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Contents

Culture of *Musa chiliocarpa* Back. in Murashige and Skoog Liquid Medium For Shoot Proliferation and Cell Types

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Abstract

The selected callus from modified MS solid medium were transferred into MS liquid medium for determination of shoot proliferation and cell types of *Musa chiliocarpa* Back. The MS liquid medium for callus proliferation was composed a combination of plant growth regulators such as auxin and cytokinin: 0.25 mg/l IAA + 1mg/l BAP, 0.25 mg/l IAA + 2mg/l BAP and 0.25 mg/l IAA + 3mg/l BAP. Similarly, 0.5 mg/l IAA and 0.75 mg/l IAA, each was mixed with 1, 2 and 3 mg/l BAP solution. The suspension MS medium supplemented with different concentrations of IAA and BAP such as 1 mg/l IAA + 1, 3 and 5 mg/l BAP; 2mg/l IAA + 1, 3 and 5 mg/l BAP and 3 mg/l IAA + 1, 3 and 5 mg/l BAP was used to determine the cell types of *M. chiliocarpa* Back. Most of cells were isodiametric and single but some were clusters, round, oval, rod, filamentous and elongated shaped.

Key words: MS medium, callus, shoot proliferation, cell types, *Musa chiliocarpa* Back.

Introduction

Banana includes a number of species and hybrids and belongs to the genus *Musa* of the family Musaceae. A family consists of 5 genera and about 150 species of wide distribution in the tropic and in the United States (Lawrence, 1951). In Myanmar, 2 genera, 15 species and 4 varieties have been stated by Hundley *et al.* (1961).

Plant tissue culture is the single of growing plant cells, tissues or organs isolated from the mother plant in an artificial media. It includes techniques and methods, appropriate to research into many botanical disciplines and several practical objectives. *In vitro* culture favors the organized and unorganized in growth. Organized growth of banana tissue *in vitro* is limited to embryo culture and shoot tip culture. *In vitro* culture of unorganized tissue in banana is almost exclusively related to the establishment of embryogenic cell culture (Sein Hla Bo, 1987).

In plant tissue culture, a small piece of plant freeing from microorganisms is used as propagule. It is also called explant. The cell

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suspension culture is a type of culture in which the multiplication of the single cell of multiplication of aggregated cells is happened, as the agitation of the liquid medium where the suspended cells consisted. The callus containing medium is agitated by rotary shaker which allowed air and liquid to mix (Sein Hla Bo, 1987). Shoot multiplication of banana is usually used for commercial practice (Imelda, 1991). Tissue culture also plays a vital role in the distribution of germplasm conservation, safe exchange of internal planting material and rapid propagation of newly selected hybrid cultivars (Gubbuk, 2004).

Materials and Methods

Cultivar of *Musa chiliocarpa* Back. (Phyee-gyan) was used for the experiment. Young suckers were collected from Vegetable and Fruit Research and Development Center (VFRDC), Yemon, Hlegu Township. This experiment was conducted during May, 2004 to January, 2007 at the farm of VFRDC.

1. Callus Culture in Liquid Medium

(a) Media Preparation

The explants from callus were placed on liquid MS medium supplemented with different concentrations and combination of BAP 1, 2 and 3 mg/l, IAA 0.25, 0.5 and 0.75 mg/l and 3% Sucrose for shoot proliferation and multiplication (Table 1). The pH of the media was adjusted to 5.8 with either 1N NaOH or 1N HCl before autoclaving. Then, the media were distributed into the 100 ml/bottle at the rate of 5 ml/bottle and autoclaved the media for 30 minutes at 121°C and 1.2 kg/cm^2.

(b) Inoculation and Incubation

The cultured bottles were maintained in the incubation room under the temperature of about 28°±2°C, light intensity 1000-2000 lux and relative humidity 30-50%.

Table 1. The different concentration of IAA and BAP and their MS liquid media for shoot multiplication

BAP \ IAA	1 mg/l	2 mg/l	3 mg/l
0.25 mg/l	Treatment 1	Treatment 2	Treatment 3
0.50 mg/l	Treatment 4	Treatment 5	Treatment 6
0.75 mg/l	Treatment 7	Treatment 8	Treatment 9

(c) Data Collection and Statistical Analysis

Number of shoots in each treatment was recorded in every subculture. Each treatment had 5 replicates. Data on the a) number of shoot per explants b) mean shoot length per explants were recorded. Data were statistically analyzed by IRRISTAT program using complete randomized design (CRD). Least significant differences (LSD) were used to compare treatments mean at 5% level of significance.

2. Cell Suspension Culture

(a) Source of Plant Materials

Two grams of fresh weight callus which perform the best in the solid medium were transferred to the suspension cultured medium by using sterilized forceps.

(b) Procedure for Suspension Culture

The medium in suspension culture was used MS medium supplemented with the mixture of different concentration of IAA and BAP (Table 2). After preparation, the media were equally distributed to the 100 ml bottles at the rate of 10 ml/bottle. The pH of the medium was adjusted to 5.8 prior to autoclaving at 121°C and 1.2 kg/cm^2 for 30 minutes. The 0.5cm-sized calli were cultured in the media. For suspension culture agitation was made by using rotary shaker at 120 rpm. Weekly observations of suspended cells from the respective medium were done under microscope. Then, the suspended cells were photomicrographed by using microscope mounted with Olympus Digital Camera.

Table 2. The concentration of IAA and BAP used in the suspension culture

Name of Hormones	Concentration				
	1 mg/l	2 mg/l	3 mg/l	4 mg/l	5 mg/l
IAA	√	√	√	-	-
BAP	√	-	√	-	√

There are 9 treatments in the experiment.

Treatment 1 = 1 mg/l IAA + 1 mg/l BAP

Treatment 2 = 1 mg/l IAA + 3 mg/l BAP

Treatment 3 = 1 mg/l IAA + 5 mg/l BAP

Treatment 4 = 2 mg/l IAA + 1 mg/l BAP

Treatment 5 = 2 mg/l IAA + 3 mg/l BAP

Treatment 6 = 2 mg/l IAA + 5 mg/l BAP

Treatment 7 = 3 mg/l IAA + 1 mg/l BAP

Treatment 8 = 3 mg/l IAA + 3 mg/l BAP

Treatment 9 = 3 mg/l IAA + 5 mg/l BAP

(c) Data Collection

The following data were collected from the respective medium.

i. the cell shape
ii. number of cells and
iii. cell types.

Results

1. Callus Culture in Liquid Medium

The result of proliferation and shoot length showed that it respond to a mixture of different concentrations of IAA and BAP.

One month after culturing, the shoot formation was developed from the cultured callus. Among 9 treatments, Treatment 6 (T6) had 13 number

of shoot response to 0.5 mg/l IAA + 3 mg/l BAP. The roots of T6 were stronger than other combinations in *M. chiliocarpa* Back. IAA 0.25 mg/l + BAP 1 mg/l had the lowest shoot number 4.1. It was observed that BAP concentrations below 3 mg/l did not produce shoot multiplication. However, increasing IAA above 0.5 mg/l and decreasing IAA below 0.5 mg/l had reduced shoot formation. Furthermore, the same results were observed with combine effect of IAA 0.75 mg/l + BAP 2 mg/l and IAA 0.75 mg/l + BAP 3 mg/l. Therefore, IAA 0.5 mg/l + BAP 3 mg/l were increased shoot elongation (6.2 cm) compare to other combinations (Table 3).

The most shoots were produced in T6. The second best result was obtained from T3. It has 10 numbers of shoots but the shoot length was only 5.9 cm. It was concluded that T6 is the best medium for liquid culture of banana (Figure 1 and 2).

2. Cell Suspension Culture

The observation was done from 1st week after shaking the callus containing media. No distinct results were obtained until 5th week after shaking process. In the 6th week observation, the started disintegration of cells was observed. The successive week observation also showed the formation of clump cells as well as single cell was clearly formed in the medium. The result was shown in Table 4. The statistical analysis revealed that T7 had the better cell suspension in the 1st week (3100 cells) and in the 2nd week, there were 3260 cells, in the 3rd week, 3550 cells and in the 4th week, cell number increased and became 4520 cells. However, the 5th week observation had the cells of 3380 and the 6th week had 3720 numbers of cells. The second best result was obtained from T1 followed by T2. The shape of the cells observed from the suspension culture was round, oval, rod, filamentous and elongated shape. Most of the cells were isodiametric and single but some were clusters. In the suspension culture, cells were found in dividing as well as nondividing in nature. The vertical and horizontal directions of cell division were also observed from this culture.

Table 3. Mean values of number of shoot elongation and multiplication

Media (MS)	Growth regulator (mg/l)	Mean no. of shoots	Mean of shoot length
T1	IAA 0.25 mg/l + BAP 1 mg/l	4.1	6.1
T2	IAA 0.25 mg/l + BAP 2 mg/l	6.2	6.0
T3	IAA 0.25 mg/l + BAP 3 mg/l	10.3	5.8
T4	IAA 0.5 mg/l + BAP 1 mg/l	3.2	6.2
T5	IAA 0.5 mg/l + BAP 2 mg/l	7.3	6.1
T6	IAA 0.5 mg/l + BAP 3 mg/l	13.6	6.2
T7	IAA 0.75 mg/l + BAP 1 mg/l	8.5	6.1
T8	IAA 0.75 mg/l + BAP 2 mg/l	9.1	5.7
T9	IAA 0.75 mg/l + BAP 3 mg/l	9.2	5.8
	F Test	**	ns
	CV%	3.0	3.7
	LSD 5%	0.3010	-

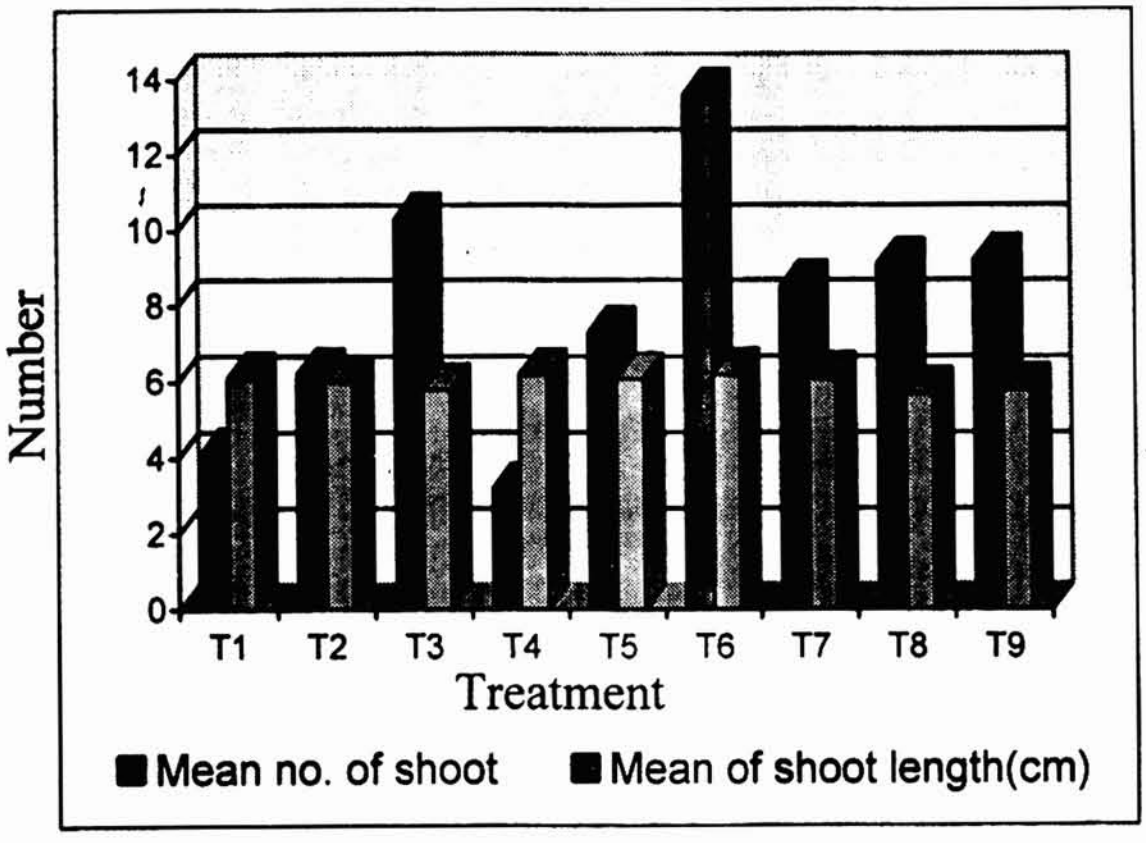
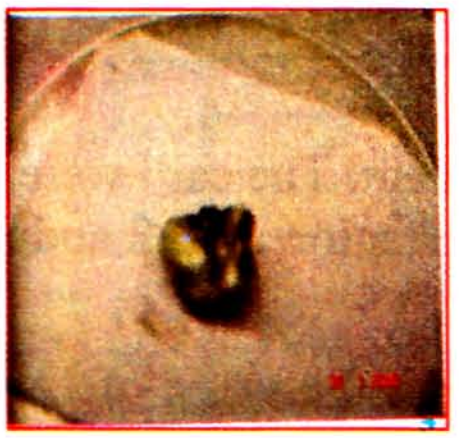


Figure 1. Number of shoots elongation and multiplication

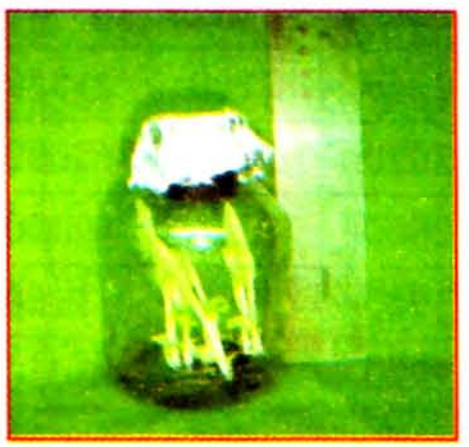


The best callus (initial)

IAA 0.5 mg/l + BAP 3 mg/l
(Shoots start formation)

Shoot elongation

IAA 0.5 mg/l + BAP 3 mg/l
(Highest shoot formation)



IAA 0.75 mg/l + BAP 3 mg/l
(Moderate shoot formation)

IAA 0.25 mg/l + BAP 1 mg/l
(Lowest shoot formation)

Figure 2. IAA+BAP shoot formation on MS liquid media

Discussion

1. Callus Culture in Liquid Medium

There are 9 treatments in this experiment. The calli were cultured on the MS basal liquid media containing a combination of 1, 2 and 3 mg/l BAP and 0.25, 0.5 and 0.75 mg/l IAA.

The experimental results indicated that the types of IAA and BAP and their concentration were significantly influenced shoot multiplication. In this experiment, the supplementation of IAA 0.5 mg/l + BAP 3 mg/l produced the best multiplication in *M. chiliocarpa* Back. plantlets. The result agreed with Gubbuk (2004) who reported that moderate concentration of BAP (below 3 mg/l) increased the shoot proliferation rate but very high concentration (above 3 mg/l) inhibited the multiplication and especially depressed shoot elongation. The combined effect of BAP and IAA increased shoot formation compared to BAP alone. A similar result was obtained by *in vitro* propagation of some new banana types *Musa spp*.

Table 4. Effect of various treatments in cell number of suspension culture

	Increased cell number					
Treatment	5th week	6th week	7th week	8th week	9th week	10th week
T1	1800	1920	2160	2240	2100	1850
T2	1860	2220	2480	3050	2900	2000
T3	1840	2170	2530	2860	2760	2370
T4	2900	3120	3430	3940	3640	3160
T5	2170	2850	3340	3920	3580	3050
T6	2530	2633	2850	3260	3200	2950
T7	3100	3260	3550	4520	3380	3220
T8	2050	2420	2860	3580	3033	3020
T9	3150	3390	3420	3670	3420	3170
F test	**	**	**	**	**	**
CV%	6.5	8.1	7.4	6.5	11.3	11.3
5% LSD	265.31	372.43	373.94	383.82	605.06	543.92

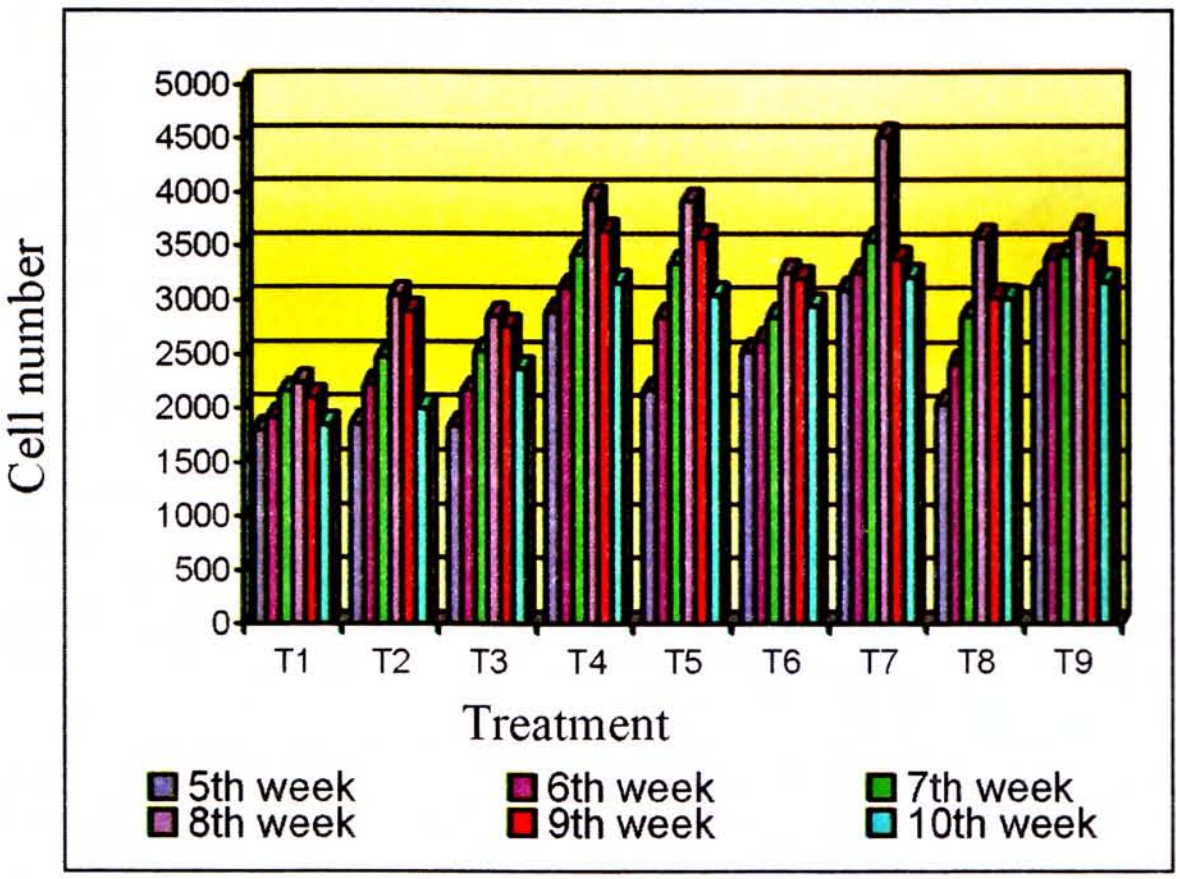


Figure 3. Effect of various treatments in cell number of suspension culture

2. Cell Suspension Culture

There are 9 treatments in this experiment. The calli were cultured on the MS basal medium supplemented with the mixture of different concentrations and combination of IAA 1, 2 and 3 mg/l and BAP 2, 3 and 5 mg/l. The result of this experiment showed that the cell separation was increased until 8^{th} week observation and then it fell in the following weeks. The separated single cells divided and formed into clumps of callus. After that, the cells were suspended and disintegrated in the medium and gradually decreased cell number was observed. It may be due to the depletion of nutrients in the cultured medium. The result of the statistical analysis showed that the cell number of the treatments were highly significant from each other. It was observed that the cells found in this experiment were various shapes and various types.

The result of this finding agreed with Khin Maung Sein (1974) who reported that the different cells in the plant can give the differences in shapes and the properties in cell culture although the differences are not significantly related to particular organs. The size of the cells from *Musa chiliocarpa* Back. was quite bigger and most of the cells were giant in size which may be one of the characters of *M. chiliocarpa* Back.

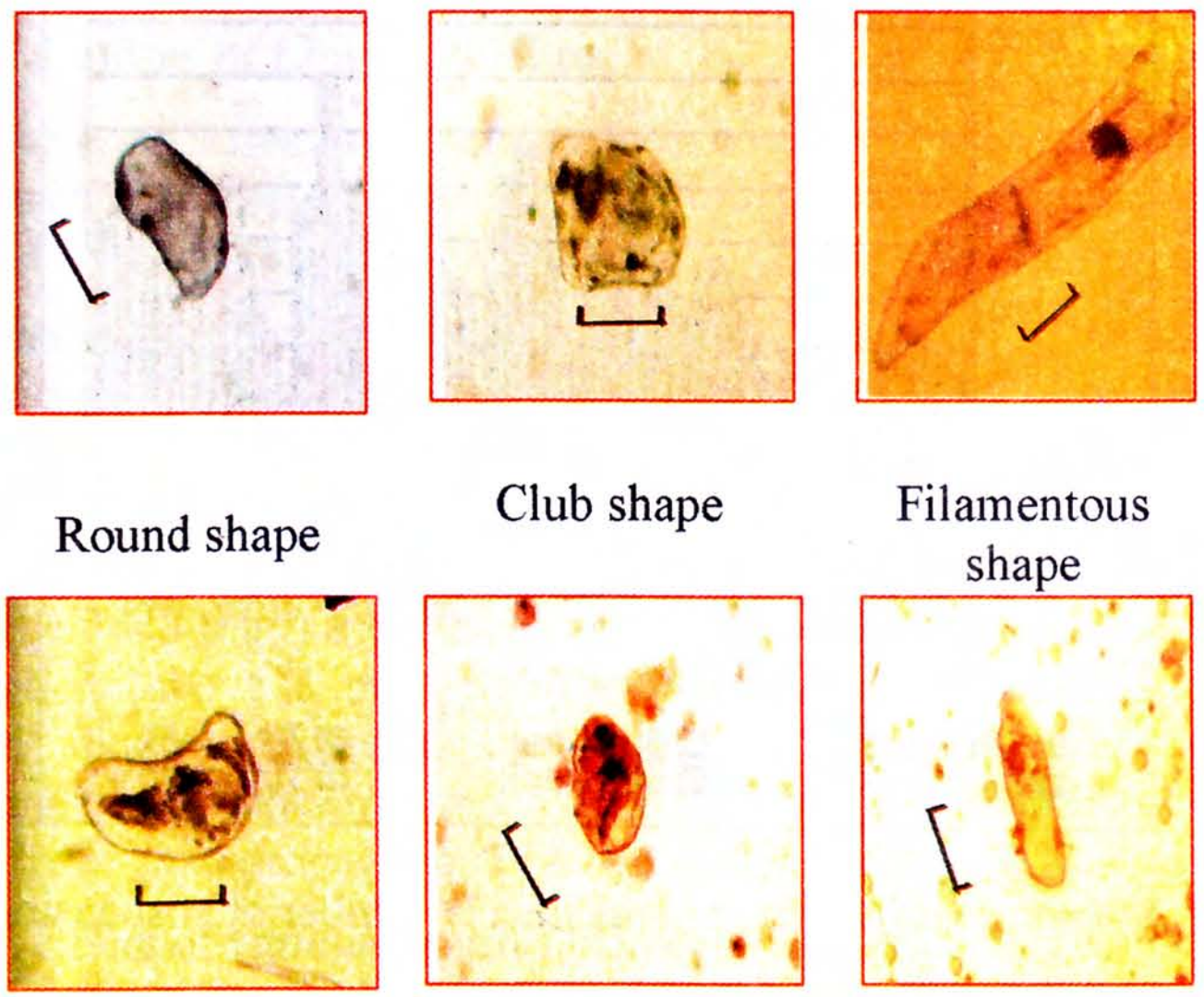
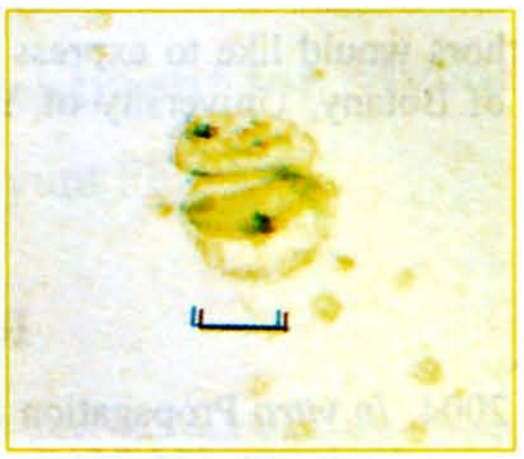


Figure 4. Oval, crescent, filamentous, round, club, elongated, rod shape and isodiametric cells from suspension culture

Dividing cell

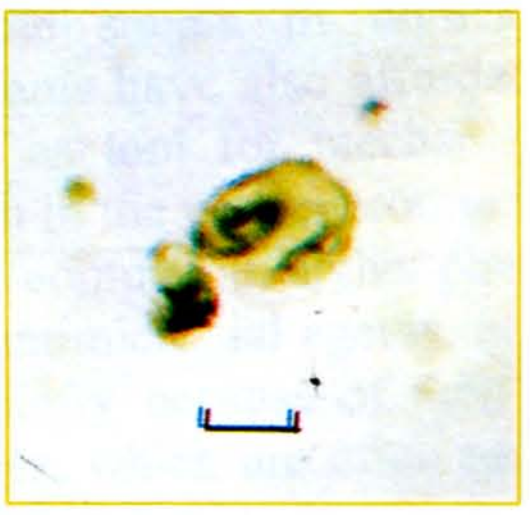


Non-dividing cell

Divided cell

Dividing cell

Dividing cell



Divided cell

Bar = 10 μ

Figure 5. Divided, non-divided and dividing cells from suspension culture

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