)[1]				
	0	0.1	0.25	0.5	0.75
VSP - 1					
% shoot formation	0	80	100	80	40
shoot length, mm	0	2.0±0.8	5.0±1.2	2.67±0.8	2.75±0.4
% root formation	0	0	0	0	0
VSP - 2					
% shoot formation	0	10	60	80	80
shoot length, mm	0	2.0±0	2.0±0.5	2.63±1.11	2.0±0.91
% root formation	0	0	0	0	0
VSP - 3					
% shoot formation	0	60	60	60	100
shoot length, mm	0	2.0±0.5	2.67±0.3	2.0±1.3	2.2±0.7
% root formation	0	0	0	0	0

[1]Means ± SE.

Shoot growth of meristems in liquid medium seemed to be faster than that in GA_3-added solid medium. In particular, it can be observed that the VSP - 1 meristems cultured on liquid medium supplemented with 0.25 mg GA_3/l grew to a length of 5.0 mm after 1.5 months (Table 2) whereas on solid medium with the same GA_3 level, the meristem shoot measured only about 4.0 mm after 4 months of culture (Table 1). This result tends to concur with that obtained in peach (Hammerschlag, 1980) and apple (Snir and Erez, 1980). The faster growth in liquid medium can be attributed to the increased absorption of nutrients by the explants. In solid medium, the

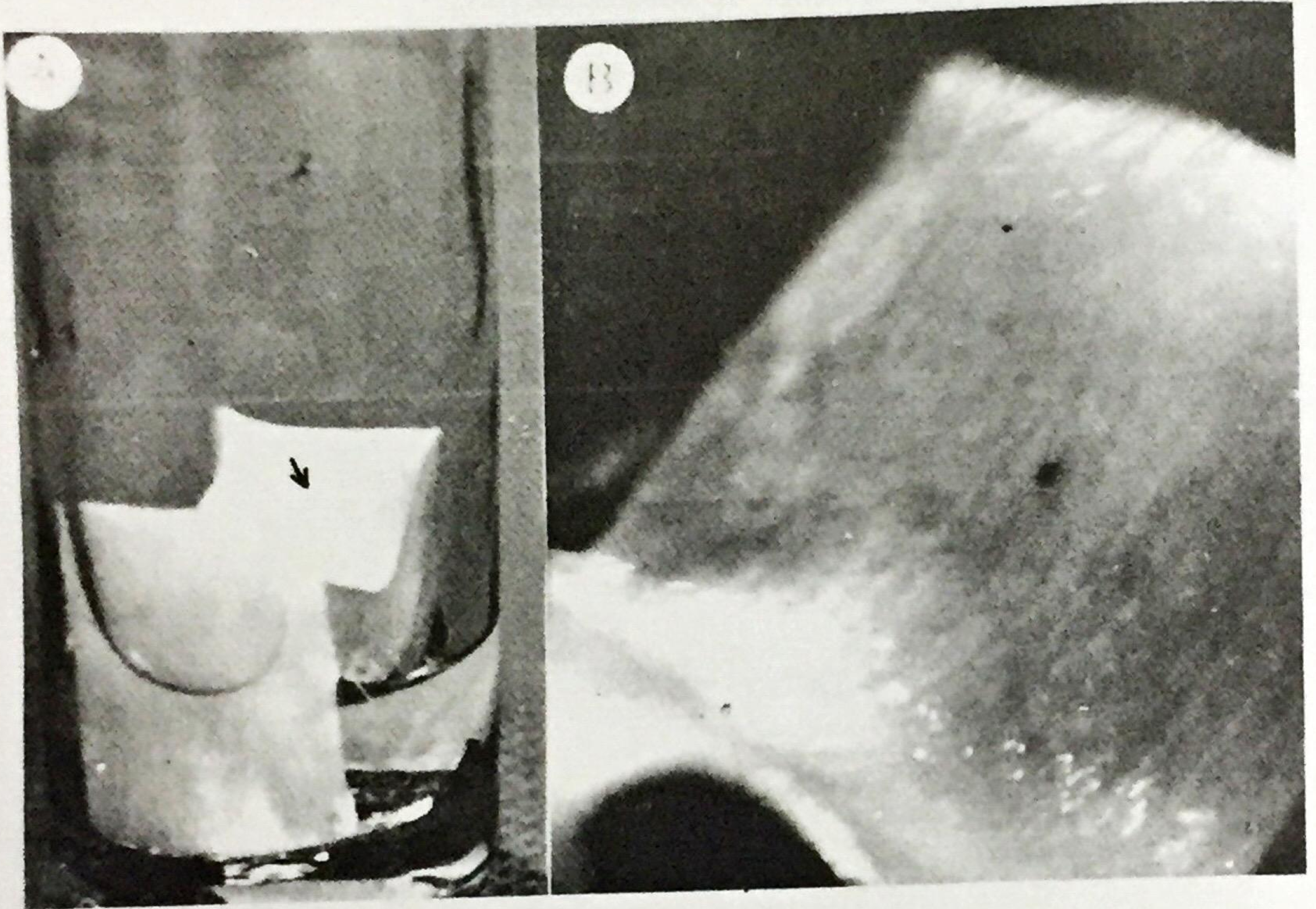

Figure 2a. (A) newly inoculated meristem on liquid medium supported by filter paper bridge, (B) a closer view of the meristem in culture.

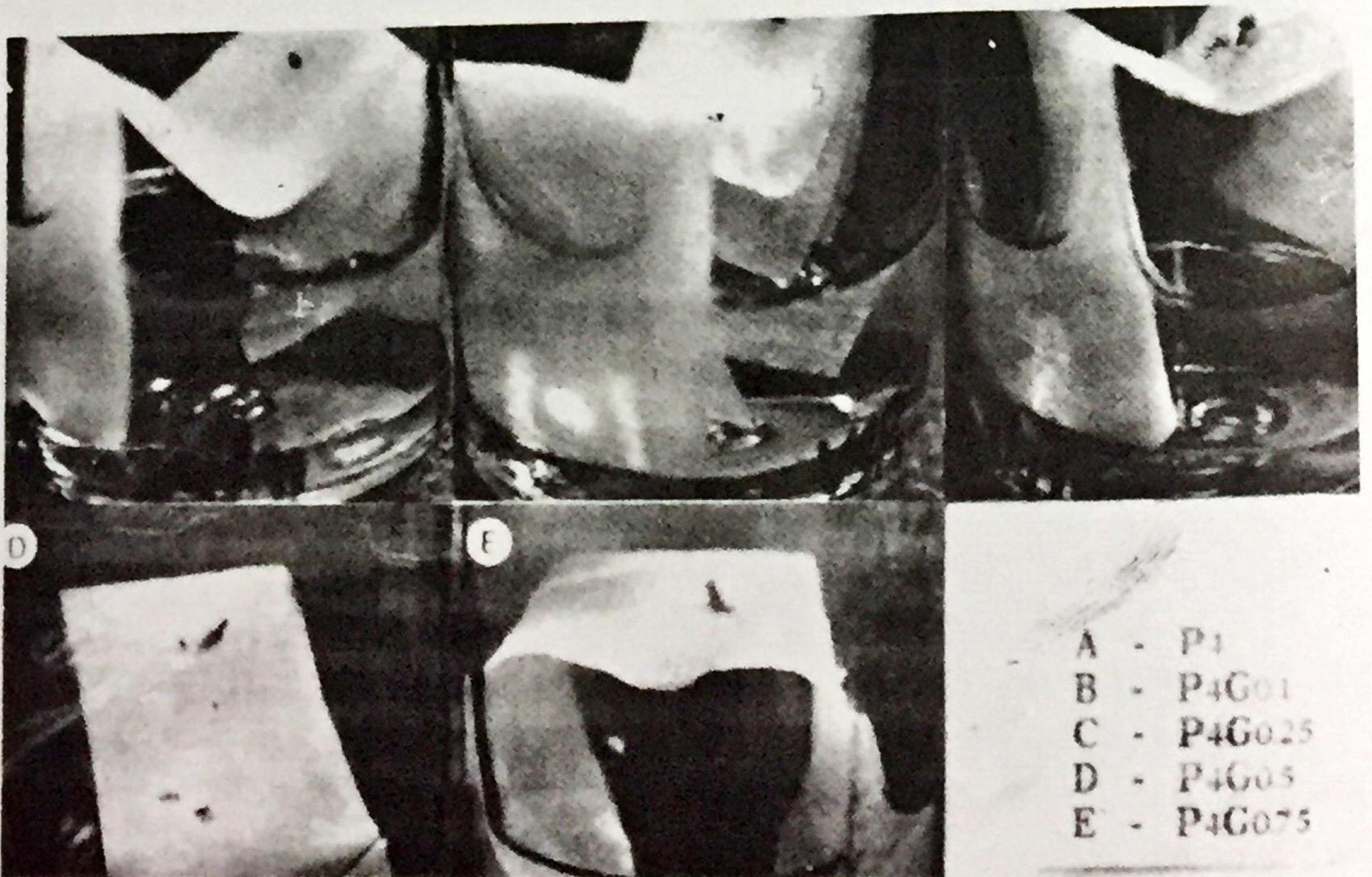

Figure 2b. VSP - 1 meristems on MS liquid medium supplemented with different levels of GA_3 after 4 weeks in culture.

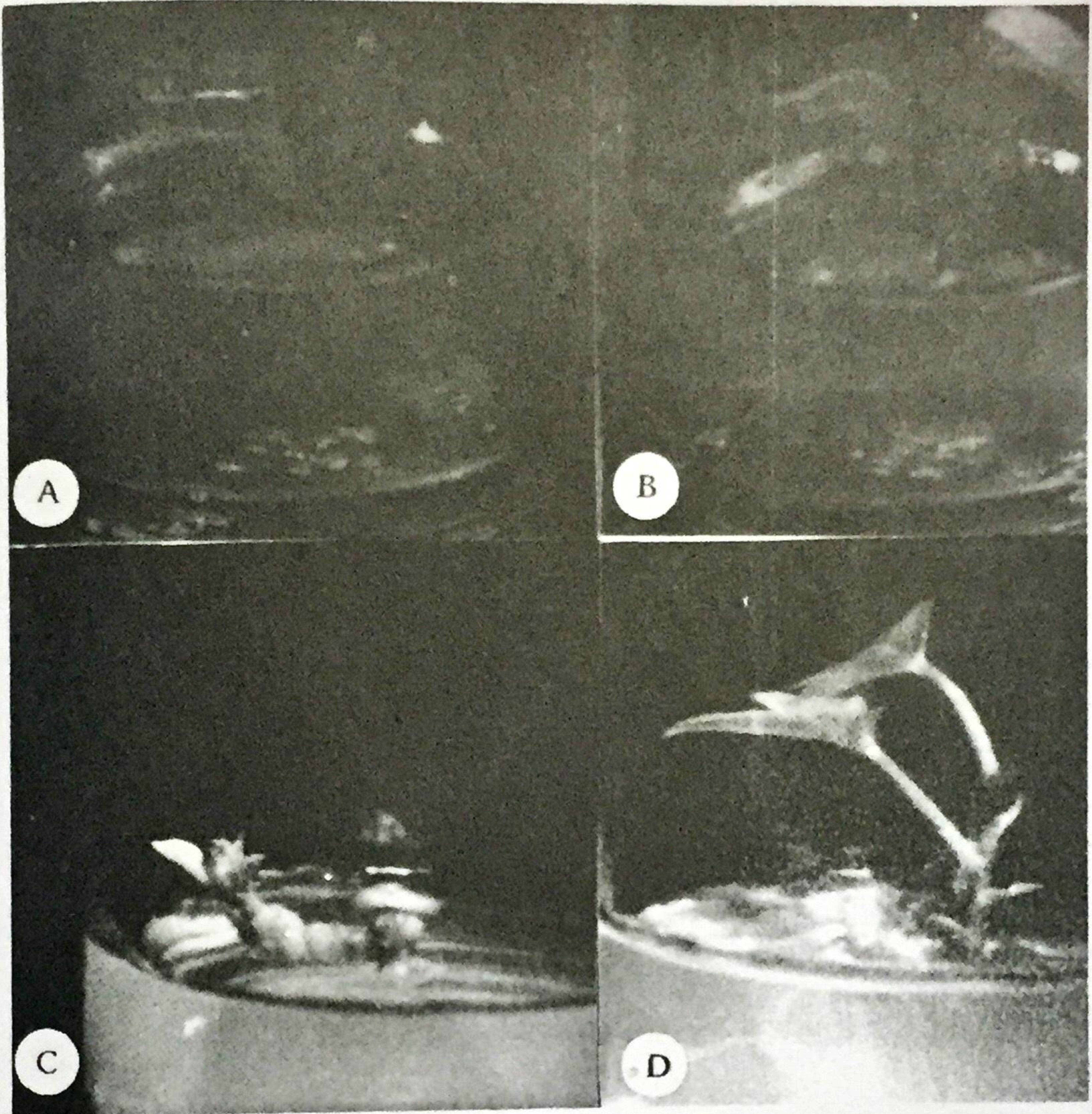

Figure 3. Stages of growth of cultured meristem. (a) growing bud from meristem transferred from liquid to regeneration medium, (b) 2 weeks after transfer, (c) development of the growing bud from meristem 1 month after transfer to solid medium with the same composition as the liquid medium, and d) 2 weeks after transfer to regeneration medium.

agar used to gel the medium may contain toxic components that can slow down shoot development (Snir and Erez, 1980). However, all meristems failed to form roots on liquid medium (Table 2). They developed roots only when transferred to the solid MS basal medium containing high sucrose concentration (Fig. 3). It was also observed that transferring the growing meristems from the liquid medium to the sucrose-enriched solid MS medium induced rapid shoot and root development.

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