RESULTS

RESULTS

Surface sterilization of the explants

0.5-1 cm long sprout tips were collected for the initial establishment of *in vitro* potato shoots. They were rinsed with detergent (1%, Teepol-20) for 2-3 min and were then washed thoroughly with tap water, followed by distilled water. These tips were subsequently surface sterilized with varying concentration of $HgCl_2$ (Table:3, 4). Shoot apices (0.2-0.4 cm) were dissected and inoculated onto MS basal medium. Lowest percentage of contamination was observed when the explants were exposed to 0.08% $HgCl_2$ in case of both the varieties. However, the duration of exposure to $HgCl_2$ varied between the varieties. In case of var DR, it required only 4 min of exposure to $HgCl_2$ while var HR showed a requirement of 6 min exposures, for achieving the lowest percentage of contamination. When the concentration of $HgCl_2$ was raised from 0.08% to 0.1 and 0.2%, browning and subsequent death of explants was observed (Table : 3 and 4).

In order to minimize the contamination further the explants were given a pre-treatment with a combination of fungicides, antibiotic and activated charcoal for a period of 4-6 hours prior to their sterilization with 0.08% $HgCl_2$ (Table:5 and Table:6). This pre-treatment reduced the percentage of contamination markedly and significantly improved the percentage survival of explants.

Establishment of shoot tip culture

Pretreated and surface sterilized shoot tips (0.2-0.4 cm long), when cultured on MS basal medium, exhibited elongation and attained a length of 4-5 cm within a period of 21 days (Plate:2 and 3).

Establishment of shoots from nodal segments

The shoots obtained from above culture were cut into single nodal segments and were utilized for establishing shoot cultures.

Table-3: Effect of surface sterilization with $HgCl_2$ on the Survival Percentage of explants of var. DR.

Concentration of $HgCl_2$ (%)	Duration (min)	% of Contamination Mean ± S.E.	% of Browning Mean ± S.E.	% of Survival Mean ± S.E.
0.05	2	@100 00 ± 0.00	-	-
0.05	4	87 00 ± 0 00	-	13 00 ± 0.0
0.08	2	80 00 ± 0.00	-	20 00 ± 0.0
0.08	4	56 66 ± 3.30	-	43 33 ± 3 3
0.1	2	56.66 ± 3.30	23.33 ± 3.30	20 00 ± 0.0
0.1	4	47.00 ± 0.00	36 30 ± 3 30	16.66 ± 3.3
0.2	2	43.33 ± 3.30	43.33 ± 3 30	13.33 ± 3.3
0 2	4	40.00 ± 0.00	60 00 ± 0.00	-

@ Values indicate ± S.E. of 3 separate results

Table - 4: Effect of surface sterilization with $HgCl_2$ on the Survival Percentage of explants of var. HR.

Concentration of $HgCl_2$ (%)	Duration (min)	% of Contamination	% of Browning	% of Survival
0.05	2	@ 100 00 ± 0 00	-	-
0 05	4	96.66 ± 3.30	-	-
0.05	6	90 00 ± 0.00	-	10.00 ± 00
0 08	2	73 33 ± 3 30	-	26 66 ± 3 30
0.08	4	60.00 ± 0.00	-	40 00 ± 0.00
0 08	6	46 66 ± 3.30	-	53 33 ± 3.30
0.1	2	60.00 ± 0 00	13.33 ± 3.30	26.66 ± 3.30
0.1	4	50.00 ± 0 00	26.66 ± 3 30	23 33 ± 3.30
0.1	6	46.66 ± 3 30	50 00 ± 0 00	13.33 ± 3.30
0.2	2	16.66 ± 3.30	63 33 ± 3 30	20 00 ± 0 00
0 2	4	3.66 ± 3 30	96.33 ± 3.30	-
0.2	6	-	100.00 ± 0.00	-

@ Values indicate ± S.E. of 3 separate results

Table-5: Survival response of explants to pre-treatment with various agents. var. DR

Treatment	Duration (hours)	% of Contamination Mean ± S.E.	% of Browning Mean ± S.E.	% of Survival Mean ± S.E.
Control	0	@58 33 ± 0.00	-	41.66 ±0 00
0.2% Rifampicin + 0 2% Bavistın + 0.1% Actıvated Charcoal	2	52 33 ± 0.00	-	47.44 ± 2.70
	4	44.44 ± 2.70	-	55.55 ± 2 70
	6	34.44 ± 2 70	28.88 ± 2.70	36 66 ± 0 00
	8	21.33 ± 2 70	78 66 ± 2 70	-
0.1% Rifampicın+ 0.1% Chloramphenicol+ 0 2% Bavistin+ 0.1% Actıvated Charcoal	2	50 00 ± 0.00	-	50 00 ± 0 00
	4	45.00 ± 0 00	-	55.00 ± 0.00
	6	34.44 ± 2.70	26.10 ± 2.70	39.40 ± 2 70
	8	11.70 ± 0.00	88.30 ± 0 00	-
0.2% Chloramphenicol+ 0.2% Bavistin+ 0 1% Activated Charcoal	2	37.00 ± 0.00	-	62.77 ± 2.70
	4	13 88 ± 2.70	-	86.10 ± 2.70
	6	20.00 ± 0 00	20.55 ± 2.70	59.55 ± 2 70
	8	14.55 ± 0.00	85.55 ± 2.70	-

*(Pre-treated explants were surface sterilized wıth 0 08% HgCl2 before inoculation).

@ Values indicate ± S.E of 3 separate results

Table - 6: Survival response of explants to pre-treatment with various agents. var. HR

Treatment	Duration (Hours)	% of Contamination Mean ± S.E.	% of Browning Mean ± S.E.	% of Survival Mean ± S.E.
Control	0	@ 46.66 ± 3.30	-	53 33 ± 3 30
0.2% Rifampicin+	2	38.33 ± 2.70	-	61 66 ± 0 00
0.2%Bavistin+	4	35.66 ± 0.00	-	64 44 ± 2.70
0.1%Activated Charcoal	6	14.33 ± 4.80	13.33 ± 0.00	72 21 ± 5 50
	8	12.22 ± 2.70	65.66 ± 0 00	22 21 ± 5.50
0.1% Rifampicin+	2	40.00 ± 0.00	-	60 00 ± 0.00
0.1% Chloramphenicol+	4	37.77 ± 2.70	-	62.21 ± 5.50
0.2% Bavistin+	6	9.33 ± 2.70	25.00 ± 0.00	66 66 ± 0 00
0.1% Activated Charcoal	8	-	52.00 ± 0.00	47 22 ± 2.70
0.2% Chloramphenicol+	2	32.66 ± 4.80	-	67.22 ± 2 70
0 2% Bavistın+	4	24.66 ± 2 70	-	75 55 ± 2.70
0 1% Activated Charcoal	6	-	6 66 ± 0 00	93.33 ± 0 00
	8	-	47 22 ± 2.70	52.77 ± 2 70

*(Pre-treated explants were surface sterilızed with 0.08% HgCl2 before ınoculation).

@ Values indicate ± S.E. of 3 separate results

Table – 7: Growth response of shoots to LS and MS basal media. var. DR and var. HR (Data recorded after 21 days)

Variety	Media used	Length of the shoots (cm) Mean ± S.E.	No. of nodes / Shoots Mean ± S.E.
DR	LS Medium	1.35 ± 0.19	2 28 ± 0.28
	MS Medium	4.16 ± 0.56	3.11 ± 0.26
HR	LS Medium	1 50 ± 0.22	2 25 ± 0.25
	MS Medium	4.80 ± 0.43	3 00 ± 0.42

Plate 2. Establishment of dissected shoot tips of var DR in MS basal medium after surface sterilization:

a. Initial explants (0.2 – 0.4 cm long)
b. Started rooting (within 3-4 days)
c. Establishment of *in vitro* shoot

In all figures, the bar = 1 Cm

Plate 2. Establishment of dissected shoot tips of var DR in MS basal medium after surface sterilization:

a. Initial explants (0.2 – 0.4 cm long)
b. Started rooting (within 3-4 days)
c. Establishment of *in vitro* shoot

Bar = 1 cm in all the plates

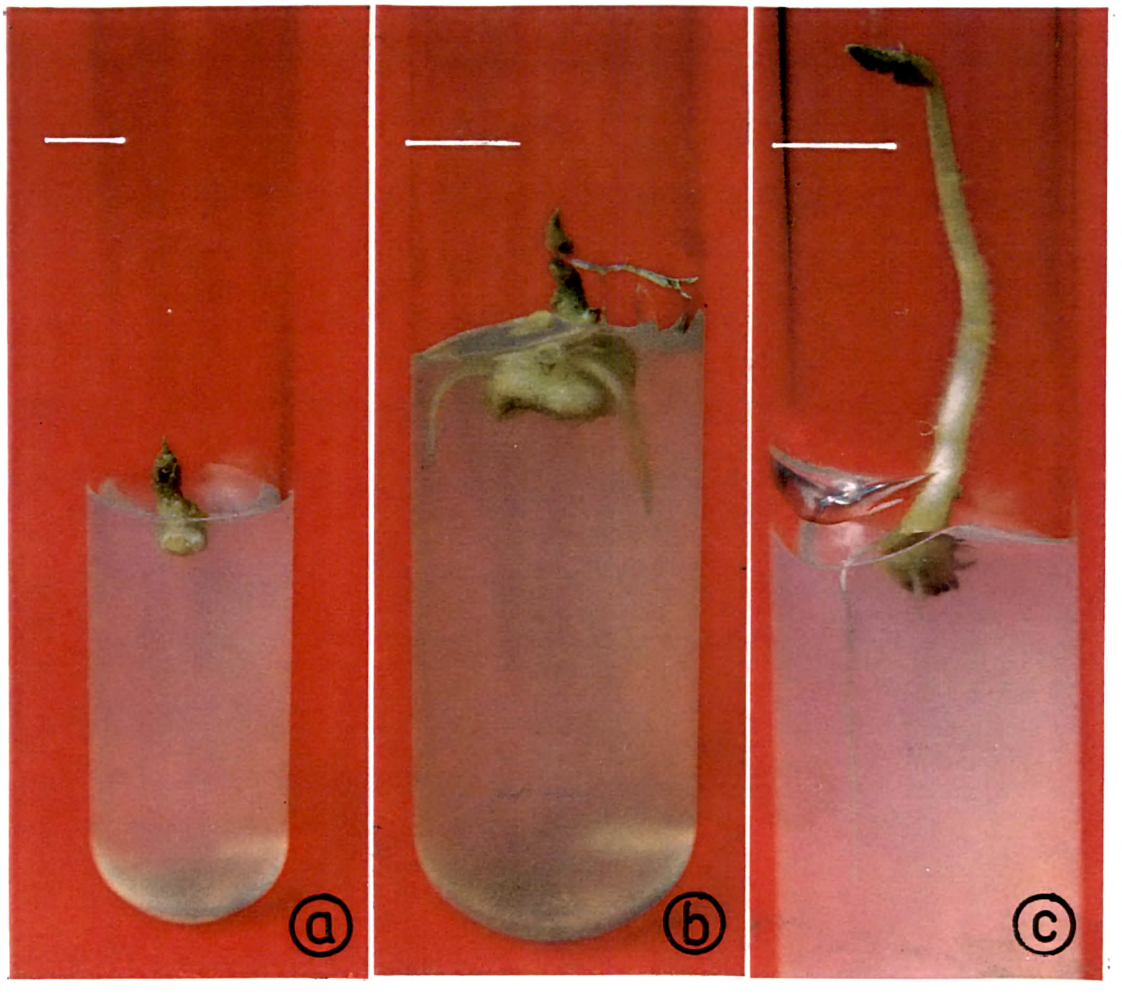
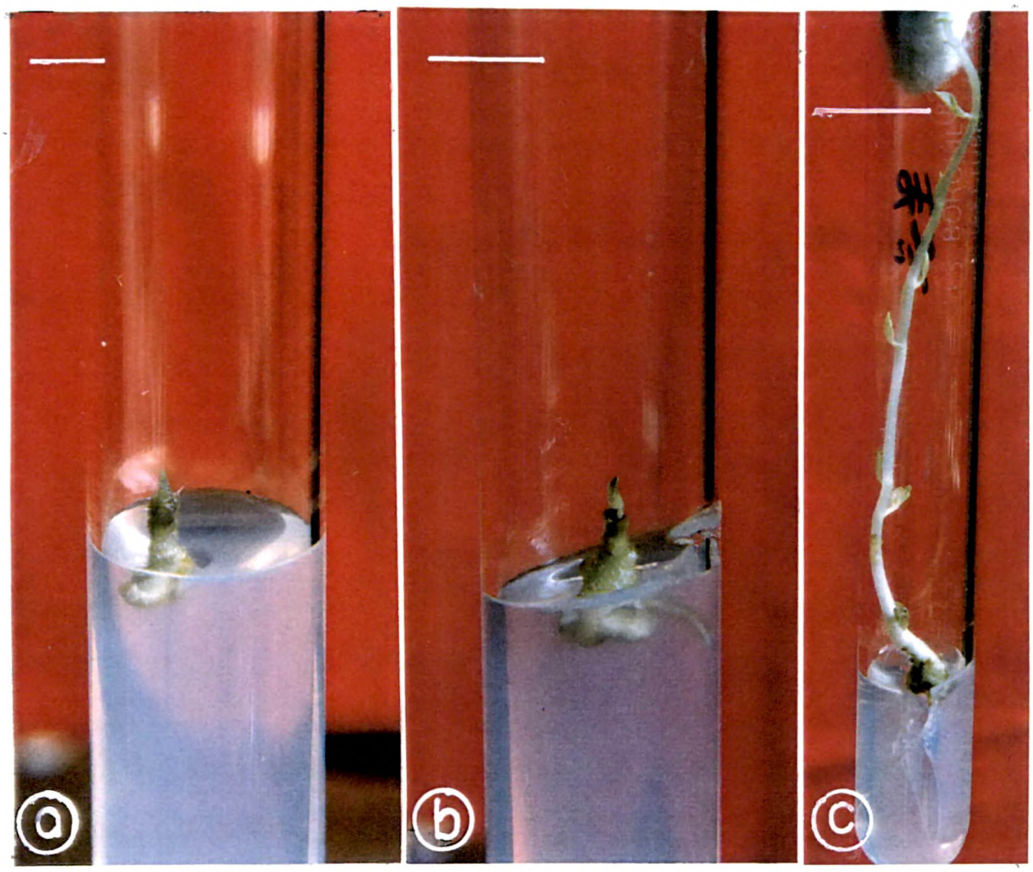

a
b
c

Plate 3. Establishment of dissected shoot tips of var HR in MS basal medium after surface sterilization:

a. Initial explants (0.2 – 0.4 cm long)
b. Started rooting (within 3-4 days)
c. Establishment of *in vitro* shoot.

In all figures, the bar = 1 Cm


a
b
c

LS and MS media were tried for establishing shoot cultures from nodal explants. Best response in the form of shoot elongation and number of nodes per shoot was observed when nodal explants were cultured on full strength MS medium (Table:7).

The effect of NAA (0.05-1.0 mg/l) and BAP (0.05-2.0 mg/l) individually and in combination on shoot elongation from nodal explants was examined. NAA alone at 0.1 mg/l significantly increased the length of shoot and number of nodes per shoot compared to the control. However, BAP individually or in combination with NAA failed to bring about any significant increase in shoot length or number of nodes per shoot. An increase in the concentration of NAA from 0.1 to 0.5 mg/l led to the production of more number of roots in both the varieties. Combinations of NAA and BAP did not show any significant effect on production of healthy shoots and leaves (Table 8 and 9).

Effect of GA_3 (0.05-0.25 mg/l), NAA (0.01-0.1 mg/l) and Ca-Pantothenate (1.0-4.0 mg/l) on growth and production of shoot from nodal explants was studied It was observed that GA_3 alone did not bring about any significant effect on length of the shoot or number of nodes per shoot, compared to the control, but a combination of GA_3, NAA and Ca-pantothenate could increase the length of the shoots significantly. In var DR, 0.05 mg/l GA_3 in combination with 0.01 mg/l NAA and 2.0 mg/l Ca-pantothenate showed significant results with respect to length of the shoots (4.0 cm), as well as mean number of nodes per shoot (3.40) compared to that of control (Table:10). Increase in concentration of GA_3 from 0.05 mg/l to 0.1 mg/l along with 0.01 mg/l NAA and 2 0 mg/l Ca-pantothenate did not have further significant effect on the mean shoot length (4.4 cm) or the mean number of nodes per shoot (3.80), but produced healthy shoots with larger green leaves. Further increase in GA_3 levels showed decrease in both the parameters in var DR

In var HR, low concentration of GA_3 (0.05 mg/l) and NAA (0.01 mg/l) along with 2.0 mg/l Ca-pantothenate, significantly improved the shoot length (3.60 cm), but had no effect on the mean number of nodes per shoot (2.20). However, an increase in the

Table - 8: Influence of BA and NAA on shoot growth from nodal explants of var. DR (Data recorded after 21 days)

Growth Regulator (mg/l)		Length of the Shoots(cm) Mean ± S.E.*	No. of Nodes/Shoot Mean± S.E.	No. of Roots/Shoot Mean ± S.E.	Size and greening of Leaves
NAA	BAP				
0	0	1.68 ± 0.36 $_{bcde}$	2.61 ± 0.52	3.33 ±1.56	Small, pale green
0.05	0	2.93 ± 0.33 $_{ab}$	3.77 ± 0.55	16.40 ± 2.94	Small, pale green
0.1	0	3.00 ± 0.46 $_{a}$	3.47 ± 0.50	28.00 ± 5.17	Small, pale green
0.5	0	2.70 ± 0.28 $_{abc}$	2.33 ± 0.21	41.00 ± 3.00	Small, pale green
1.0	0	1.96 ± 0.32 $_{abcde}$	4.81 ± 0.45	34.60 ± 9.61	Small, pale green
0	0.05	1.68 ± 0.28 $_{bcde}$	3.12 ± 0.63	0.00 ± 0.00	Small green
0	0.1	1.91 ± 0.30 $_{abcde}$	3.26± 0.49	5.33 ± 2.60	Small green
0	0.5	1.52 ± 0.26 $_{de}$	2 64 ± 0.34	1.33 ± 0.33	Medium green
0	1.0	1.46 ± 0.35 $_{de}$	3.07± 0.59	0.00 ± 0.00	Small green
0	2.0	1.57 ± 0.21 $_{de}$	2.47 ± 0.19	0.00 ± 0.00	Small green
0.05	0.05	1.52 ± 0.30 $_{de}$	2.83 ± 0.60	8.60 ± 3.02	Small green
0.05	0.1	2.02 ± 0.28 $_{abcde}$	2.00 ± 0.22	5.20 ± 1.46	Small green
0.05	0.5	1.88 ± 0.23 $_{bcde}$	1.93 ± 0.30	4.66 ± 0.88	Very small green
0.05	1.0	1.40 ± 0.33 $_{de}$	2.00 ± 0.36	0.00 ± 0.00	Very small pale green
0.05	2.0	1.52 ± 0.24 $_{de}$	2.31 ± 0.33	0.00 ± 0.00	Small pale green
0.1	0.05	0.71 ± 0.21 $_{e}$	1.66 ± 0.33	10.25 ± 2.25	Very small pale green
0.1	0.1	0.87 ± 0.16 $_{de}$	2.16 ± 0.30	9.50± 3.77	Very small pale green
0.1	0.5	1.60 ± 0.37 $_{cde}$	2.60 ± 0.42	2.66 ± 0.33	Very small pale green
0.1	1.0	2.97 ± 0.46 $_{ab}$	2.87 ± 0.40	6.33 ± 1.85	Small green
0.1	2.0	2.15 ± 0.56 $_{abcde}$	2.75 ± 0.45	12.50 ± 8.56	Small green

*Values with the same superscript letter are not significantly different at P>0.05 (Duncan's Multiple range test)

Table-9: Influence of BAP and NAA on shoot growth from nodal explants of Var HR.
(Data recorded after 21 days)

Growth Regulator		Length of the Shoots(cm) Mean ± S.E.*	No. of Nodes/Shoot Mean ± S.E.	No. of Roots/Shoot Mean ± S.E.	Size and greening of Leaves
NAA (mg/l)	BAP (mg/l)				
0	0	$1.59 \pm 0.24_{bcd}$	1.63 ± 0.24	12.80 ± 2.26	Small pale green
0.05	0	$2.09 \pm 0.33_{b}$	2.07 ± 0.26	11.40 ± 2.01	Small pale green
0.1	0	$3.21 \pm 0.39_{a}$	2.15 ± 0.31	11.00 ± 1.16	Small pale green
0.5	0	$3.02 \pm 0.41_{a}$	2.23 ± 0.26	29.00 ± 3.6	Small pale green
1.0	0	$1.88 \pm 0.25_{bc}$	1.92 ± 0.19	31.20 ± 3.24	Small pale green
0	0.05	$1.28 \pm 0.21_{bcd}$	1.44 ± 0.17	2.00± 0.40	Small pale green
0	0.1	$1.56 \pm 0.17_{bcd}$	1.50 ± 0.15	2.00 ± 0.40	Small pale green
0	0.5	$1.22\ 0.20_{bcd}$	1.44 0.17	2.00 ± 0.00	Small pale green
0	1.0	$1.29 \pm 0.22_{bcd}$	1.37 ± 0.18	1.00 ± 0.00	Small pale green
0	2.0	$1.45 \pm 0.26_{bcd}$	1.54 ± 0.20	00 ± 0.00	Small pale green
0.05	0.05	$1.60 \pm 0.33_{bcd}$	1.75 ± 0.25	8.80 ± 0.80	Very small pale green
0.05	0.1	$1.31 \pm 0.18_{bcd}$	1.66 ± 0.14	7.80 ± 2.53	Very small pale green
0.05	0.5	$1.46 \pm 0.25_{bcd}$	1.66 ± 0.23	9.00 ± 2.85	Very small pale green
0.05	1.0	$1.47 \pm 0.19_{bcd}$	1.85 ± 0.20	6.80 ± 1.98	Very small pale green
0.05	2.0	$1.40 \pm 0.18_{bcd}$	1.22 ± 0.14	7.20 ± 1.52	Very small pale green
0.1	0.05	$1.16 \pm 0.22_{bcd}$	1.60 ± 0.24	13.25 ± 3.42	Very small pale green
0.1	0.1	$1.22 \pm 0.29_{bcd}$	1.28± 0.18	11.2 ±1.93	Very small pale green
0.1	0.5	$1.04 \pm 0.21_{bcd}$	1.28 ± 0.18	12.75 ± 2.68	Very small pale green
0.1	1.0	$2.12 \pm 0.36_{b}$	2.07 ± 0.26	6.75 1.93	Very small pale green
0.1	2.0	$1.70 \pm 0.34_{bcd}$	1.80 ± 0.24	7.60 ± 1.56	Very small pale green

*Values with the same superscript letter are not significantly different at P>0.05 (Duncan's Multiple range test)

Table - 10: Effect of GA_3, NAA and Ca-pantothanate on shoot growth of Potato. Var. DR. (Data recorded after 21 days of inoculation)

Growth Regulator			Length of the Shoot(cm) Mean ± S.E.	No. of Nodes/Shoot Mean ± S.E.*	Size and greening of Leaves
GA_3 (mg/l)	NAA (mg/l)	Ca-pantothanate (mg/l)			
0	0	0	1.2 ± 0.20	1.4 ± 0.40 $_{ab}$	Small pale
0.05	0	0	1.7 ± 0.20	2.0 ± 0.31 $_{abc}$	Small pale
0.1	0	0	2.4 ± 0.24	2.2 ± 0.20 $_{abc}$	Small pale
0.25	0	0	1.2 ± 0.37	1.4 ± 0.60 $_{ab}$	Small pale
0	0.01	0	2.2±0.58	2.0±0.44 $_{abc}$	Small pale
0	0.05	0	2.2±0.20	1.6±0.24 $_{ab}$	Small pale
0	0.1	0	2.0±0.63	1.4±0.67 $_{ab}$	Small pale
0.05	0.01	0	1.8±0.48	1.6±0.50$_{ab}$	Small pale
0.05	0.05	0	3.2±0.37	2.4±0.50 $_{abc}$	Small pale
0.05	0.1	0	3.0±0.31	2.2±0.37 $_{abc}$	Small pale
0.1	0.01	0	3.4±0.50	2.6±0.50 $_{bcd}$	Small pale
0.1	0.05	0	2.6±0.24	1.6±0.24 $_{ab}$	Small pale
0.1	0.1	0	3.8±0.37	2.0±0.44 $_{abc}$	Small pale
0.25	0.01	0	1.6±0.50	1.6±0.24 $_{ab}$	Small pale
0.25	0.05	0	2.8±0.37	1.8±0.20 $_{ab}$	Small pale
0.25	0.1	0	3.0±0.77	1.8±0.37 $_{ab}$	Small pale
0.05	0.01	1	2.4±0.50	2.6±0.67 $_{bcd}$	Large Green
0.05	0.01	2	4.0±0.83	3.4±0.74 $_{cd}$	Large Green
0.05	0.01	4	2.6±0.67	2.0±0.31 $_{abc}$	Large Green
0.05	0.05	1	2.4±0.60	2.2±0.37$_{abc}$	Large Green
0.05	0.05	2	3.6±0.74	2.0±0.63 $_{abc}$	Large Green
0.05	0.05	4	2.8±0.96	1.8±0.37 $_{ab}$	Large Green
0.05	0.1	1	2.2±0.48	1.4±0.24 $_{ab}$	Large Green
0.05	0.1	2	3.6±0.81	2.4±0.24 $_{abc}$	Large Green

Table 10 Contd.....

Growth Regulator			Length of the Shoot(cm) Mean ± S.E.	No. of Nodes/Shoot Mean ± S.E.*	Size and greening of Leaves
GA$_3$ (mg/l)	NAA (mg/l)	Ca-pantothanate (mg/l)			
0.05	0.1	4	2.2±0.58	1.6±0.40 $_{ab}$	Large Green
0.1	0.01	1	3.2±0.73	1.6±0.40 $_{ab}$	Large Green
0.1	0.01	2	4.4±1.02	3.8±0.96 $_{d}$	Large Green
0.1	0.01	4	2.2±0.37	1.8±0.37 $_{ab}$	Large Green
0.1	0.05	1	2.6±0.50	1.6±0.40 $_{ab}$	Large Green
0.1	0.05	2	3.6±1.02	2.2±0.20 $_{abc}$	Large Green
0.1	0.05	4	2.6±0.85	1.8±0.48 $_{ab}$	Large Green
0.1	0.1	1	3.4±0.91	2.4±0.50 $_{abc}$	Large Green
0.1	0.1	2	3.9±0.84	1.8±0.37 $_{ab}$	Large Green
0.1	0.1	4	2.5±0.47	1.6±0.40 $_{ab}$	Large Green
0.25	0.01	1	2.9±0.64	1.8±0.37 $_{ab}$	Large Green
0.25	0.01	2	3.4±0.50	2.2±0.37 $_{abc}$	Large Green
0.25	0.01	4	2.8±0.25	1.6±0.24 $_{ab}$	Large Green
0.25	0.05	1	2.8±0.12	1.6±0.24 $_{ab}$	Large Green
0.25	0.05	2	3.1±0.33	2.6±0.24 $_{bcd}$	Large Green
0.25	0.05	4	2.1±0.29	1.6±0.24 $_{ab}$	Large Green
0.25	0.1	1	1.6±0.33	1.0±0.31 $_{a}$	Large Green
0.25	0.1	2	2.8±0.25	1.8±0.20 $_{ab}$	Large Green
0.25	0.1	4	1.5±0.22	1.2±0.20 $_{ab}$	Large Green

*Values with the same superscript letter are not significantly different at P>0.05 (Duncan's Multiple range test)

concentration of GA_3 from 0.05 to 0.25 mg/l along with 0.01 mg/l NAA and 2.0 mg/l Ca-pantothenate showed significant improvement in both mean length of the shoot (5.1 cm) and mean number of nodes per shoot (3.40), compared to the control (Table:11). The size and colour of leaves did not show any improvement in var HR under the influence of above medium (Plate : 4).

Growth of *in vitro* shoots of potato on liquid stationary culture

Nodal explants were cultured on MS liquid stationary culture medium containing IBA (0.1-2.0 mg/l), ADS (0.1-2.0 mg/l) and BAP (1,1.5 mg/l), individually and in combinations. In var DR, IBA alone did not show any significant increase in the length of the shoots or in number of nodes per shoot, but vigorous rooting was observed in all the concentrations tried. ADS alone could produce larger and greener leaves than the control, but did not show any increase in other parameters. 0.5 mg/l IBA and 0.5 mg/l ADS, in combination with 1.5 mg/l BAP showed significant increase in the mean length of the shoots (8.0 cm) as well as in the mean number of nodes per shoot (6.00) than that of control (Table:12). However, shoots were thin and unhealthy. Basal callusing was observed at higher concentration of BAP (Plate: 5 and 6).

Similar results were observed in var HR, where maximum shoot length (6.6 cm) was seen when the medium was supplemented with 1.5 mg/l BAP and 0.5 mg/l IBA (Table:13). Shoots produced in this medium were very thin and basal callusing was also observed (Plate: 7 and 8).

Growth of *in vitro* shoot as affected by a combination of GA_3 (0.05-0.25 mg/l), NAA (0.01, 0.05 mg/l), Ca-pantothenate (2.0 mg/l), Thiamine-HCl (1.0 mg/l) and Pyridoxine-HCl (1.0 mg/l) was also examined on MS liquid stationary medium. It was observed that, 1.0 mg/l Thiamine-HCl, along with 0.1 mg/l GA_3, 0.01 mg/l NAA and 2 mg/l Ca-pantothenate showed significant effect on mean shoot length (7.1 cm) as well as on mean number of nodes per shoot (5.4), than the control, in var DR (Table:14; Plate: 9).

Table - 11: Effect of GA_3,NAA and Ca-pantothanate on shoot growth of Potato. var HR.
(Data recorded after 21 days)

Rrowth Regulator			Length of the Shoot (cm) Mean± S.E.	No. of Nodes/Shoot Mean± S.E.	Nature of Leaves
GA_3 (mg/l)	NAA (mg/l)	Ca-pantothanate (mg/l)			
0	0	0	1.2±0.12	1.2±0.37	Pale leaves
0.05	0	0	2.0±0.22	1.8±0.20	Pale leaves
0.1	0	0	1.5±0.27	1.2±0.37	Pale leaves
0.25	0	0	2.2±0.20	1.4±0.24	Pale leaves
0	0.01	0	1.3±0.20	.8±0.37	Pale leaves
0	0.05	0	1.5±0.27	.6±0.40	Pale leaves
0	0.1	0	1.6±0.29	1.0±0.31	Pale leaves
0.05	0.01	0	2.2±0.12	1.6±0.24	Pale leaves
0.05	0.05	0	2.2±0.25	1.6±0.24	Pale leaves
0.05	0.1	0	2.5±0.41	2.2±0.37	Pale leaves
0.1	0.01	0	2.2±0.46	1.4±0.24	Pale leaves
0.1	0.05	0	2.8±0.64	2.4±0.50	Pale leaves
0.1	0.1	0	2.8±0.40	2.2±0.37	Pale leaves
0.25	0.01	0	3.7±0.84	2.4±0.50	Pale leaves
0.25	0.05	0	3.0±0.35	2.0±0.31	Pale leaves
0.25	0.1	0	2.8±0.46	2.0±0.44	Pale leaves
0.05	0.01	1	1.9±0.24	1.4±0.40	Large Green
0.05	0.01	2	3.6±0.91	2.2±0.80	Large Green
0.05	0.01	4	2.5±0.74	1.0±0.44	Large Green
0.05	0.05	1	2.8±0.20	2.0±0.00	Large Green
0.05	0.05	2	3.7±0.51	2.4±0.50	Large Green
0.05	0.05	4	2.7±0.33	2.2±0.37	Large Green
0.05	0.1	1	2.6±0.10	2.2±0.20	Large Green
0.05	0.1	2	3.5±1.08	2.4±0.67	Large Green
0.05	0.1	4	3.0±0.35	2.0±0.44	Large Green

Table 11 Contd...

Rrowth Regulator			Length of the Shoot (cm) Mean± S.E.	No. of Nodes/Shoot Mean± S.E.	Nature of Leaves
GA_3 (mg/l)	NAA (mg/l)	Ca-pantothanate (mg/l)			
0.1	0.01	1	3.5±0.35	2.6±0.24	Large Green
0.1	0.01	2	3.8±0.25	2.8±0.20	Large Green
0.1	0.01	4	2.9±0.18	2.2±0.20	Large Green
0.1	0.05	1	2.7±0.37	1.8±0.37	Large Green
0.1	0.05	2	4.4±0.82	3.0±0.54	Large Green
0.1	0.05	4	2.7±0.75	1.8±0.58	Large Green
0.1	0.1	1	2.4±0.36	1.2±0.37	Large Green
0.1	0.1	2	2.5±0.15	2.0±0.31	Large Green
0.1	0.1	4	2.0±0.47	1.8±0.58	Large Green
0.25	0.01	1	5.0±0.89	2.6±0.40	Large Green
0.25	0.01	2	5.1±1.08	3.4±0.40	Large Green
0.25	0.01	4	4.2±0.37	3.2±0.20	Large Green
0.25	0.05	1	3.8±0.62	3.0±0.44	Large Green
0.25	0.05	2	4.2±0.64	3.0±0.54	Large Green
0.25	0.05	4	2.2±0.40	1.6±0.40	Large Green
0.25	0.1	1	2.7±0.25	1.8±0.20	Large Green
0.25	0.1	2	3.0±0.22	1.6±0.24	Large Green
0.25	0.1	4	2.2±0.25	.8±0.37	Large Green

Table -12: Growth of *in vitro* shoots of Potato in liquid stationary culture under the influence of BA, IBA and ADS var DR (Data recorded after 21 days of inoculation)

Growth Regulator (mg/l)			Length of shoots(cm)	No. of nodes/shoot	No. of Roots/shoot	Remarks
BA	IBA	ADS	Mean ± S.E.*	Mean ± S.E.*	Mean ± S.E.*	
0	0	0	4.7 ± 0.51 bcde	2.8 ±0.20 ef	4.4 ±1.16	–
0	0.1	0	4.1 ± 0.67 cde	2.8 ±0.37 ef	13.4 ± 3.31	Vigorous rooting
0	0.5	0	7.8 ± 0.94 abc	3.5 ± 0.50 def	14.8 ± 4.87	Vigorous rooting
0	1.0	0	5.0 ± 1.03 bcde	2.4 ±0.50 f	11.0 ± 6.18	Vigorous rooting
0	2.0	0	3.6 ± 0.99e	2.3 ± 0.47 f	10.5 ± 4.94	Vigorous rooting
0	0	0.1	3.8 ± 0.81 de	2.5 ± 0.64 ef	9.0 ± 2.19	Large green leaves
0	0	0.5	4.9 ± 1.22 bcde	3.0 ± 0.54 ef	8.6 ±1.07	Large green leaves
0	0	1.0	3.8 ± 0.20 de	2.8 ± 0.20 ef	9.6 ± 1.36	Large green leaves
0	0	2.0	4.9 ± 0.84 bcde	3.0 ± 0.54 ef	7.6 ± 0.92	Large green leaves
1	0.5	0	7.5 ± 0.86 abcd	6.5 ±0.86 a	-	Basal callusing
1	0.5	0.5	6.1 ± 1.42 abcde	3.0 ± 0.57 def	-	Basal callusing
1	0.5	2.0	6.6 ± 2.33 abcde	4.0 ± 1.0 bcdef	3.0 ± 0.00	Basal callusing thin shoot
1	1.0	0	5.7 ± 1.67 bcde	3.5 ± 0.86 def	-	Basal callusing thin shoot
1	1.0	0.5	7.1 ± 1.47 abcde	5.5 ±1.25 abc	10.5 ± 1.65	Basal callusing thin shoot
1	1.0	2.0	7.1 ± 0.82 abcde	3.75 ± 0.47 cdef	7.3 ± 2.05	Basal callusing thin shoot

Table - 12 Contd...

Growth Regulator (mg/l)			**Length of shoots(cm) Mean ± S.E.***	**No. of nodes/shoot Mean ± S.E.***	**No. of Roots/shoot Mean ± S.E.***	**Remarks**
BA	**IBA**	**ADS**				
1.5	0.5	0	7.5 ± 1.51 $_{abc}$	3.2 ± 0.37 $_{def}$	-	Basal callusing thin shoot
1.5	0.5	0.5	8.0 ±1.08 $_{ab}$	6.0 ± 0.70 $_{ab}$	-	Basal callusing thin shoot
1.5	0.5	2.0	6.7 ± 1.49 $_{abcde}$	4.5 ± 0.28 $_{bcde}$	-	Basal callusing thin shoot
1.5	1.0	0	7.0 ± 1.0 $_{abcde}$	3.33 ± 0.33 $_{def}$	-	Basal callusing thin shoot
1.5	1.0	0.5	9.8 ± 0.96 $_{a}$	5.0 ± 0.40 $_{ab}$	8.8 ± 2.42	Basal callusing thin shoot
1.5	1.0	2.0	7.1 ±1.08 $_{abcde}$	4.25 ± 0.47 $_{bcdef}$	4.5 ± 2.17	Basal callusing thin shoot

*Values with the same superscript letter are not significantly different at P>0.05 (Duncan's Multiple range test)

Table -13: Growth of *in vitro* shoots of Potato in liquid stationary culture under the influence of BA, IBA and ADS var HR (Data recorded after 21 days of inoculation)

Growth Regulator (mg/l)			Length of shoots(cm)	No. of nodes/shoot	No. of Roots/shoot	Remarks
BA	IBA	ADS	Mean ± S.E.*	Mean ± S.E.*	Mean ± S.E.*	
0	0	0	1.8 ±0.25 e	1.4 ±0.24 f	2.40 ±0.67 ab	–
0	0.1	0	3.3 ±0.25 cd	2.4 ±0.24 bcdef	4.80 ±1.15 bc	Vigorous rooting
0	0.5	0	4.1 ±0.24 abcd	3.0 ±00 abcdef	9.00 ±1.22 de	Vigorous rooting
0	1.0	0	3.8 ±0.60 bcd	2.2 ±0.37 cdef	9.60 ±1.24 de	Vigorous rooting
0	2.0	0	3.0 ±0.75 d	4.0 ±1.08 a	20.6 ±2.85 f	Vigorous rooting
0	0	0.1	3.9 ±0.40 bcd	2.0 ±0.31 def	7.00 ±1.34 cd	Large green leaves
0	0	0.5	3.8 ±0.25 bcd	2.6 ±0.50 abcdef	7.60 ±0.97 cde	Large green leaves
0	0	1.0	3.6 ±0.29 bcd	2.6 ±0.24 abcdef	10.2 ±1.31 de	Large green leaves
0	0	2.0	4.3 ±0.94 abcd	2.6 ±0.60 abc	8.80 ±0.86 de	Large green leaves
1	0.5	0	5.0 ±0.65 abcd	3.6 ±0.24 ef	00.0 ±00 a	Basal callusing
1	0.5	0.5	3.5 ±0.27 bcd	1.8 ±0.20 abcdef	00.0 ±00 a	Basal callusing
1	0.5	2.0	3.7 ±1.86 bcd	3.0 ±1.0 bcdef	00.0 ±00 a	Basal callusing thin shoot
1	1.0	0	4.0 ±0.83 bcd	2.4 ±0.50 abcde	2.80 ±0.58 ab	Basal callusing thin shoot
1	1.0	0.5	6.1 ±1.07 ab	3.0 ±0.54 abcde	9.80 ±1.46 de	Basal callusing thin shoot
1	1.0	2.0	4.5 ±0.61 abcd	3.2 ±0.37 ab	10.8 ±1.59 e	Basal callusing thin shoot

Table 13 Contd....

Growth Regulator (mg/l)			**Length of shoots(cm) Mean ± S.E.***	**No. of nodes/shoot Mean ± S.E.***	**No. of Roots/shoot Mean ± S.E.***	**Remarks**
BA	**IBA**	**ADS**				
1.5	0.5	0	6.6 ±1.06 $_a$	3.8 ±0.48 $_{ab}$	00.0 ±00 $_a$	Basal callusing thin shoot
1.5	0.5	0.5	5.8 ±0.80 $_{abc}$	3.8 ±0.37 $_{ab}$	00.0 ±00 $_a$	Basal callusing thin shoot
1.5	0.5	2.0	3.9 ±0.65 $_{bcd}$	2.6 ±0.24 $_{abcdef}$	00.0 ±00 $_a$	Basal callusing thin shoot
1.5	1.0	0	4.3 ±0.83 $_{abcd}$	3 3 ±0.33 $_{abcde}$	00.0 ±00 $_a$	Basal callusing thin shoot
1.5	1.0	0.5	3.5 ±0.54 $_{bcd}$	2.7 ±0.25 $_{abcdef}$	00.0 ±00 $_a$	Basal callusing thin shoot
1.5	1.0	2.0	4.8 ±0.90 $_{abcd}$	3.4 ±0.60 $_{abcd}$	00.0 ±00 $_a$	Basal callusing thin shoot

*Values with the same superscript letter are not significantly different at P>0.05 (Duncan's Multiple range test)

Table - 14: Growth response of *in vitro* shoots from nodal explants to various additives in liquid stationary culture. var DR. (Data recorded after 21 days of inoculation)

Growth Regulator (mg/l)					Length of the Shoot (cm) Mean ± S.E.*	Number of Nodes/ Shoot Mean ± S.E.*	Remarks
GA_3	NAA	Ca-panto thanate	Thiamine -Hcl	Pyridoxin -Hcl			
0.10	0.01	2	0	0	$4.6 \pm 0.11_{abc}$	$3.0 \pm 0.01_{a}$	–
0.05	0.01	2	1.0	0	$3.9 \pm 0.18_{a}$	$3.0 \pm 00_{a}$	Large, green leaves, healthy shoots
0.1	0.01	2	1.0	0	$7.1 \pm 0\ 48_{e}$	$5.4 \pm 0.67_{c}$	Large, green leaves, healthy shoots
0.25	0.01	2	1.0	0	$5.8 \pm 0.76_{cde}$	$3.2 \pm 0.20_{ab}$	Large, green leaves, healthy shoots
0.05	0.05	2	1.0	0	$4.1 \pm 0.29_{ab}$	$2.8 \pm 0.20_{a}$	Large, green leaves, healthy shoots
0.1	0.05	2	1.0	0	$6.7 \pm 0.33_{de}$	$3.4 \pm 0.24_{abc}$	Large, green leaves, healthy shoots
0.25	0.05	2	1.0	0	$5.9 \pm 0.48_{cde}$	$3.4 \pm 0.24_{abc}$	Large, green leaves, healthy shoots
0.05	0.01	2	0	1.0	$4.5 \pm 0.41_{abc}$	$3.4 \pm 0.24_{abc}$	Medium, green leaves, healthy shoots
0.1	0.01	2	0	1.0	$5.4 \pm 0.62_{abcd}$	$3.2 \pm 0.20_{ab}$	Medium, green leaves, healthy shoots

Table 14 (Contd....)

Growth Regulator (mg/l)					Length of the Shoot (cm) Mean ± S.E.*	Number of Nodes/ Shoot Mean ± S.E.*	Remarks
GA_3	NAA	Ca-panto thanate	Thiamine -Hcl	Pyridoxin -Hcl			
0.25	0.01	2	0	1.0	$5.0 \pm 0.41_{abc}$	$3.2 \pm 0.48_{ab}$	Medium, green leaves, healthy shoots
0.05	0.05	2	0	1.0	$4.5 \pm 0.41_{abc}$	$3.2 \pm 0.20_{ab}$	Medium, green leaves, healthy shoots
0.1	0.05	2	0	1.0	$5.6 \pm 0.57_{bcde}$	$3.4 \pm 0.24_{abc}$	Medium, green leaves, healthy shoots
0.25	0.05	2	0	1.0	$4.9 \pm 0.43_{abc}$	$3.6 \pm 0.24_{abc}$	Medium, green leaves, healthy shoots
0.05	0.01	2	1.0	1.0	$4.0 \pm 0.52_{ab}$	$3.2 \pm 0.20_{ab}$	Large, green leaves, healthy shoots
0.1	0.01	2	1.0	1.0	$5.8 \pm 0.37_{cde}$	$4.2 \pm 0.37_{bc}$	Large, green leaves, healthy shoots
0.25	0.01	2	1.0	1.0	$4.9 \pm 0.48_{abc}$	$3.4 \pm 0.24_{abc}$	Large, green leaves, healthy shoots
0.05	0.05	2	1.0	1.0	$4.3 \pm 0.60_{abc}$	$3.0 \pm 0.31_{a}$	Large, green leaves, healthy shoots
0.1	0.05	2	1.0	1.0	$5.2 \pm 0.53_{abcd}$	$3.8 \pm 0.37_{abc}$	Large, green leaves, healthy shoots
0.25	0.05	2	1.0	1.0	$4.8 \pm 0.37_{abc}$	$3.4 \pm 0.40_{abc}$	Large, green leaves, healthy shoots

*Values with the same superscript letter are not significantly different at $P>0.05$ (Duncan's Multiple range test)

Plate 4. (a) Healthy shoot growth of var DR in MS semisolid medium supplemented with GA_3 (0.1 mg/l), NAA (0.01 mg/l) and Ca-pantothanate (2 mg/l).

(b) Healthy shoot growth from single node cuttings of potato var DR showing rooting and larger green leaves, established in MS semi solid medium supplemented with GA_3 (0.1 mg/l), NAA (0.01 mg/L) and Ca-pantothenate (2 mg/l).

(c) Shoot growth of potato var HR showing rooting and maximum number of nodes produced in MS semisolid medium supplemented with GA_3 (0.25 mg/l), NAA (0.01 mg/l) and Ca-pantothenate (2 mg/l).

In all figures, the bar = 1 Cm


a
b
c

Plate 5. (a) Growth of *in vitro* shoots of potato var DR in liquid stationary culture in MS medium supplemented with various levels IBA, showing thin shoot growth and vigorous rooting.

(b) Growth of *in vitro* shoots of potato var DR in liquid stationary culture on MS medium supplemented with various levels ADS and BAP, showing larger green leaves.

In all figures, the bar = 1 Cm


a
b
BOROSIL

Plate 6. (a), (b) and (c) : Growth of *in vitro* shoots of potato var DR in liquid stationary culture on MS medium supplemented with combination of BAP, IBA and ADS showing thin shoots and basal callusing

In all figures, the bar = 1 Cm

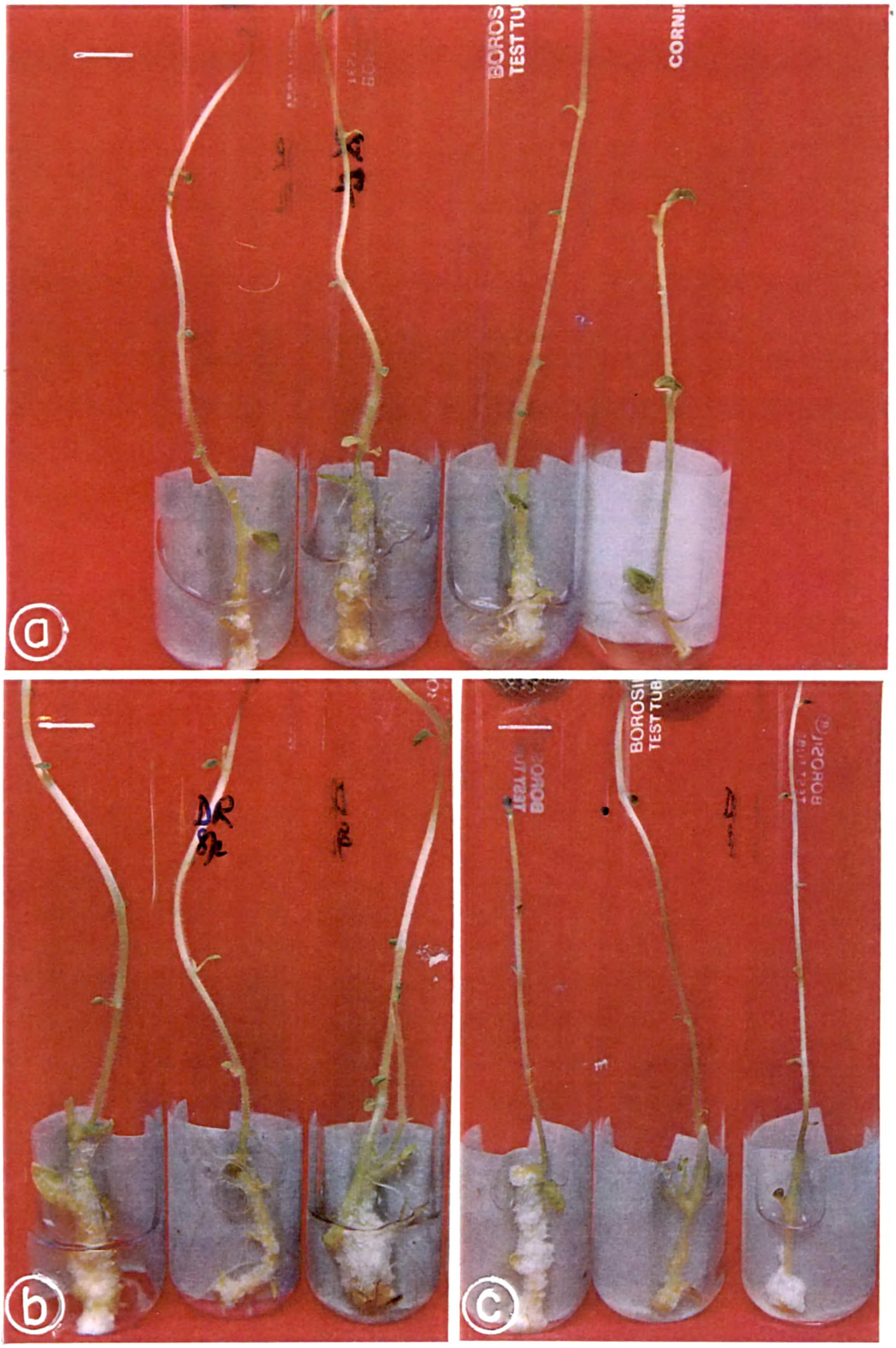

a
b
c

Plate 7. (a) Growth of *in vitro* shoots of potato var HR in liquid stationary culture on MS medium supplemented with various levels of IBA, showing thin shoots and vigorous rooting.

(b) Growth of *in vitro* shoots of potato var HR in liquid stationary culture on MS medium supplemented with various levels of ADS showing large green leaves, but less number of nodes.

In all figures, the bar = 1 Cm


a
BOROSIL®
TEST TUBE
b

Plate 8. (a) and (b) Growth of *in vitro* shoots of potato var HR in liquid stationary culture on MS medium supplemented with combination of BAP, IBA and ADS showing thin shoots and basal callusing.

In all figures, the bar = 1 Cm

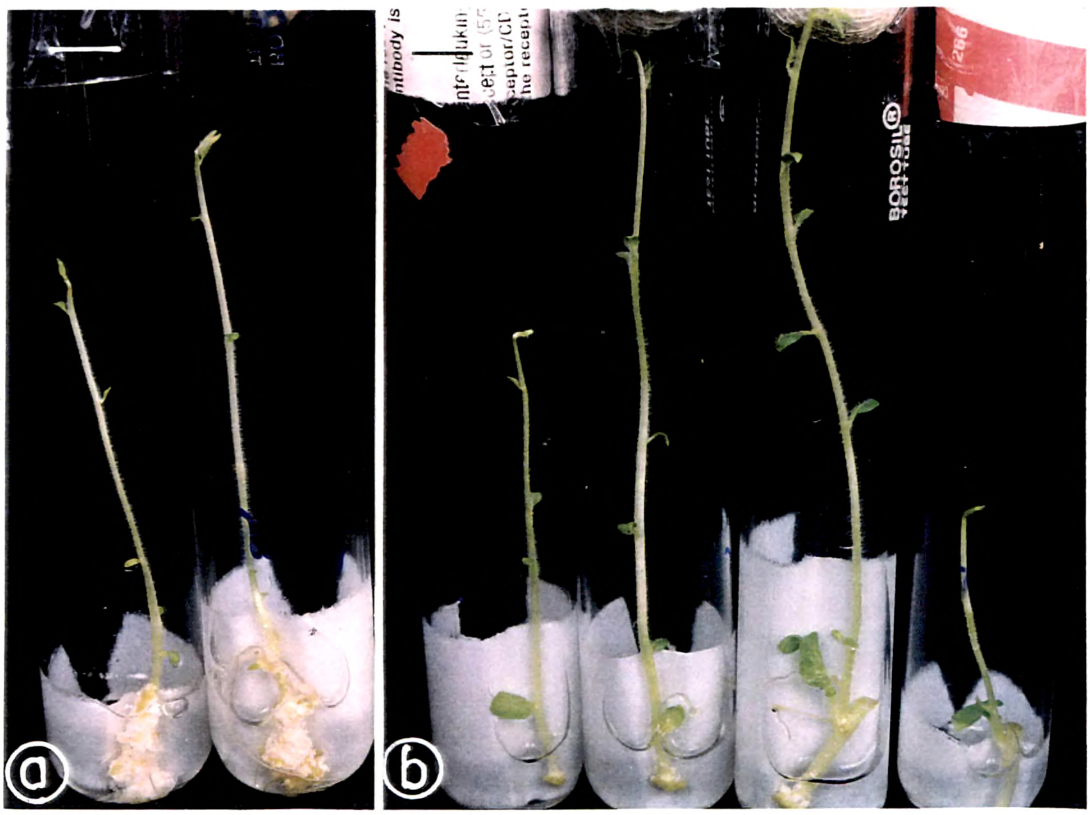
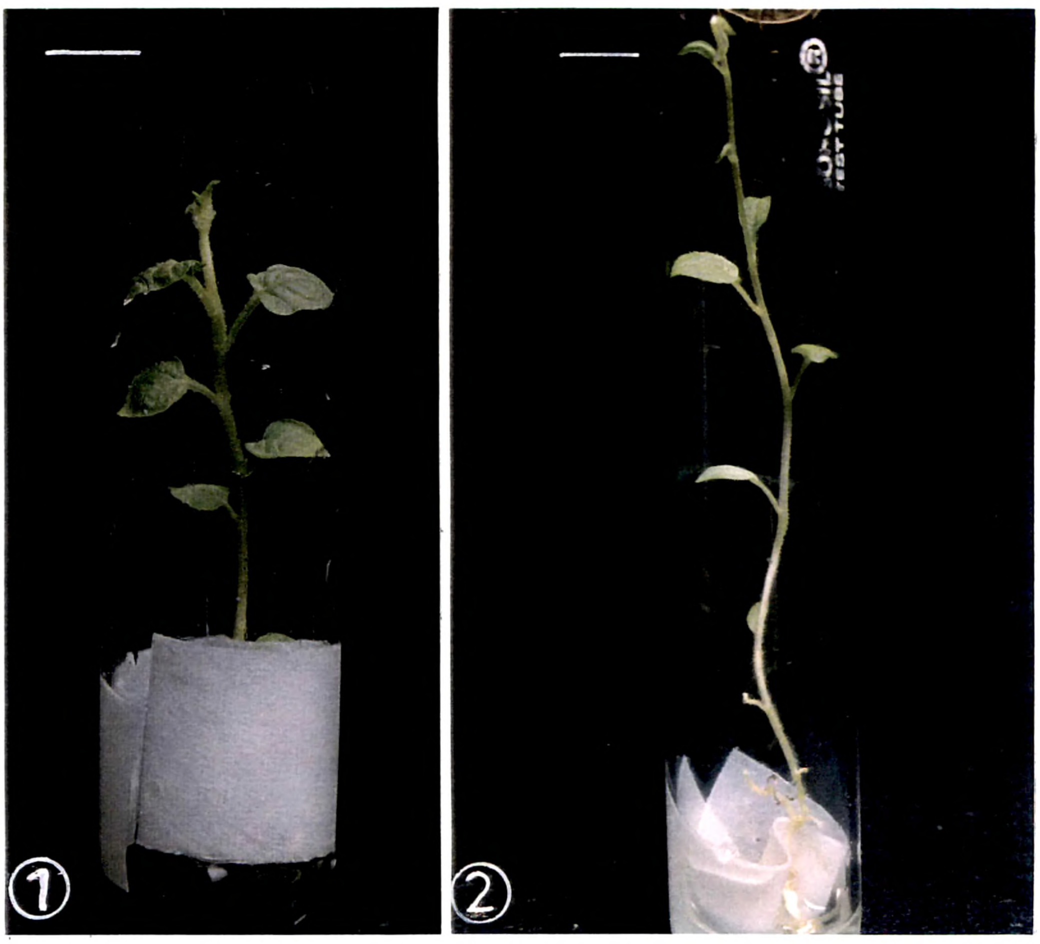

a
b

Plate 9. Growth of *in vitro* shoots of potato var DR in liquid stationary culture on MS medium supplemented with:

(a) 0.05 mg/l GA_3, 0.01 mg/l NAA, 2 mg/l Ca-pantothenate and 1.0 mg/l Thiamine-HCl.

(b) 0.1 mg/l GA_3, 0.01 mg/l NAA, 2 mg/l Ca-pantothenate and 1.0 mg/l Thiamine-HCl showing healthy shoots growth with large green leaves.

In all figures, the bar = 1 Cm


①
②

Healthy shoots with larger, green leaves were observed in this medium. Addition of Pyridoxine-HCl did not show any further increase in shoot length or number of nodes per shoot.

In var HR, maximum mean shoot length (7.2 cm) and mean number of nodes per shoot (5.6) were observed when the medium was supplemented with 1.0 mg/l Thiamine-HCl, 0.25 mg/l GA_3, 0.01 mg/l NAA and 2.0 mg/l Ca-pantothenate (Table:15). Addition of Pyridoxine-HCl did not show any further effect in this respect (Plate: 10).

Liquid medium showed better response in all aspects than the solid medium. After 21 days of inoculation, shoots reached to a height of 7.1 cm in liquid medium while on semisolid medium after 21 days it was only 4.3 cm in var DR (Table: 16) The mean number of nodes per shoot in liquid medium was 5.4 while on semisolid medium it was only 3.6 (Table·16).

Similar results were observed in var HR, where length of the shoot was 7.2 cm and 5.1 cm in liquid and semi-solid medium respectively and the mean number of nodes per shoot was 5.6 and 3.6 in liquid and semi-solid medium respectively (Table:17). Leaves were bigger, green and shoots were healthy in liquid medium.

Shoot production of potato in liquid agitated culture

Highest mean number of shoots (10.6) was obtained when pieces of cultured shoots with three nodes were grown in agitated MS liquid medium containing 0.25 mg/l GA_3, 0.01 mg/l NAA, 2.0 mg/l Ca-pantothenate and 1.0 mg/l Thiamine-HCl in var DR, where mean length of the shoots (4.48cm) and mean number of nodes per shoot (3.11) were also significantly higher, compared to the control (Table:18; Plate: 11).

In var HR, maximum mean number of shoots (8.6) with a significantly higher mean length of shoot (3.38cm) were observed when shoot segments were cultured in the medium supplemented with 0.25 mg/l GA_3, 0.05 mg/l NAA, 2.0 mg/l Ca-pantothenate and 1.0 mg/l Thiamine-HCl (Table:19). This method yielded healthy shoots with rapid growth (Plate: 12).

Table - 15: Growth response of *in vitro* shoots from nodal explants to various additives in liquid stationary culture. var HR. (Data recorded after 21 days of inoculation)

Growth Regulator (mg/l)					Length of the Shoot (cm) Mean ± S.E.*	Number of Nodes/Shoot Mean ± S.E.*	Remarks
GA_3	NAA	Ca-pantothanate	Thiamine-Hcl	Pyridoxin-Hcl			
0.25	0.01	2	0	0	$5.2 \pm 0.21_{bc}$	$4.0 \pm 0.13_{cd}$	–
0.05	0.01	2	1.0	0	$3.2 \pm 0.12_{a}$	$2.6 \pm 0.24_{a}$	Large, green leaves, healthy shoots
0.1	0.01	2	1.0	0	$5.4 \pm 1.15_{bc}$	$3.8 \pm 0.37_{bcd}$	Large, green leaves, healthy shoots
0.25	0.01	2	1.0	0	$7.2 \pm 0.66_{c}$	$5.6 \pm 0.40_{d}$	Large, green leaves, healthy shoots
0.05	0.05	2	1.0	0	$4.2 \pm 0.25_{ab}$	$3.6 \pm 0.24_{abc}$	Large, green leaves, healthy shoots
0.1	0.05	2	1.0	0	$5.4 \pm 0.48_{bc}$	$4.0 \pm 0.31_{cd}$	Large, green leaves, healthy shoots
0.25	0.05	2	1.0	0	$5.3 \pm 0.51_{bc}$	$4.0 \pm 0.31_{cd}$	Large, green leaves, healthy shoots
0.05	0.01	2	0	1.0	$4.5 \pm 0.35_{ab}$	$3.6 \pm 0.24_{abc}$	Medium, green leaves, healthy shoots
0.1	0.01	2	0	1.0	$5.3 \pm 0.64_{bc}$	$4.2 \pm 0.48_{cd}$	Medium, green leaves, healthy shoots

Table 15 (Contd..)

Growth Regulator (mg/l)					Length of the Shoot (cm) Mean ± S.E.*	Number of Nodes/Shoot Mean ± S.E.*	Remarks
GA_3	NAA	Ca-pantothanate	Thiamine-Hcl	Pyridoxin-Hcl			
0.25	0.01	2	0	1.0	$5.3 \pm 0.71_{bc}$	$3.6 \pm 0.24_{abc}$	Medium, green leaves, healthy shoots
0.05	0.05	2	0	1.0	$4.8 \pm 0.76_{ab}$	$4.0 \pm 0.63_{cd}$	Medium, green leaves, healthy shoots
0.1	0.05	2	0	1.0	$5.7 \pm 0.90_{bc}$	$3.8 \pm 0.20_{bcd}$	Medium, green leaves, healthy shoots
0.25	0.05	2	0	1.0	$6.0 \pm 0.67_{bc}$	$4.4 \pm 0.24_{cd}$	Medium, green leaves, healthy shoots
0.05	0.01	2	1.0	1.0	$4.2 \pm 0.46_{ab}$	$2.8 \pm 0.37_{ab}$	Large, green leaves, healthy shoots
0.1	0.01	2	1.0	1.0	$5.2 \pm 0.30_{bc}$	$4.0 \pm 0.00_{cd}$	Large, green leaves, healthy shoots
0.25	0.01	2	1.0	1.0	$6.0 \pm 0.27_{bc}$	$4.8 \pm 0.20_{d}$	Large, green leaves, healthy shoots
0.05	0.05	2	1.0	1.0	$5.1 \pm 0.43_{ab}$	$4.2 \pm 0.37_{cd}$	Large, green leaves, healthy shoots
0.1	0.05	2	1.0	1.0	$5.9 \pm 0.85_{bc}$	$4.0 \pm 0.31_{cd}$	Large, green leaves, healthy shoots
0.25	0.05	2	1.0	1.0	$5.5 \pm 0.47_{bc}$	$4.2 \pm 0.37_{cd}$	Large, green leaves, healthy shoots

*Values with the same superscript letter are not significantly different at P>0 05 (Duncan's Multiple range test)

Table:16. Comparison of shoot growth on solid and liquid medium. var HR (Data recorded after 21 days)

Characters observed / parameters	Solid medium Mean ± S.E.	Liquid medium Mean ± S.E.
Length of the shoots	4.3 ± 0.96	7.1 ± 0.48
Number of nodes per shoot	3.6 ± 0.46	5.4 ± 0.67
Quality of the shoot	+ +	+ + +
Leaves	Small, pale	Large, green

Table:17. Comparison between solid and liquid medium for shoot multiplication of potato. var HR (Data recorded after 21 days)

Characters observed / parameters	Solid medium Mean ± S.E.	Liquid medium Mean ± S.E.
Length of the shoots	5.1 ± 0.98	7.8 ± 0.66
Number of nodes per shoot	3.4 ± 0.40	5.6 ± 0.40
Quality of the shoot	+ +	+ + +
Leaves	Small, pale	Large, green

Table-18: Production of *in vitro* shoots from stem segments in liquid agitated culture under the influence of GA_3, NAA, Ca-pantothanate and Thiamine-HCl. var DR. (Data recorded after 21 days of inoculation)

Growth Regulator (mg/l)				No. of Shoot/flask Mean ±S.E.*	Length of Shoot Mean ± S.E.*	No. of Nodes/Shoot Mean ± S.E.*	No. of Roots/Flask Mean ± S.E.*	Remarks
GA3	NAA	Ca-pantothanate	Thiamine-HCl					
0	0	0	0	$4.4 \pm 0.60_{a}$	$1.59 \pm 0.22_{de}$	$2.81 \pm 0.22_{abc}$	$9.6 \pm 1.63_{a}$	Large, green leaves, healthy shoots
0.05	0.01	2	1.0	$4.6 \pm 0.24_{a}$	$1.10 \pm 0.18_{e}$	$2.36 \pm 0.33_{ce}$	$14.8 \pm 3.99_{ab}$	Large, green leaves, healthy shoots
0.1	0.01	2	1.0	$5.2 \pm 0.20_{ab}$	$1.82 \pm 0.19_{cde}$	$2.0 \pm 0.22_{e}$	$18.8 \pm 2.31_{bc}$	Large, green leaves, healthy shoots
0.15	0.01	2	1.0	$6.0 \pm 0.31_{bcd}$	$3.18 \pm 0.19_{a}$	$2.37 \pm 0.17_{cd}$	$24.4 \pm 2.65_{cd}$	Large, green leaves, healthy shoots
0.2	0.01	2	1.0	$6.6 \pm 0.50_{cd}$	$2.86 \pm 0.19_{a}$	$2.50 \pm 0.11_{bcd}$	$30.8 \pm 2.87_{de}$	Large, green leaves, healthy shoots
0.25	0.01	2	1.0	$10.6 \pm 0.40_{e}$	$4.48 \pm 0.20_{g}$	$3.11 \pm 0.16_{a}$	$23.2 \pm 1.80_{c}$	Large, green leaves, healthy shoots
0.05	0.05	2	1.0	$4.4 \pm 0.24_{a}$	$1.15 \pm 0.14_{e}$	$1.62 \pm 0.20_{e}$	$25.6 \pm 1.28_{cd}$	Large, green leaves, healthy shoots
0.1	0.05	2	1.0	$4.8 \pm 0.20_{ab}$	$2.08 \pm 0.27_{bcd}$	$2.23 \pm 0.21_{ce}$	$34.0 \pm 1.41_{c}$	Large, green leaves, healthy shoots
0.15	0.05	2	1.0	$5.6 \pm 0.24_{abc}$	$2.44 \pm 0.24_{abc}$	$2.47 \pm 0.22_{bcd}$	$34.8 \pm 1.06_{e}$	Large, green leaves, healthy shoots
0.2	0.05	2	1.0	$5.0 \pm 0.77_{ab}$	$2.62 \pm 0.32_{ab}$	$2.61 \pm 0.18_{abcd}$	$25.6 \pm 1.72_{cd}$	Large, green leaves, healthy shoots
0.25	0.05	2	1.0	$7.2 \pm 0.37_{d}$	$3.88 \pm 0.29_{f}$	$3.03 \pm 0.21_{ab}$	$41.6 \pm 1.80_{f}$	Large, green leaves, healthy shoots

*Values with the same superscript letter are not significantly different at P>0.05 (Duncan's Multiple range test)

Table-19: Production of invitro shoots from stem segments in liquid agitated culture under the influence of GA_3, NAA, Ca-pantothanate and Thiamine-HCl. var HR. Data recorded after 21 days of inoculation)

Growth Regulator (mg/l)				No. of Shoot/flask Mean ± S.E.*	Length of Shoot Mean ± S.E.*	No. of Nodes/Shoot Mean ± S.E.*	No. of Roots/Flask Mean ± S.E.*	Remarks
GA_3	NAA	Ca-pantothanate	Thiamine-HCl					
0	0	0	0	$4.0 \pm 0.44_{a}$	$1.8 \pm 0.26_{cd}$	$2.16 \pm 0.11_{bcd}$	$9.4 \pm 1.40_{a}$	Large, green leaves, healthy shoots
0.05	0.01	2	1.0	$4.8 \pm 0.20_{ab}$	$1.85 \pm 0.27_{cd}$	$2.30 \pm 0.13_{bcd}$	$10.4 \pm 0.67_{a}$	Large, green leaves, healthy shoots
0.1	0.01	2	1.0	$4.8 \pm 0.20_{ab}$	$2.12 \pm 0.24_{cd}$	$2.07 \pm 0.13_{cd}$	$9.0 \pm 1.78_{a}$	Large, green leaves, healthy shoots
0.15	0.01	2	1.0	$5.6 \pm 0.67_{bc}$	$2.42 \pm 0.20_{bc}$	$2.30 \pm 0.10_{bcd}$	$10.4 \pm 1.74_{a}$	Large, green leaves, healthy shoots
0.2	0.01	2	1.0	$6.2 \pm 0.37_{cd}$	$3.39 \pm 0.27_{a}$	$2.68 \pm 0.16_{ab}$	$9.2 \pm 0.86_{a}$	Large, green leaves, healthy shoots
0.25	0.01	2	1.0	$7.2 \pm 0.48_{de}$	$2.94 \pm 0.23_{ab}$	$2.72 \pm 0.14_{ab}$	$9.0 \pm 1.64_{a}$	Large, green leaves, healthy shoots
0.05	0.05	2	1.0	$5.2 \pm 0.20_{abc}$	$1.48 \pm 0.19_{d}$	$1.88 \pm 0.11_{d}$	$10.8 \pm 1.46_{a}$	Large, green leaves, healthy shoots
0.1	0.05	2	1.0	$5.6 \pm 0.24_{bc}$	$1.94 \pm 0.23_{cd}$	$2.13 \pm 0.13_{cd}$	$17.8 \pm 1.35_{b}$	Large, green leaves, healthy shoots
0.15	0.05	2	1.0	$6.0 \pm 0.31_{bcd}$	$2.33 \pm 0.27_{bc}$	$2.58 \pm 0.17_{abc}$	$20.2 \pm 2.95_{b}$	Large, green leaves, healthy shoots
0.2	0.05	2	1.0	$7.6 \pm 0.67_{ef}$	$2.41 \pm 0.20_{bc}$	$2.44 \pm 0.13_{bcd}$	$19.8\ 1.88_{b}$	Large, green leaves, healthy shoots
0.25	0.05	2	1.0	$8.6 \pm 0.40_{f}$	$3.38 \pm 0.26_{a}$	$2.90 \pm 0.17_{a}$	$28.2 \pm 3.26_{c}$	Large, green leaves, healthy shoots

*Values with the same superscript letter are not significantly different at P>0 05 (Duncan's Multiple range test)

Plate 10. Growth of *in vitro* shoots of potato var HR in liquid stationary culture on MS medium supplemented with :

(a) 0.1 mg/l GA_3, 0.01 mg/l NAA, 2 mg/l Ca-pantothenate and 1 mg/l Thiamine-HCl

(b) 0.25 mg/l GA_3, 0.01 mg/l NAA, 2 mg/l Ca-pantothenate and 1 mg/l Thiamine-HCl.

showing healthy shoot growth with large green leaves.

In all figures, the bar = 1 Cm

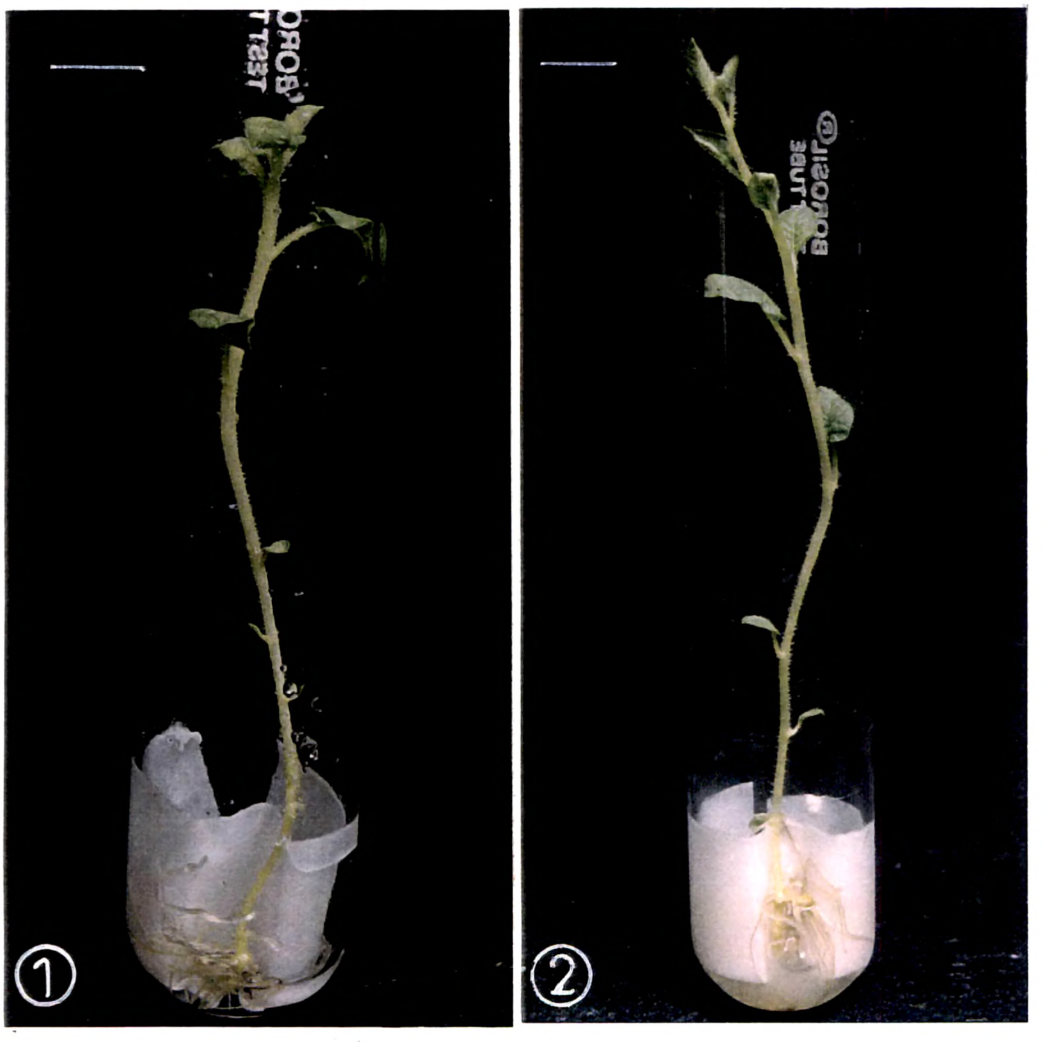

①
②

Plate 11. Shoot production of potato var DR in liquid agitated culture in MS medium supplemented with :

(a) Control – no growth regulator

(b) 0.25 mg/l GA_3, 0.01 mg/l NAA, 2 Ca-pantothenate and 1 mg/l Thiamine HCl – showing maximum number of shoots/flask.

In all figures, the bar = 1 Cm

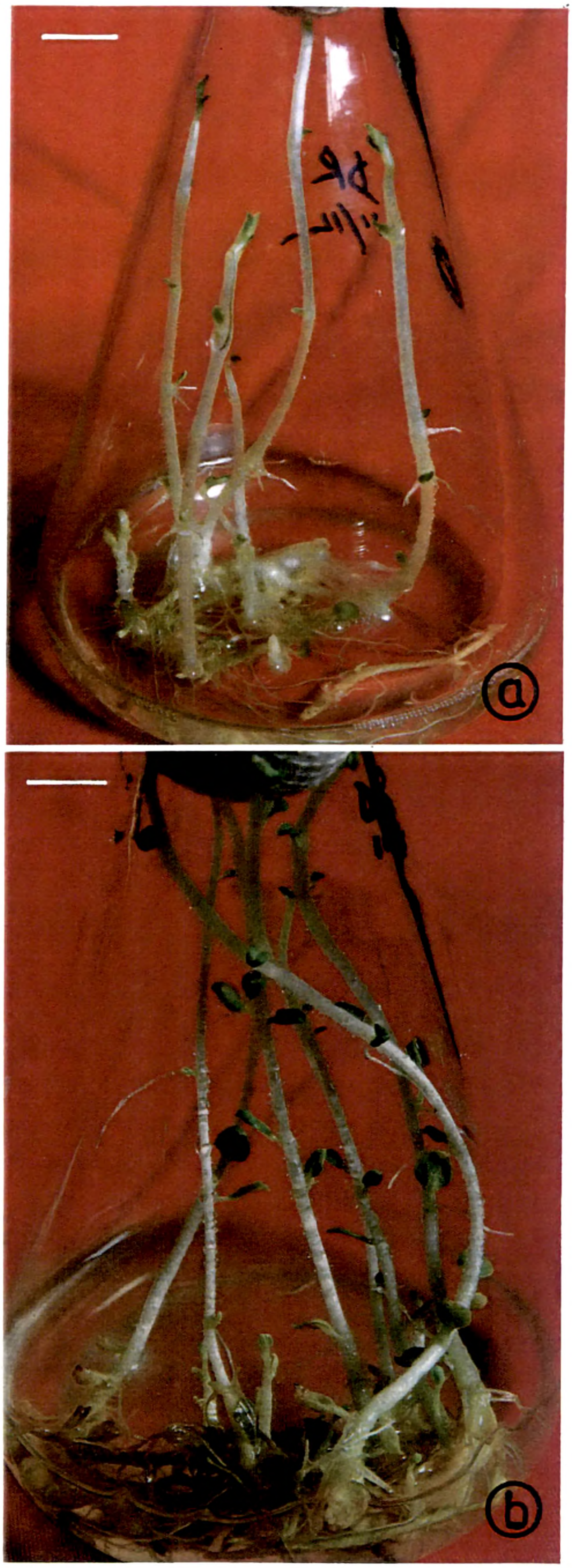
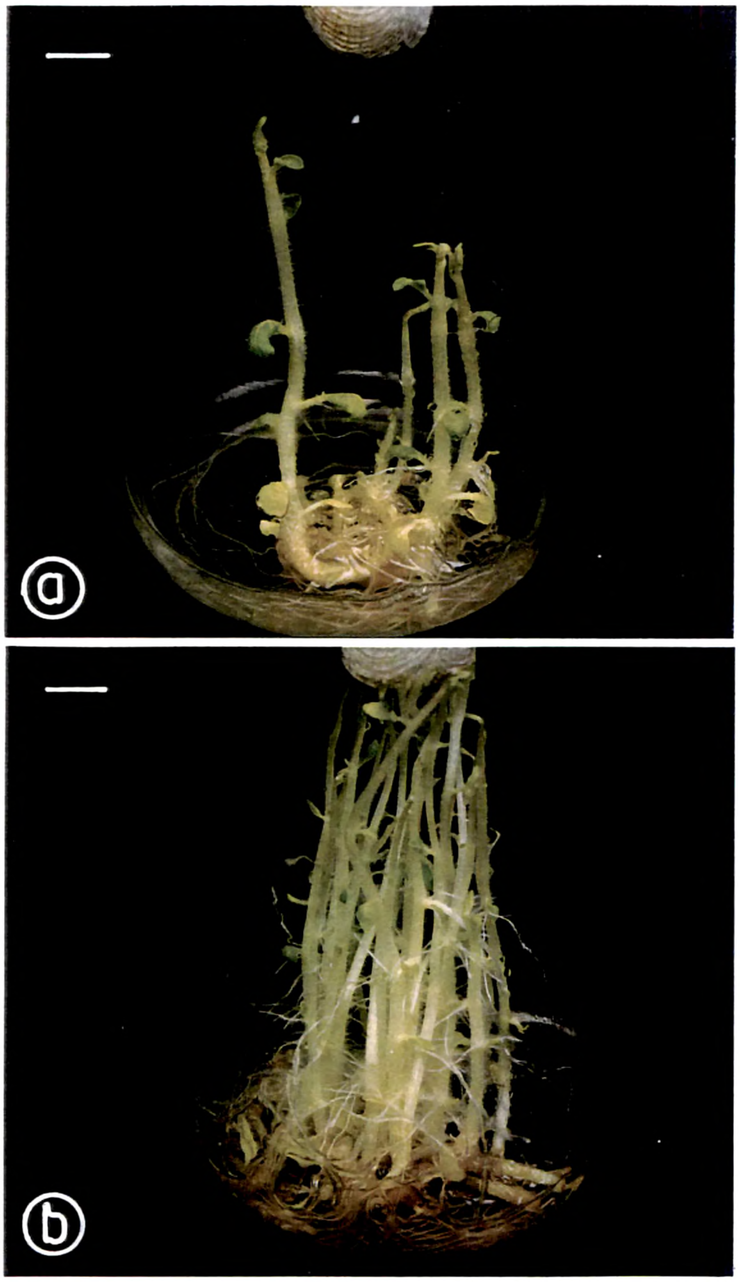

a
b

Plate 12. Shoot production of potato var HR in liquid agitated culture in MS medium supplemented with :

(a) Control – no growth regulator.

(b) 0.25 mg/l GA_3, 0.05 mg/l NAA, 2 mg/l Ca-pantothenate and 1 mg/l Thiamine-HCl – showing maximum number of shoots / flask.

In all figures, the bar = 1 Cm


a
b

Shoot production of potato, under various levels of $AgNO_3$ (0.5-20.0 mg/l) when studied, it was observed that in var DR, high concentration of $AgNO_3$ (15 mg/l and above) inhibited the shoot length. Low concentration of $AgNO_3$ (0.5 mg/l) did not bring about any increase in the number of shoots, length of shoots or number of nodes per shoot compared to control. However, the quality of the shoots was better under the influence of $AgNO_3$ as compared to that of control (Table:20). $AgNO_3$ also helped in the production of dark green leaves with increased width of leaf lamina (Plate: 13, 14 and 15).

In var HR, $AgNO_3$ at 0.5 mg/l concentration showed significant increase in the mean number of shoots per flask (10.6) as well as produced healthy green shoots with dark green and larger leaves (Table:21). Higher concentration of $AgNO_3$ decreased the length of the shoots as compared to the control.

Since the effect of high concentration of $AgNO_3$ on growth of shoots was similar to that of an anti-gibberellin (inhibitor of gibberellin biosynthesis i.e. dwarfing the potential of GA_3), GA_3 was incorporated at higher levels in the medium to reverse this effect (Table:20,21). It was interesting to note that GA_3 at 10 mg/l could subjugate the inhibitory effect of $AgNO_3$. However, in later experiments, these shoots exposed to $AgNO_3$ (10 mg/l) and GA_3 (10 mg/l) failed to produce tubers in the tuberization medium.

Microtuber Production

To optimize the sucrose concentration for tuberization, various levels of sucrose (3-12%) were tried along with BAP and CCC. In var DR 3% sucrose could not produce any tuber. An increase in the sucrose concentration from 3-8% showed positive effect on tuberization. Sucrose at 8% (w/v) level produced maximum mean number of tubers (10.66) per flask (Table: 22). The average diameter and fresh weight of tubers was 0.85 cm and 0.313 gm, respectively. Further increase in sucrose concentration did not improve the number or size of the tubers. Var HR also showed a response similar to var DR at 8% (w/v) sucrose concentration (Table:23). Hence, in all subsequent experiments on tuberization sucrose was employed at a concentration of 8% (w/v).

Table-20: Effect of $AgNO_3$ alongwith GA_3, NAA, Ca-pantothanate and Thiamine-HCl on shoot multiplication. var DR. (Data recorded after 21 days of inoculation)

Concentration of $AgNO_3$ (mg/l)	Growth Regulator (mg/l)				No. of shoots/flask Mean ± S.E.*	Length of shoot (cm) Mean ± S.E.*	No. of nodes Mean ± S.E.*	Remarks
	GA_3	NAA	Ca-panto thanate	Thiamine-HCl				
0.0	0.1	0.01	2	1.0	10.0 ±0.70 $_{ef}$	6.40 ±1.42 $_{a}$	4.70 ±0.73 $_{a}$	Large, green leaves, healthy shoots
0.5	0.1	0.01	2	1.0	9.60 ±0.50 $_{e}$	5.00 ±0.64 $_{abc}$	3.25 ±0.52 $_{abc}$	Large, dark green leaves, healthy shoots
1.0	0.1	0.01	2	1.0	5.00 ±0.31 $_{bc}$	3.00 ±1.20 $_{bc}$	4.00 ±0.57 $_{abc}$	Large, dark green leaves, healthy shoots
2.5	0.1	0.01	2	1.0	4.20 ±0.37 $_{bc}$	3.10 ±1.26 $_{bc}$	2.75 ±1.18 $_{abc}$	Large, dark green leaves, healthy shoots
5.0	0.1	0.01	2	1.0	5.60 ±0.50 $_{c}$	4.41 ±0.66 $_{abc}$	2.40 ±0.67 $_{bc}$	Large, dark green leaves, healthy shoots
10.0	0.1	0.01	2	1.0	4.40 ±0.40 $_{bc}$	1.80 ±0.46 $_{c}$	3.33 ±0.33 $_{abc}$	Large, dark green leaves, healthy shoots
15.0	0.1	0.01	2	1.0	3.60 ±0.50 $_{b}$	2.75 ±0.47 $_{bc}$	1.50 ±0.28 $_{c}$	Large, dark green leaves, healthy shoots
20.0	0.1	0.01	2	1.0	2.00 ±0.31 $_{a}$	2 25 ±0.75 $_{bc}$	1.50 ±0.50 $_{bc}$	Large, dark green leaves, healthy shoots
10.0	2.0	0.01	2	1.0	7.80 ±0.37 $_{d}$	5.50 ±0.59 $_{ab}$	3.55 ±0.41 $_{abc}$	Large, dark green leaves, healthy shoots
10.0	4.0	0.01	2	1.0	9.00 ±0.31 $_{de}$	5.25 ±0.51 $_{ab}$	3.37 ±0.53 $_{abc}$	Large, dark green leaves, healthy shoots
10.0	8.0	0.01	2	1.0	9.00 ±0.54 $_{de}$	5.20 ±0.72 $_{ab}$	3.42 ±0.29 $_{abc}$	Large, dark green leaves, healthy shoots
10.0	10.0	0.01	2	1.0	11.0 ±0.54 $_{f}$	5.77 ±0.86 $_{ab}$	4 00 ±0.57 $_{ab}$	Large, dark green leaves, healthy shoots

*Values with the same superscript letter are not significantly different at $P>0.05$ (Duncan's Multiple range test)

Table-21: Shoot production under the influence of $AgNO_3$ alongwith GA_3, NAA and Ca-pantothanate. var. HR (Data recorded after 21 days)

Concentration of $AgNO_3$ (mg/l)	Growth Regulator (mg/l)			No. of shoots/flask Mean ± S.E.*	Length of shoot (cm) Mean ±S.E.*	No. of nodes Mean ± S.E.*	Remarks
	GA_3	NAA	Ca-pantothanate				
0	0.1	0.01	2	$8.2 \pm 0.48_d$	$5.625 \pm 1\ 68_{ab}$	$5.0 \pm 1.13_a$	–
0.5	0.1	0.01	2	$10.6 \pm 0.50_f$	$7.83 \pm 0.53_a$	$4.41 \pm 0\ 58_{abc}$	Large green leaves, healthy shoots
1.0	0.1	0.01	2	$7.8 \pm 0.37_d$	$5.75 \pm 0.77_{ab}$	$4.37 \pm 0.62_{abc}$	Large green leaves, healthy shoots
2.5	0.1	0.01	2	$7.8 \pm 0.37_d$	$4.37 \pm 1.14_{bc}$	$3.37 \pm 0.96_{abc}$	Large green leaves, healthy shoots
5.0	0.1	0.01	2	$4.8 \pm 0.80_c$	$2.0 \pm 0.25_c$	$2.0 \pm 0.00_{abc}$	Large green leaves, healthy shoots
10.0	0.1	0.01	2	$4.4 \pm 0.60_{bc}$	$2.2 \pm 0.48_c$	$2.0 \pm 0.00_{abc}$	Large green leaves, healthy shoots
15.0	0.1	0.01	2	$3.2 \pm 0.37_b$	$3.33 \pm 0.33_{bc}$	$2.33 \pm 0.33_{abc}$	Plants height inhibited
20.0	0.1	0.01	2	$1.2 \pm 0.20_a$	$1.5 \pm 00_c$	$3.0 \pm 00_{abc}$	Plants height inhibited
10.0	2.0	0.01	2	$3.4 \pm 0.24_{bc}$	$7.33 \pm 0.66_{ab}$	$4.0 \pm 00_{abc}$	–
10.0	4.0	0.01	2	$8.6 \pm 0.50_{de}$	$4.11 \pm 0.37_{bc}$	$3.25 \pm 0.36_{abc}$	–
10.0	8.0	0.01	2	$8.6 \pm 0.50_{de}$	$5.72 \pm 0.77_{ab}$	$3.71 \pm 0.28_{abc}$	–
10.0	10.0	0.01	2	$9.8 \pm 0.58_{ef}$	$5.45 \pm 0.85_{ab}$	$4.57 \pm 0.71_{ab}$	–

*Values with the same superscript letter are not significantly different at $P>0.05$ (Duncan's Multiple range test)

Table-22: Effect of varying levels of Sucrose along with 5mg / l BA and 500 mg/l CCC on microtuber production of potato. var. DR (Data recorded after 60 days)

Sucrose concentration (%)	No. of tubers			Total no. of tubers Mean S.E.*	Average diameter (cm) Mean± S.E.*	Average fresh weight (gm) Mean S.E.*	Total diameter (cm) Mean S.E.*	Total fresh weight (gm) Mean S.E.*
	A*	B*	C*					
3	0.00±0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00±0.00	0.00±0.00	0.00 ± 0.00
6	0.00 ± 0.00	0.00 ± 0.00	2.33 ± 0.23 $_{ab}$	2.33 ± 0.33 $_a$	0.78 ± 0.01$_a$	0.176±0.00$_a$	1.83 ± 0.28 $_a$	0.40 ± 0.04 $_a$
8	0.66 ± 0.33 $_a$	4.00 ± 0.57 $_b$	6.00 ± 0.00 $_b$	10.66 ± 0.66 $_c$	0.85±0.01$_b$	0.313±0.01$_b$	9.13 ± 0.68 $_c$	3.35 ± 0.33 $_b$
10	0.00 ± 0.00	2.66 ± 0.33 $_a$	2.60 ± 0.33 $_b$	5.33 ± 0.33 $_b$	0.86±0.02$_b$	0.295±0.01$_b$	4.60 ± 0.32 $_b$	1.57 ± 0.12 $_a$
12	0.00±.0.00	0.00 ± 0.00	1.66 ± 0.33 $_a$	1.66 ± 0.33 $_a$	0.75±0.02$_a$	0.207±0.01$_a$	1.23 ± 0.21 $_a$	0.35 ± 0.08 $_a$

*Values with the same superscript letter are not significantly different at P>0.05 (Duncan's Multiple range test)

Table-23: Effect of varying levels of sucrose along with BAP (5 mg/L) and CCC (5 mg/L) on microtuber of potato. var. HR (Data recorded after 60 days)

Sucrose concentration (%)	No. of tubers			Total no. of tubers Mean S.E.*	Average diameter (cm) Mean± S.E.*	Average fresh weight (gm) Mean S.E.*	Total diameter (cm) Mean S.E.*	Total fresh weight (gm) Mean S.E.*
	A	B*	C*					
3	0.00	0.00 $_a$	0.00 $_a$	0.00 $_a$	0.00 $_a$	0.00 $_a$	0.00 $_a$	0.00 $_a$
6	0.00	0.00 $_a$	6.75±.47 $_d$	6.75±.47 $_c$	.746±.00 $_b$	.215±.00 $_b$	5.052±. 31 $_c$	1.455±.09 $_c$
8	1.0±00	3.25±.47 $_b$	6.25±.47 $_d$	10.50±.50 $_d$	.855±.06 $_b$	.329±.03 $_d$	8.925± .56 $_d$	3.420±.24 $_d$
10	0.00	2.5±.28 $_b$	4.25±.47 $_c$	6.75±.47 $_c$	.816±.02 $_b$	.30±.00 $_{cd}$	5.475± .22 $_c$	2.02±.12 $_c$
12	0.00	0.00 $_a$	1.50±.28 $_b$	1.50±.28 $_b$	.875±.22 $_b$	.262±.00 $_{bc}$	1.175±.21 $_b$	.388±.06 $_b$

*Values with the same superscript letter are not significantly different at P>0.05 (Duncan's Multiple range test)

Plate 13. Shoot production of potato var DR in liquid agitated culture in MS medium supplemented with –

(a) Control - 0.25 mg/l GA_3, 0.01 mg/l NAA, 2 mg/l Ca-pantothenate and 1 mg/l Thiamine-HCl.

(b) 0.25 mg/l GA_3, 0.01 mg/l NAA, 2 mg/l Ca-pantothenate, 1 mg/l Thiamine-HCl and 0.5 mg/L $AgNO_3$ – showing healthy shoot growth with larger green leaves.

In all figures, the bar = 1 Cm

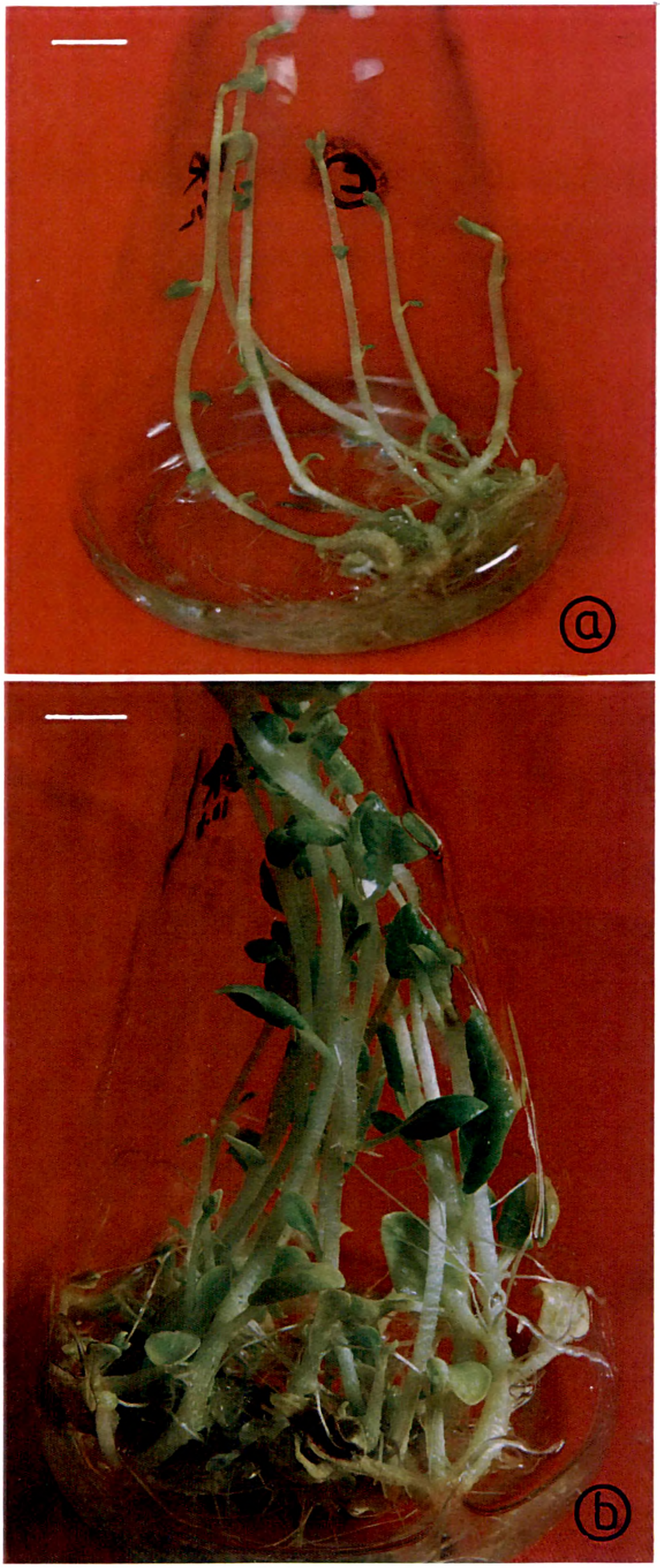

a
b

Plate 14. Shoot production of potato var DR in liquid agitated culture in MS medium supplemented with –

(a) 0.25 mg/l GA_3, 0.01 mg/l NAA, 2 mg/l Ca-pantothenate, 1 mg/l Thiamine HCl and 1.0 mg/l $AgNO_3$.

(b) 0.25 mg/l GA_3, 0.01 mg/l NAA, 2 mg/l Ca-pantothenate, 1 mg/l Thiamine HCl and 2.5 mg/l $AgNO_3$.

In all figures, the bar = 1 Cm


a
b

Plate 15. Shoot production of potato var DR in liquid agitated culture in MS medium supplemented with –

(a) 0.25 mg/l GA_3, 0.01 mg/l NAA, 2 mg/l Ca-pantothenate and 1 mg/l Thiamine-HCl. and 10 mg/l $AgNO_3$.

(b) 0.25 mg/l GA_3, 0.01 mg/l NAA, 2 mg/l Ca-pantothenate, 1 mg/l Thiamine-HCl and 20 mg/L $AgNO_3$

In all figures, the bar = 1 Cm


a
b

Plate 16. Shoot production of potato var HR in liquid agitated culture in MS medium supplemented with –

(a) Control – 0.25 mg/l GA_3, 0.05 mg/l NAA, 2 mg/l Ca-pantothenate and 1 mg/l Thiamine-HCl.

(b) 0.25 mg/l GA_3, 0.05 mg/l NAA, 2 mg/l Ca-pantothenate, 1 mg/l Thiamine-HCl and 0.5 mg/l $AgNO_3$

In all figures, the bar = 1 Cm

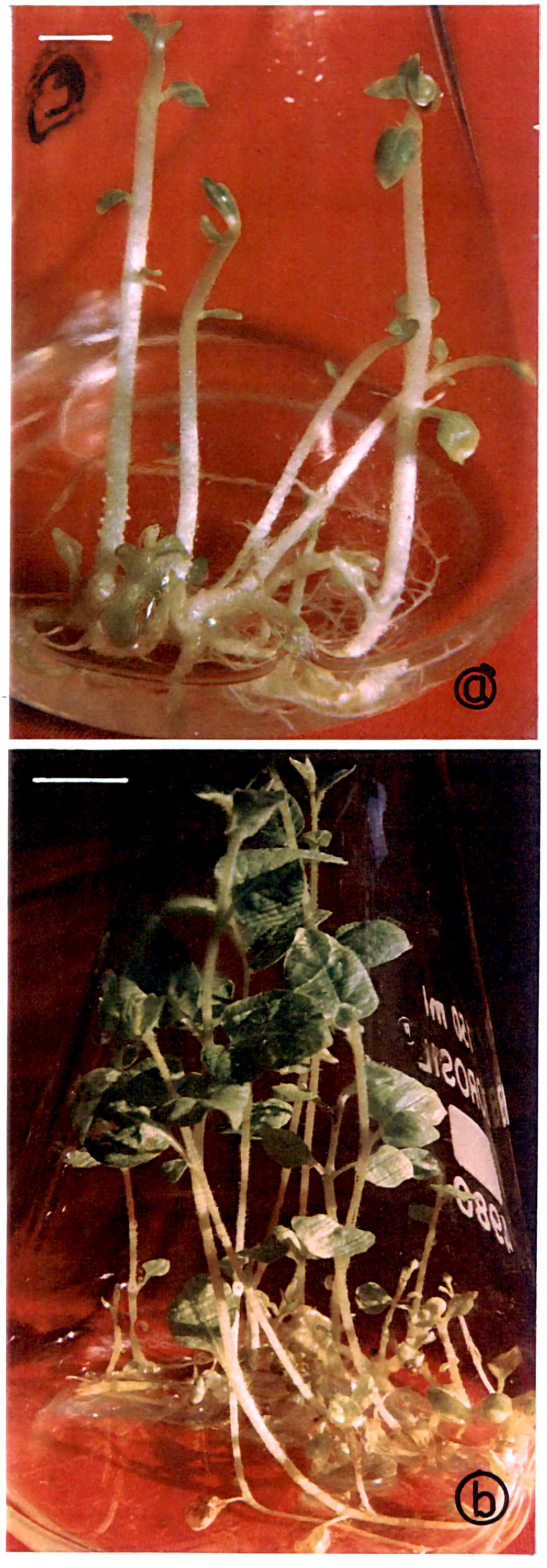

a
b

Plate 17. Shoot production of potato var HR in liquid agitated culture in MS medium supplemented with –

(a) 0.25 mg/l GA_3, 0.05 mg/l NAA, 2 mg/l Ca-pantothenate and 1 mg/l Thiamine-HCl and 1.0 mg/l $AgNO_3$.

(b) 0.25 mg/l GA_3, 0.05 mg/l NAA, 2 mg/l Ca-pantothenate, 1 mg/l Thiamine-HCl and 2.5 mg/l $AgNO_3$

In all figures, the bar = 1 Cm


a
b

Plate 18. Shoot production of potato var HR in liquid agitated culture in MS medium supplemented with –

(a) 0.25 mg/l GA_3, 0.05 mg/l NAA, 2 mg/l Ca-pantothenate and 1 mg/l Thiamine-HCl and 10 mg/l $AgNO_3$

(b) 0.25 mg/l GA_3, 0.05 mg/l NAA, 2 mg/l Ca-pantothenate, 1 mg/l Thiamine-HCl and 20 mg/L $AgNO_3$.

In all figures, the bar = 1 Cm


a
b

Effect of temperature and light on microtuber production was studied. When cultures of var DR were kept at 23° ± 2° C and 16 hour photo period for tuberization, greenish coloured microtubers were formed. The mean total number of tubers per flask was very less (3.8) and average diameter and fresh weight of tubers was 0.73 cm and 0.43 gm respectively. (Table:24). Total number of tubers increased (7.6) when cultures were kept under continuous dark condition at 23° ± 2° C, but average diameter (0.76 cm) and average fresh weight (0.34 gm) did not show any improvement. However, in BOD incubator at 15 ± 2°C under continuous dark condition, tuber number significantly increased (13.60). The average tuber diameter (0.83 cm) and fresh weight (0.449 gm) were also higher, compared to those produced at 23 ± 2° under continuous dark condition (Table:24). Microtubers produced under continuous dark condition were whitish in colour (Plate: 19).

In var HR, total number of tubers per flask (5.00) were induced at 23 ± 2°C under 16 hour photo-period. The average diameter and fresh weight of tubers was 0.70 cm and 0.229 gm respectively (Table:25). Total number of tubers did not increase when cultures were kept at 23 ± 2°C under continuous dark condition. Whereas, at 15 ± 2°C, under continuous dark condition there was a significant increase in all the aspects. The total number of tubers was 11.0 per flask with average diameter of 0.86 cm and average fresh weight of 0.409 gm (Table:25; Plate: 20 and 21).

Types of tuberization media on microtuber production

1. Single node culture on solid tuberization medium

Single nodes of var DR, when cultured on solid tuberization medium with 8%(w/v) sucrose, produced only one tuber from each nodal explants (Plate: 22). These tubers were small in size and their average diameter and average fresh weight were 0.64 cm and 0.336 gm respectively (Table:26). When the concentration of sucrose was raised to 10% (w/v), both the total diameter and total fresh weight of tubers got reduced markedly. In var HR, average diameter of tubers produced in solid tuberization medium supplemented with 8% (w/v) sucrose was 0.60 cm and average fresh weight 0.178 gm (Table:27; Plate: 24).

Table- 24: Effect of temperature and light on microtuber production of potato var. DR.

Temperature °C	Light/dark condition	Total no. of tubers Mean S.E.*	Average diameter (cm) Mean S.E.*	Average fresh weight (gm) Mean S.E.*	Total diameter (cm) Mean S.E.*	Total fresh weight (gm) Mean S.E.*	Remarks
23±2	Light	$3.8 \pm .37_a$	.732±.03	$.436 \pm .03_b$	$2.74 \pm .17_a$	$1.617 \pm .10_a$	Small, greenish
23±2	Dark	$7.6 \pm .50_b$	.765±.01	$.348 \pm .01_a$	$5.80 \pm .36_b$	$1.529 \pm .04_a$	Small, whitish
15±2	Dark	$13.6 \pm .67_c$	.828±.04	$.449 \pm .01_c$	$11.16 \pm .43_c$	$6.056 \pm .09_c$	Large, whitish.

*Values with the same superscript letter are not significantly different at P>0.05 (Duncan's Multiple range test)

Table-25: Effect of temperature and light on microtuber production. var. HR.
(Data recorded after 60 days)

Temp. °C	Light/ Dark condition	Total no. of tubers/flask Mean ±S.E.*	Av. dia (cm) Mean ± S.E. *	Av. F.W. (gm) Mean ±S.E.*	Total dia (cm) Mean ±S.E.*	Total F.W. (gm) Mean ± S.E.*	Remarks
23±2	Light	$5.0 \pm 0.89_a$	$0.70 \pm 0.03_a$	$0.229 \pm 0.00_a$	$3.26 \pm 0.08_a$	$1.299 \pm 0.05_a$	Very small, green
23±2	Dark	$6.0 \pm 1.09_a$	$0.67 \pm 0.01_a$	$0.228 \pm 0.00_a$	$4.53 \pm 0.17_b$	$1.214 \pm 0.04_a$	Very small, white
15±2	Dark	$11.0 \pm 1.0_b$	$0.86 \pm 0.02_b$	$0.409 \pm 0.03_b$	$9.86 \pm 0.03_c$	$4.390 \pm 0.19_b$	Large, white

*Values with the same superscript letter are not significantly different at P>0.05 (Duncan's Multiple range test)

Table-26: Microtuber production using different types of culture medium. var DR.
(Data recorded after 60 days)

Media used	Sucrose conc. (%)	No. of tubers Mean ± S.E.	Total dia (cm) Mean ± S.E.	Total F.W. (gm) Mean ± S.E.	Av. dia (cm) Mean ± S.E.	Av. F.W. (gm) Mean ± S.E.	Quality of tubers	Remarks
Solid tuberization media	8	$14.2 \pm 0.66_c$	$9.2 \pm 0.11_c$	$4.76 \pm 0.19_c$	$0.64 \pm 0.03_{ab}$	$0.336 \pm 0.01_b$	++	Plant height inhibited, one tuber in each shoot on the top, small in size.
	10	$7.8 \pm 0.66_a$	$4.23 \pm 0.20_a$	$2.45 \pm 0.17_a$	$0.54 \pm 0.02_a$	$0.321 \pm 0.03_b$		
Solid multiplication media (3%) + liquid tuberization media (8%)	8	$19.0 \pm 0.86_d$	$11.7 \pm 1.20_d$	$4.18 \pm 0.39_{bc}$	$0.61 \pm 0.01_a$	$0.220 \pm 0.02_{ab}$	+	Number is more but size is very small.
	10	$14.0 \pm 1.04_c$	$7.50 \pm 0.23_b$	$2.12 \pm 0.09_a$	$0.53 \pm 0.02_a$	$0.156 \pm 0.01_a$		
Liquid propagation media (3%) → Decanted → Add liquid tuberization media (8%)	8	$11.0 \pm 1.18_b$	$8.66 \pm 0.06_b$	$5.72 \pm 0.03_d$	$0.78 \pm 0.04_b$	$0.540 \pm 0.06_c$	+++	Though number is less but good size and shape.
	10	$7.2 \pm 0.73_a$	$5.36 \pm 0.12_a$	$3.62 \pm 0.49_b$	$0.74 \pm 0.04_b$	$0.520 \pm 0.09_c$		

*Values with the same superscript letter are not significantly different at $P>0.05$ (Duncan's Multiple range test)

Table-27. Microtuber production using different types of culture medium. var HR. (Data recorded after 60 days)

Media used	Sucrose conc. (%)	No. of tubers Mean ± S.E.	Total dia (cm) Mean ± S.E.*	Total F.W. (gm) Mean ± S.E.*	Av. dia (cm) Mean ± S.E.*	Av. F.W. (gm) Mean ± S.E.*	Quality of tubers	Remarks
Solid tuberization media	8	$14.50 \pm 0.22_c$	$7.1 \pm 0.11_c$	$2.37 \pm 0.02_b$	$0.60 \pm 0.04_b$	$0.178 \pm 0.00_a$	++	Plant height inhibited, one tuber in each shoot on the top, small in size.
	10	$8.83 \pm 0.40_a$	$4.53 \pm 0.20_b$	$1.54 \pm 0.06_a$	$0.60 \pm 0.05_b$	$0.193 \pm 0.01_a$		
Solid multiplication media (3%) + liquid tuberization media (8%)	8	$18.16 \pm 0.74_d$	$10.0 \pm 0.20_d$	$3.26 \pm 0.03_c$	$0.62 \pm 0.02_b$	$0.200 \pm 0.01_{ab}$	+	Number is more but size is very small.
	10	$9.83 \pm 0.16_b$	$5.43 \pm 0.32_b$	$1.66 \pm 0.07_a$	$0.67 \pm 0.06_b$	$0.191 \pm 0.00_a$		
Liquid propagation media (3%) → Decanted → Add liquid tuberization media (8%)	8	$10.33 \pm 0.61_b$	$10.1 \pm 0.05_d$	$3.56 \pm 0.07_c$	$0.89 \pm 0.05_c$	$0.347 \pm 0.02_c$	+++	Though number is less but good size and shape.
	10	$7.66 \pm 0.21_a$	$3.33 \pm 0.08_a$	$2.12 \pm 0.05_b$	$0.45 \pm 0.02_a$	$0.289 \pm 0.01_b$		

*Values with the same superscript letter are not significantly different at P>0.05 (Duncan's Multiple range test)

Plate 19. Induction of Microtubers of var DR under –

(a) Light condition at 23 ± 2°C.

(b) Continuous dark condition at 15 ± 2°C.

In all figures, the bar = 1 Cm

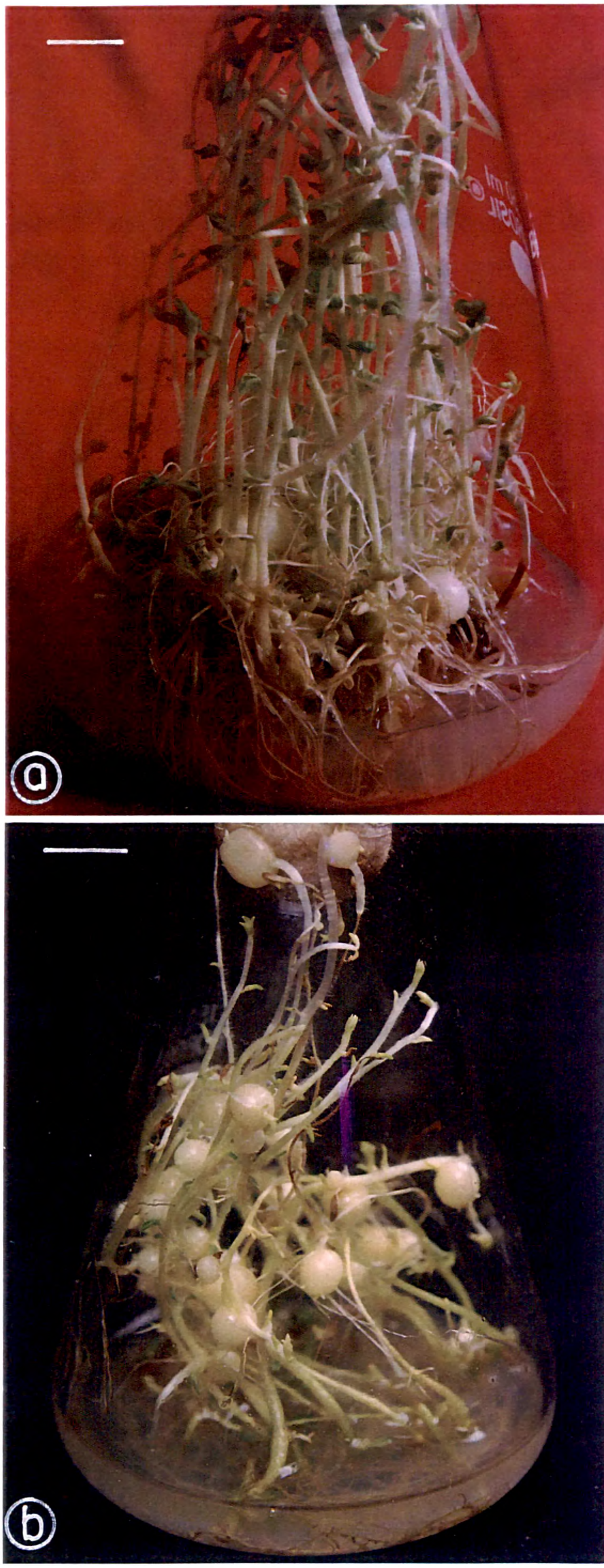

a
b

Plate 20. Induction of microtubers of var HR under –

(a) Light condition at $23 \pm 2°C$.

(b) Continuous dark condition at $15 \pm 2°C$

In all figures, the bar = 1 Cm

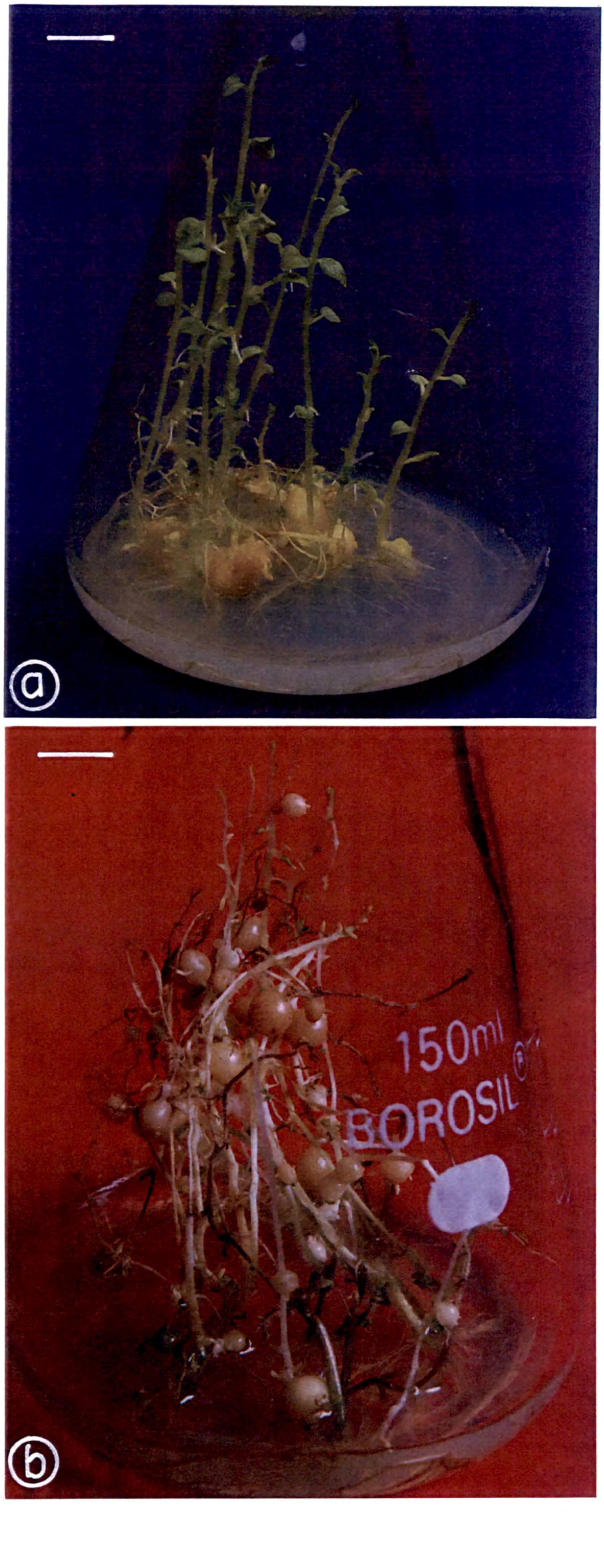

a
150ml
BOROSIL
b

Plate 21. (a) and (b) Cultures kept under continuous dark condition at 15 ± 2°C. in BOD incubator for microtuber induction.


a
b

2. Shoot production from single nodes on solid propagation medium and the induction of microtubers by over layering with liquid tuberization medium (bi-layer method)

Single nodes, when cultured on solid propagation medium supplemented with 3% (w/v) sucrose, produced shoots within a period of 21 days. These shoots when supplied with liquid tuberization medium containing 8% (w/v) sucrose started producing microtubers within a period of another 45 days. Though the number of tubers produced per flask was more than that in solid tuberization medium, average diameter and fresh weight did not show any significant increase (Plate: 23a and 25a). In var DR, the average diameter of the tubers was 0.61 cm and average fresh weight 0.22 gm while in var HR average diameter and average fresh weight was 0.62 cm and 0.200 gm respectively (Table: 26 and 27).

3. Shoot production from stem segments on liquid agitated culture and the induction of microtubers by replacing with liquid tuberization medium

Shoot segments with 3 nodes on culturing in liquid medium with 3% (w/v) sucrose, produced healthy shoots within 14 days. when the shoot multiplication medium was decanted and liquid tuberization medium with 8% (w/v) sucrose was added to these shoots produced larger tubers (Plate: 23b and 25b). These tubers had an average diameter of 0.78 cm and average fresh weight of 0.54 gm in var DR (Table - 26). Var HR produced tubers with an average diameter and average fresh weight of 0.89 cm and 0.347 gm respectively (Table:27).

Microtuber production under the influence of varying concentration of BAP and CCC individually and in combination was examined (Plate: 26 and 27). All the levels of BAP (0.1-10.0 mg/l) and CCC (100 mg/l-1000 mg/l) tried alone and in combinations, showed significant variations in their results. BAP alone did not produce any 'A' grade (>0.70 gm) tuber in DR and HR varieties. Maximum mean number of tubers (11.66) with a total diameter of 12.47 cm and total fresh weight of 5.04 gm was produced in a medium supplemented with 5 mg/l BAP and 500 mg/l CCC, in var DR, (Table:28). Further increase in the levels of BAP or CCC did not show any significant increase in all the parameters observed.

Table-28: Effect of various concentration of BAP, CCC and 8% Sucrose on microtuber production. var. DR. (Data recorded after 60 days)

Growth Regulator		No. of tubers(grade)			Total no. of tubers/flask Mean ± S.E.*	Total diameter of the tubers (cm) Mean ± S.E.*	Total fresh weight of the tubers (gm) Mean ± S.E.*
BAP (mg/l)	CCC (mg/l)	A*	B*	C*			
0	0	00	1.66± 0.66 $_{ab}$	0.66 ± .33 $_{a}$	2.33 ± 0.33	1.93 ± 0.08	0.57 ± 0.05
0.1	0	00	1.66±0.33 $_{ab}$	3.00 ± .57 $_{abcd}$	4.66 ± 0.33	3.50 ± 0.20	1.19 ±0.08
1	0	00	1.66±0.33 $_{ab}$	2.66 ± .88 $_{abcd}$	4.33 ±0.66	4.00 ± 0.12	1.15 ±0 .05
2.5	0	00	2.33±0.33 $_{abcd}$	3.66 ± .88 $_{abcdef}$	6.00 ± 0.57	4.36 ± 0.08	1.50 ± 0.12
5.0	0	00	1.66±0.33 $_{ab}$	2.00 ± .57 $_{ab}$	3.66 ± 0.33	3.20 ± 0.05	1.13 ± 0.06
7.5	0	00	3.33±0.33 $_{abcde}$	3.33 ± .33 $_{abcde}$	6.66 ± 0.33	5.80 ± 0.15	2.13 ± 0.07
10.0	0	00	3.33±0.88 $_{abcde}$	4.33 ± 1.20 $_{bcdef}$	7.66 ± 0.66	6.13 ± 0.64	4.45 ± 0.28
0.1	100	00	2.0 0±0.57 $_{abc}$	7.00 ± .57 $_{fg}$	9.00 ± 0.57	7.13 ± 0.83	1.91 ± 0.42
0.1	250	00	2.00 ± 0.57 $_{abc}$	4.00 ± 1.0 $_{abcdef}$	6.00± 0.57	4.70 ± 0.37	1.27 ± 0.23
0.1	500	00	2.60 ± 0.66 $_{abcd}$	6.00 ± 1.15 $_{defg}$	8.66 ±0.66	5.49 ± 0.44	1.91 ±0.27
0.1	750	00	2.00± 0.57 $_{abc}$	3.66 ± .33 $_{abcdef}$	5.66 ± 0.33	4.88 ± 0.16	1.49 ± 0.22
0.1	1000	00	2.00± 0.57 $_{abc}$	6.00 ± .57 $_{defg}$	8.00 ± 0.57	5.10 ± 0.55	2.14 ± 0.13

Table 28 Cont.....

Growth Regulator		No. of tubers(grade)			Total no. of tubers/flask Mean ± S.E.*	Total diameter of the tubers (cm) Mean ± S.E.*	Total fresh weight of the tubers (gm) Mean ± S.E.*
BAP (mg/l)	CCC (mg/l)	A*	B*	C*			
1.0	100	00	2.30 ± 0.33 abcd	6.00 ± .57 defg	8.33 ± 0.33	6.95 ± 0.12	1.98 ± 0.06
1.0	250	00	3.60 ± 0.66 abcdef	6.00 ± 1.52 defg	9.66 ± 0.88	6.96 ± 0.17	2.28 ± 0.15
1.0	500	00	4.00 ± 0.57 bcdef	5.33 ± .66 bcdefg	9.33 ± 0.33	7.37 ± 0.33	3.00 ± 0..11
1.0	750	00	4.00 ±0.57 bcdef	5.66 ± 1. cdefg	9.66 ±1.2	7.67 ± 0.36	3.17 ± 0.23
1.0	1000	00	1.60 ± 0.33 ab	6.00 ± .57 defg	7.66 ± 0.33	5.76 ± 0.23	1.85 ± 0.02
2.5	100	00	4.60 ± 0.88 def	5.33 ± .88 bcdefg	10.0 ± 0.00	8.72 ± 0.28	3.18 ± 0.07
2.5	250	00	2.00 ± 0.57 abc	8.66 ± 1.2 g	10.66 ± 1.76	8.20 ± 1.70	2.69 ±0.38
2.5	500	00	5.30 ± 1.20 ef	4.33 ± 1.45 bcdef	9.66 ± 0.33	8.10 ± 0.95	3.07 ± 0.12
2.5	750	00	4.30 ± 0.88 cdef	5.66 ± 1.76 cdefg	9.66 ± 1.33	8.36 ± 1.30	2.58 ±0.58
2.5	1000	00	2.00 ± 0.57 abc	6.00 ± .57 defg	8.00 ± 0.00	6.38 ± 0.53	1.80 ± .026
5.0	100	1.0 0± .57 bcd	4.60 ± 0.88 def	3.33 ± .88 abcde	9.00 ± 1.00	7.97 ± 0.29	3.29 ± 0.10
5.0	250	0.66 ± .33 abc	4.60 ± 0.88 def	5.00 ± 1.0 bcdef	10.33 ± 1.20	9.11 ± 0.96	3.98 ± 0.17
5.0	500	1.66±.33 d	6.00 ± 0.57 f	4.00 ± .57 abcdef	11.66 ± 0.66	12.47 ± 0.26	5.04 ± 0.07
5.0	750	0.66 ± .33 abc	4.30 ± 0.88 cdef	6.66 ± .33 efg	11.66 ± 0.66	10.0 ± 0.15	3.49 ± 0.09
5.0	1000	0.33 ± .33 ab	2.60 ± 0.66 abcd	6.66 ± .33 efg	10.00 ± 0.57	7.63 ± 0.54	2.75 ± 0.06

Table 28 Cont.....

Growth Regulator		No. of tubers(grade)			Total no. of tubers/flask Mean ± S.E.*	Total diameter of the tubers (cm) Mean ± S.E.*	Total fresh weight of the tubers (gm) Mean ± S.E.*
BAP (mg/l)	CCC (mg/l)	A*	B*	C*			
7.5	100	$1.66 \pm .33_{d}$	$3.33 \pm 0.33_{abcde}$	4.00 ± 1.15^{abcdef}	9.00 ± 1.15	9.40 ± 0.61	3.49 ± 0.27
7.5	250	$1.33 \pm .33_{cd}$	$4.00 \pm 0.57_{bcdef}$	$3.66 \pm .66_{abcdef}$	9.00 ± 0.57	9.21 ± 0.26	3.21 ± 0.07
7.5	500	$1.33 \pm .33_{cd}$	$4.00 \pm 0.57_{bcdef}$	$4.00 \pm 1.15_{abcdef}$	9.33 ± 0.88	8.31 ± 0.92	2.91 ± .45.45
7.5	750	$1.00 \pm 00_{bcd}$	$2.30 \pm 1.45_{abcd}$	$5.33 \pm 1.85_{bcdefg}$	8.66 ± 0.88	7.32 ± 0.77	2.35 ± 0.23
7.5	1000	$1.00 \pm .57_{bcd}$	$2.00 \pm 0.57_{abc}$	$7.00 \pm 1.15_{fg}$	10.0 ± 1.52	6.94 ± .19	2.14 ± 0.06
10.0	100	$00 \pm 00_{a}$	$3.00 \pm 1.15_{abcde}$	$5.66 \pm 1.85_{cdefg}$	8.66 ± 2.02	4.86 ± 0.49	1.55 ± 0.31
10.0	250	$0.66 \pm .33_{abc}$	$2.00 \pm 0.57_{abc}$	$5.00 \pm .57_{bcdef}$	7.66 ± 0.33	5.97 ± 0.89	1.73 ± 0.33
10.0	500	$1.33 \pm .33_{cd}$	$2.00 \pm 1.0_{abc}$	$2.66 \pm 1.2_{abcd}$	6.00 ± 1.52	5.03 ± 0.38	2.00 ± 0.07
10.0	750	$0.00 \pm 00_{a}$	$3.00 \pm 0.57_{abcde}$	$3.66 \pm 1.66_{abcdef}$	6.66 ± 1.20	5.10 ± 0.40	1.39 ± 0.19
10.0	1000	$0.33 \pm .33_{ab}$	$1.33 \pm 0.33_{a}$	$2.33 \pm 1.2_{abc}$	4.00 ± 1.00	2.76 ± 2.0	1.69 ± 0.32

*Values with the same superscript letter are not significantly different at P>0 05 (Duncan's Multiple range test)

Plate 22. Microtuber production in solid tuberization medium from single node cuttings of var DR.

(a) 8% sucrose

(b) 10% sucrose.

In all figures, the bar = 1 Cm

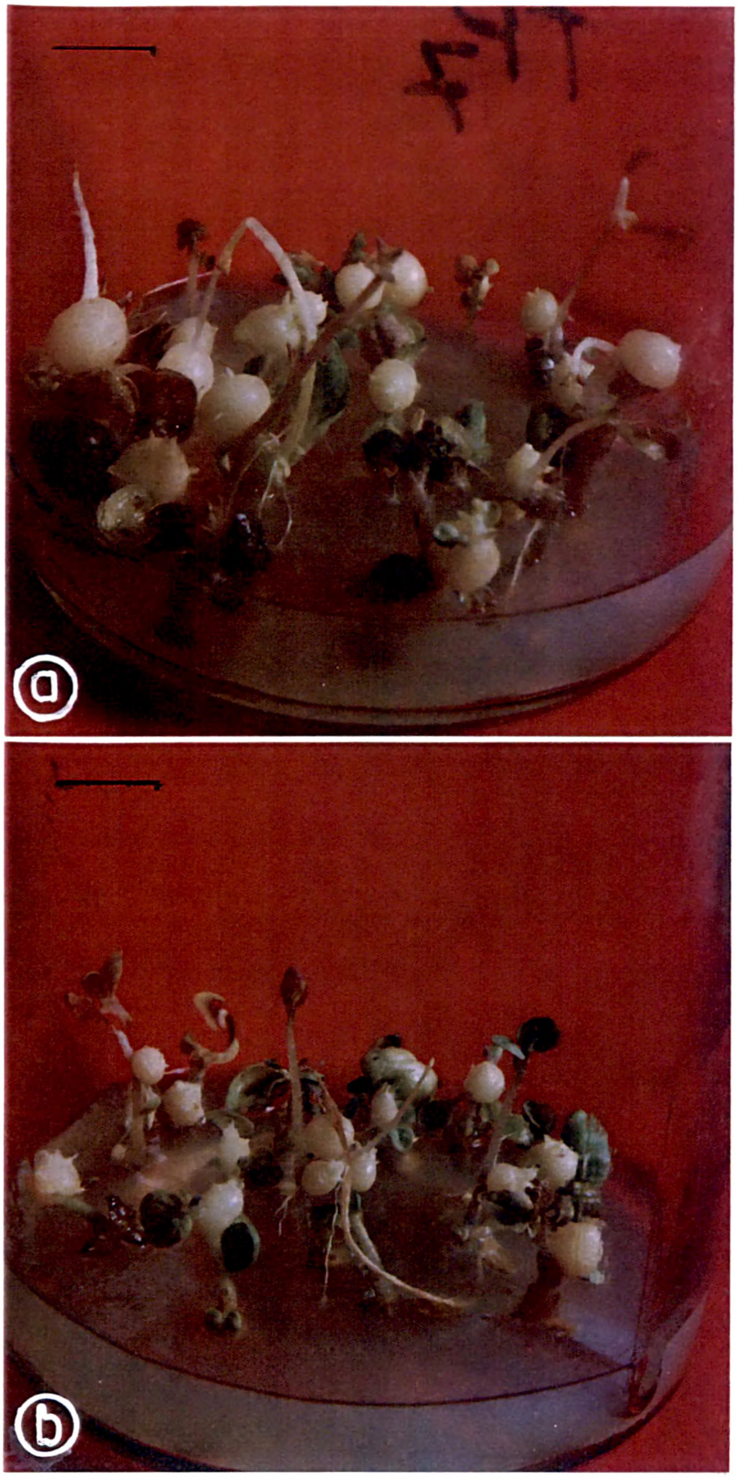

a
b

Plate 23 (a) Microtuber production in bilayer medium (solid propagation medium and liquid tuberization medium) var DR

(b) Microtuber production in lıquid tuberizatıon medıum of var DR.

In all figures, the bar = 1 Cm

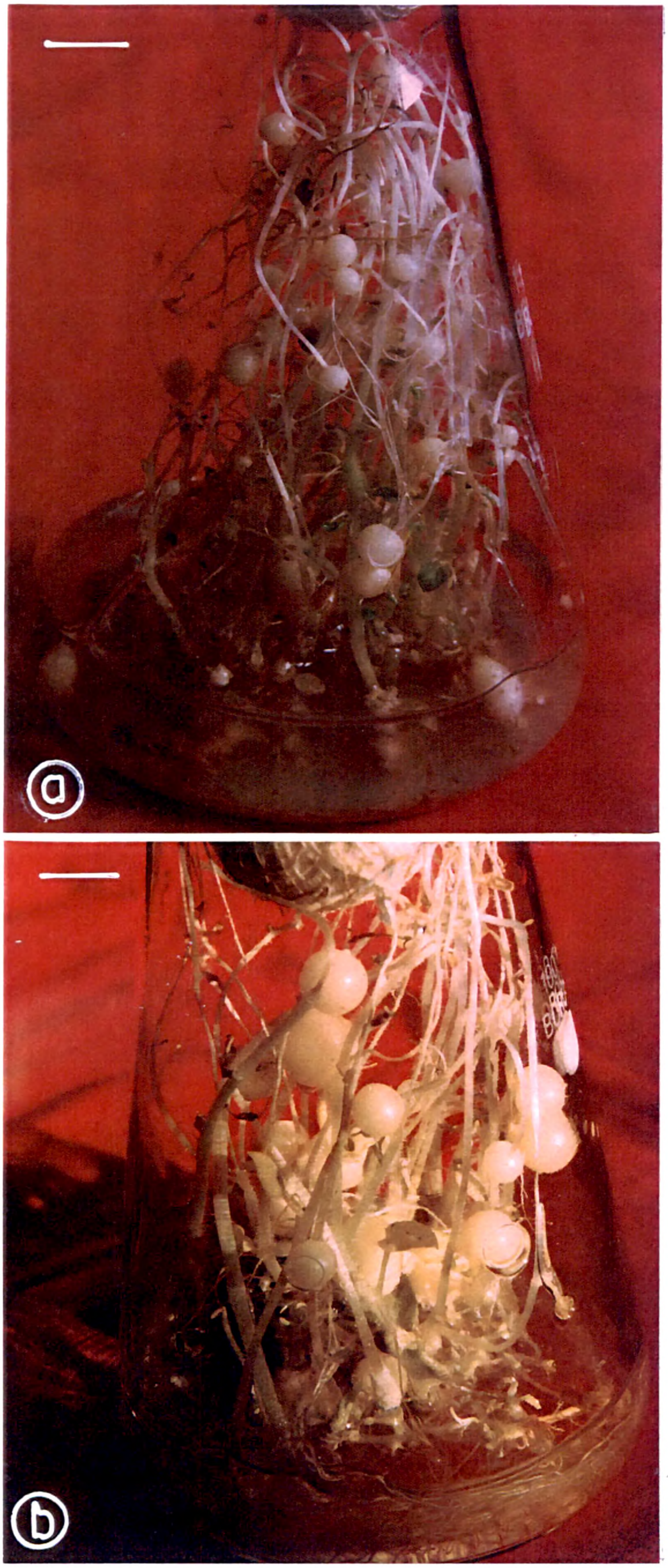

a
b

Plate 24. Microtuber production in solid tuberization medium from single node cuttings of var HR –

(a) 8% sucrose

(b) 10% sucrose.

In all figures, the bar = 1 Cm

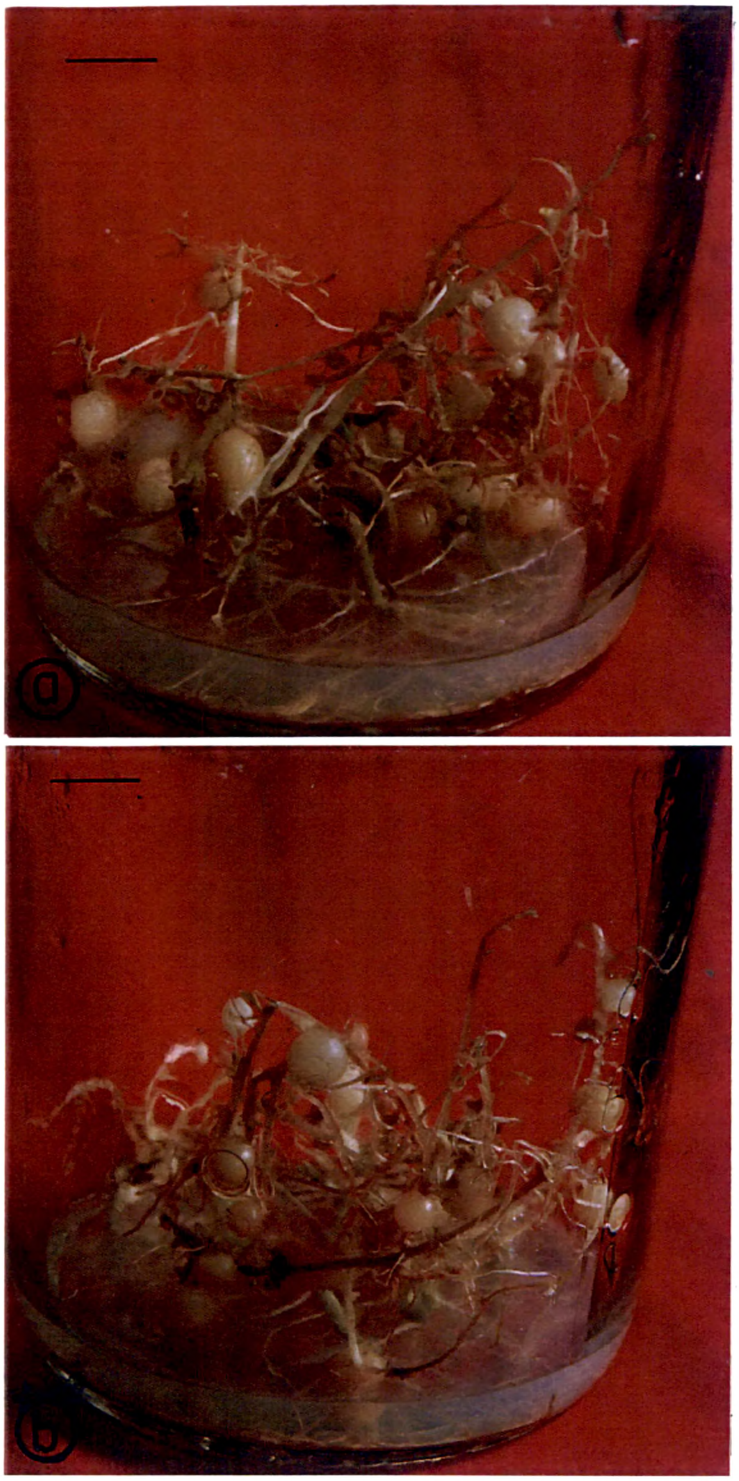

a
b

Plate 25. (a) Microtuber production ın bilayer medium (solıd propagation medium and liquid tuberization medium) var HR.

(b) Microtuber production in liquid tuberizatıon medium. var HR.

In all figures, the bar = 1 Cm

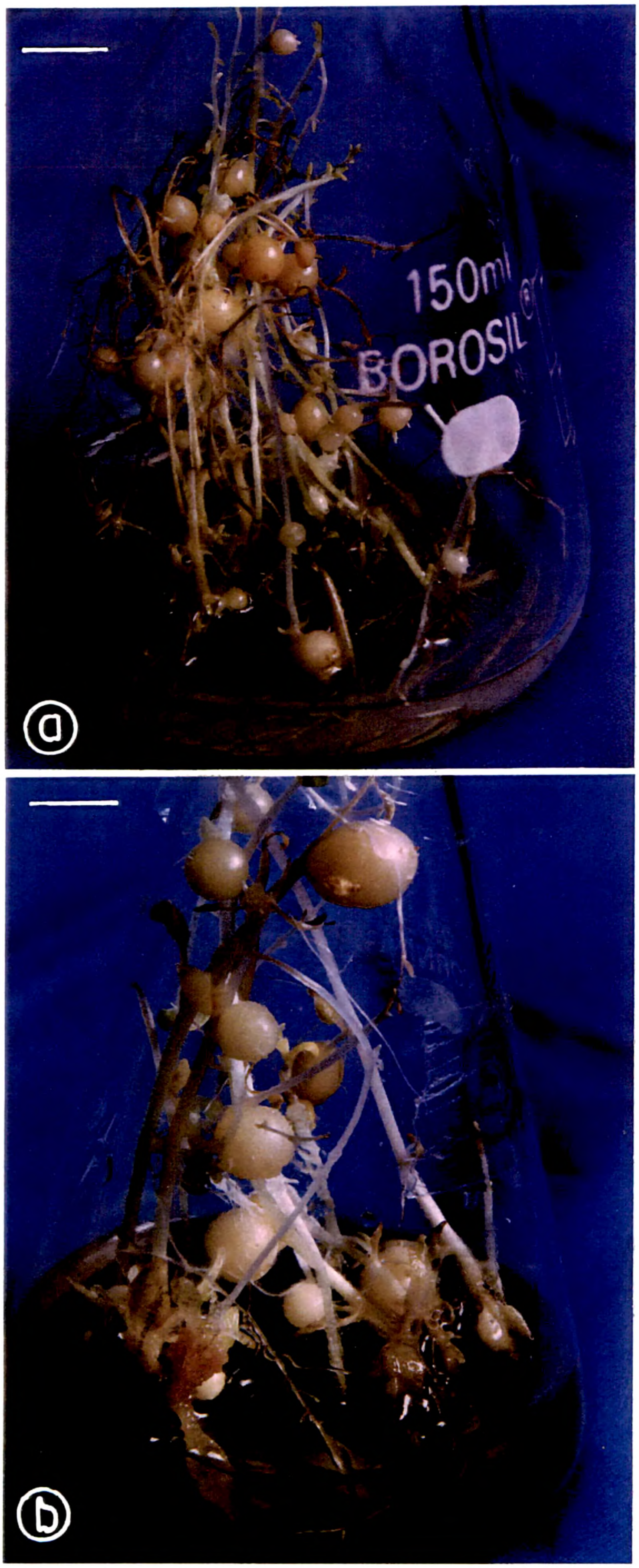

150ml
BOROSIL
a
b

Plate 26. (a) Induction of microtubers in MS liquid medium supplemented with 8% sucrose, 5 mg/l BAP and 500 mg/l CCC var DR.

(b) Harvested microtubers kept on a petridish var DR.

In all figures, the bar = 1 Cm

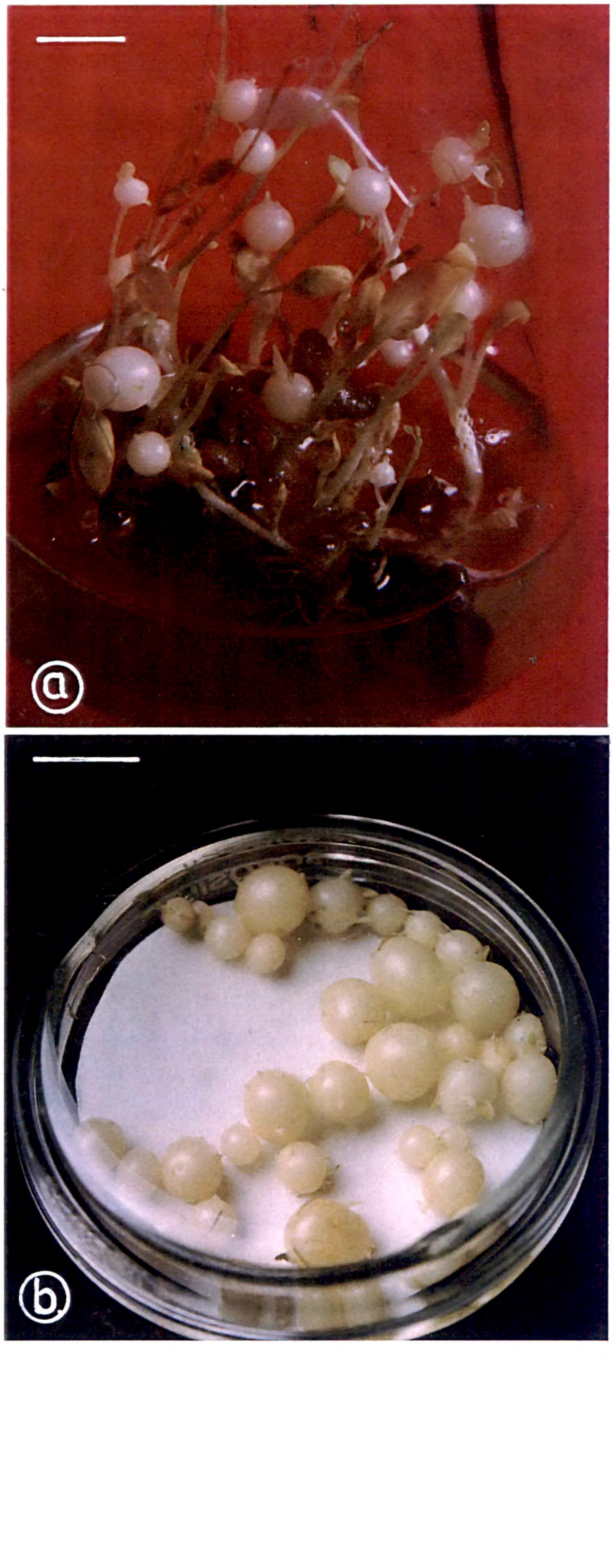
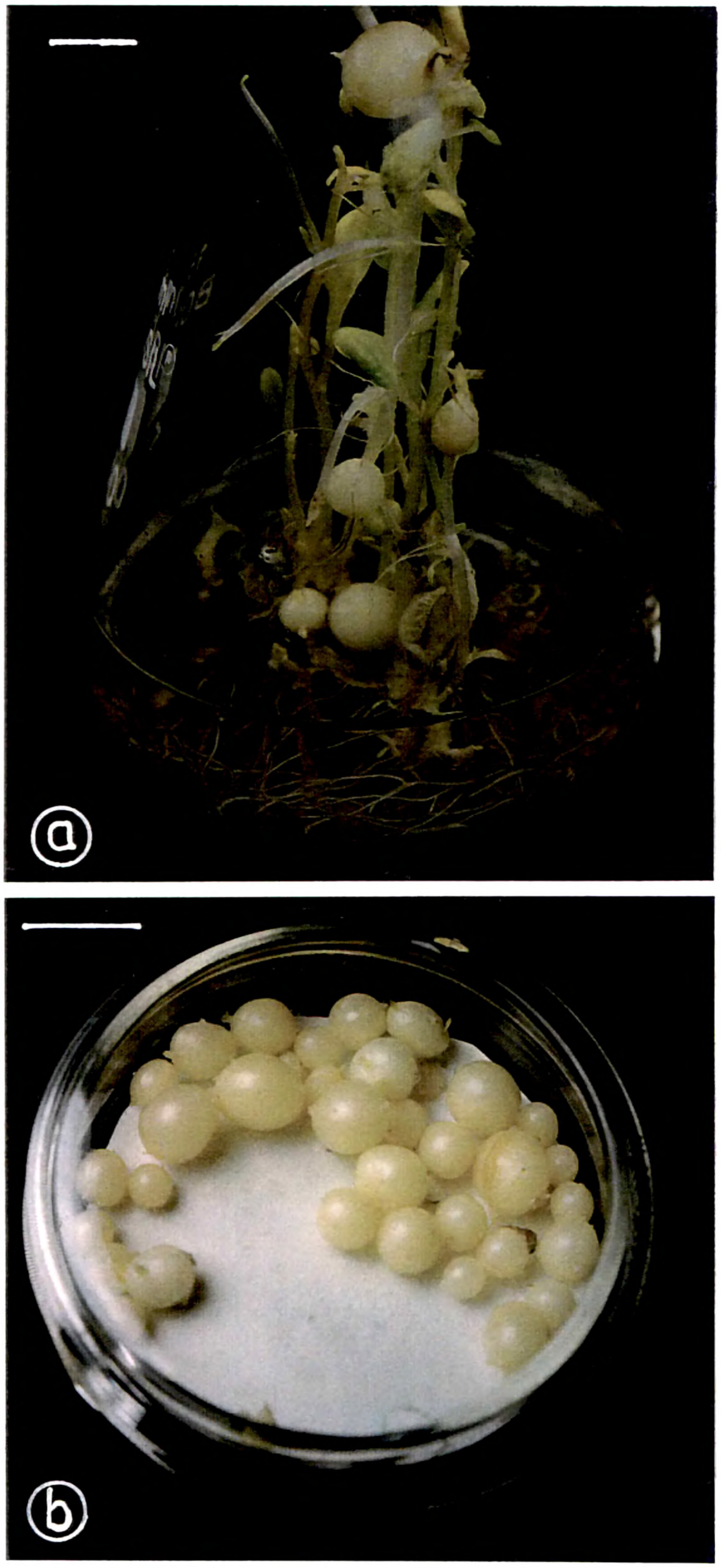

a
b

Plate 27. (a) Induction of microtubers in MS liquid medium supplemented with 8% sucrose, 7.5 mg/l BAP and 750 mg/l CCC. var HR.

(b) Harvested microtubers kept on a petridish. var HR.

In all figures, the bar = 1 Cm


a
b

In var HR, increase in concentration of BAP up to 7.5 mg/l and CCC up to 750 mg/l showed a positive effect on the mean total number (15.33), total diameter (11.9) and total fresh weight (4.97 gm) of the tubers (Table:29).

Effect of Silver nitrate ($AgNO_3$) on microtuber production

Various concentrations of $AgNO_3$ (0.5-20.0 mg/l) were incorporated in the propagation and tuberization medium in order to study its effect on tuberization (as per the flow chart in the following page).

Shoots used for tuberization, derived from liquid propagation medium without $AgNO_3$

When shoots derived from liquid propagation medium (without $AgNO_3$) were exposed to tuberization medium containing 10 and 15 mg/l $AgNO_3$, they produced large number of tubers in var DR (Plate: 28 and 29). The total diameter and total fresh weight also exhibited a significant increase at 10 and 15 mg/l $AgNO_3$ compared to the control (Table:30). Var HR also exhibited a similar response (Plate: 30 and 31) to various concentrations of $AgNO_3$ (Table:31; Chart: 1 and 2).

Shoots used for tuberization, derived from liquid propagation medium supplemented with 0.5 mg/l $AgNO_3$

Incorporation of $AgNO_3$ at 0.5 mg/l in the propagation medium resulted in the production of green healthy shoots. These shoots on exposure to the tuberization medium containing varying concentrations of $AgNO_3$ produced large sized micro tubers. The number of tubers, total diameter and total fresh weight of the tubers per flask increased steadily with increase in the concentration of $AgNO_3$ up to 15 mg/l. Maximum total number (14.0) and more higher grades (A and B) of tubers (total 10.0) were obtained under the influence of 15 mg/l $AgNO_3$ in var DR (Plate: 32 and 33). Total diameter (14.35 cm) and total fresh weight (9.37 gm) of tubers have also been significantly increased by 15 mg/l AgNO3 as compared to those of control (Table:32).

$AgNO_3$ on microtuberization of potato

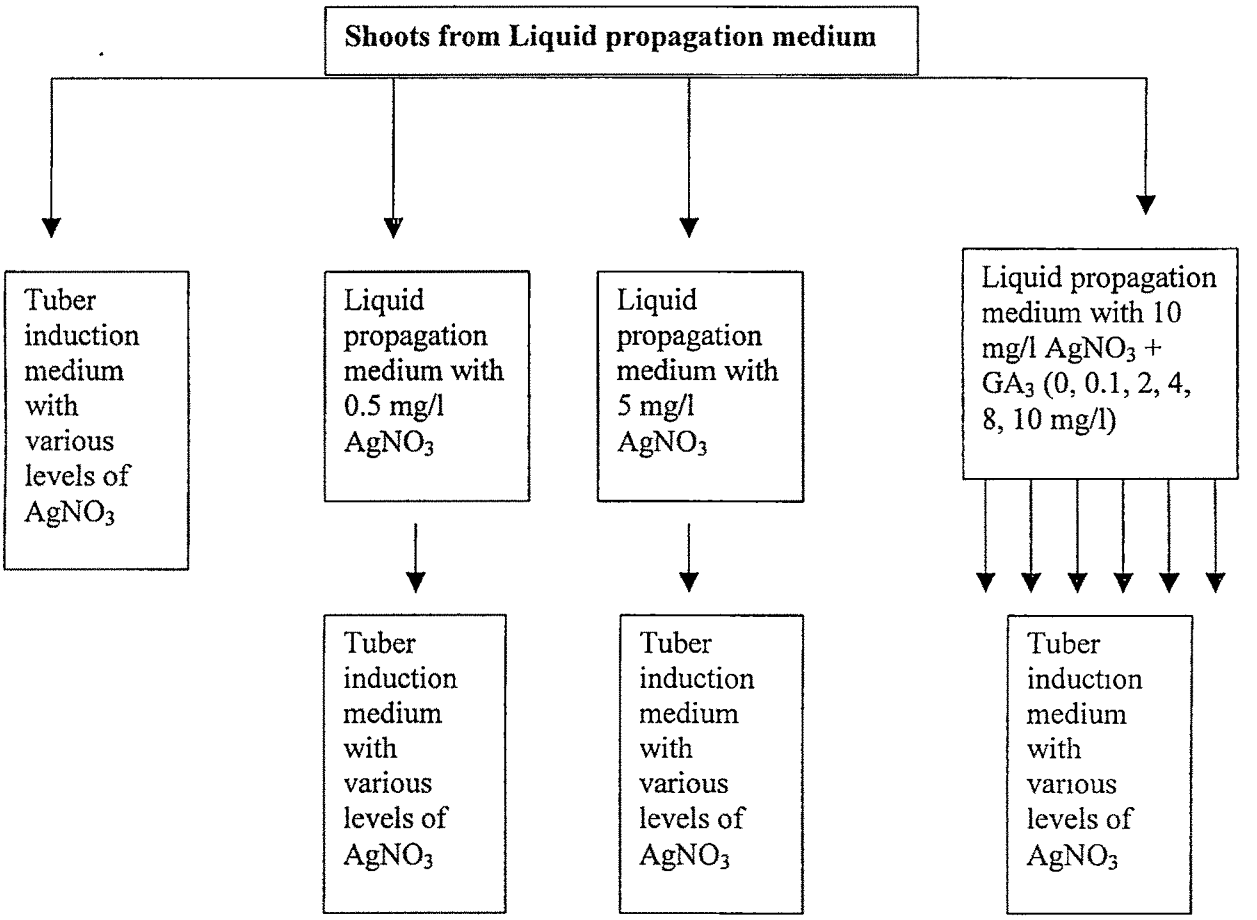


Table-29: Effect of various concentration of BAP, CCC and 8% Sucrose on microtuber production. var. HR. (Data recorded after 60 days)

Growth Regulator		No. of tubers(grade)			Total no. of tubers/flask Mean ± S.E.	Total diameter of the tubers (cm) Mean ± S.E.	Total fresh weight of the tubers (gm) Mean ± S.E.
BAP (mg/l)	CCC (mg/l)	A	B	C			
0	0	00	0.00	0.00	0.00	0.00	0.00
0.1	0	00	0.00	2.00 ± 0.57	2 00 ± 0.57	1.43 ± 0.37	0.49 ± 0.12
1	0	00	0.00	2.00 ± 0.57	2.00 ± 0.57	1.46 ± 0.43	0.42 ± 0.13
2.5	0	00	1.00 ± 0.00	2.33 ± 0.33	3.33 ± 0.33	2.70 ± 0.35	0.87 ± 0.08
5.0	0	00	1.66 ± 0.33	3.33 ± 0.66	4.66 ± 0.66	4.43 ± 0.41	1.34 ± 0.12
7.5	0	00	2.33 ± 0.33	3.33 ± 0.33	5.66 ± 0.33	4.73 ± 0.31	1.65 ± 0.03
10.0	0	00	2.00 ± 0.57	3.66 ± 0.88	5.66 ± 0.88	4.60 ± 0.75	1.65 ± 0.20
0.1	100	00	2.00 ± 0.00	3.00 ± 0.57	5.00 ± 0.57	4.16 ± 0.55	1.78 ± 0.30
0.1	250	00	1.66 ± 0.33	3.33 ± 0.88	5.00 ± 0.57	4.30 ± 0.43	1.45 ± 0.06
0.1	500	00	3.00 ± 0.57	2.66 ± 0.33	5.66 ± 0.66	5.00 ± 0.61	1.88 ± 0.21
0.1	750	00	1.66 ± 0.33	4.66 ± 0.88	6.33 ± 0.66	5.13 ± 0.37	1.73 ± 0.06
0.1	1000	00	1.66 ± 0.66	6.00 ± 1.15	7.66 ± 1.33	5.89 ± 0.99	1.99 ± 0.38

Table 29 (Contd...)

Growth Regulator		No. of tubers(grade)			Total no. of tubers/flask Mean ± S.E.	Total diameter of the tubers (cm) Mean ± S.E.	Total fresh weight of the tubers (gm) Mean ± S.E.
BAP (mgl/l)	CCC (mg/l)	A	B	C			
1.0	100	00	1.66 ± 0.33	4.66 ± 1.33	6.33 ± 1.20	4.93 ± 0.76	1.82 ± 0.44
1.0	250	00	2.66 ± 0.33	3.66 ± 0.88	6.33 ± 0.66	4.76 ± 0.67	1.88 ± 0.05
1.0	500	00	1.33 ± 0.33	5.33 ± 0.33	6.66 ± 0.33	5.33 ± 0.24	1.66 ± 0.12
1.0	750	00	1.00 ± 0.00	4.66 ± 1.20	5.66 ± 1.20	4.53 ± 0.68	1.58 ± 0.16
1.0	1000	00	1.33 ± 0.33	4.33 ± 0.33	6.66 ± 0.66	5.23 ± 0.28	1.85 ± 0.03
2.5	100	00	1.33 ± 0.33	2.33 ± 0.33	3.66 ± 0.33	3.26 ± 0.33	1.40 ± 0.31
2.5	250	00	1.66 ± 0.33	4.66 ± 0.33	6.33 ± 0.33	5.13 ± 0.44	1.80 ± 0.08
2.5	500	00	2.00 ± 0.57	5.33 ± 1.20	7.33 ± 0.66	5.80 ± 0.32	1.94 ± 0.05
2.5	750	00	1.33 ± 0.33	3.66 ± 0.33	5.00 ± 0.57	3.93 ± 0.50	1.32 ± 0.15
2.5	1000	00	1.00 ± 0.00	3.00 ± 0.57	4.00 ± 0.57	3.10 ± 0.43	1.09 ± 0.09
5.0	100	0.66 ± 0.33	1.66 ± 0.66	6.00 ±1.15	8.33 ±0.88	6.76 ±0.74	2.49 ± 0.38
5.0	250	0.66 ± 0.33	2.00 ± 0.57	7.00 ±0.57	9.66 ±0.33	7.66 ±0.35	2.80 ± 0.38
5.0	500	0.33 ± 0.33	4.00 ± 0.57	7.33 ±0.88	11.66 ±0.88	9.53 ±0.63	3.72 ± 0.33
5.0	750	1.00 ± 0.57	4.60 ± 0.33	8.66 ±0.88	14.33 ±1.20	12.5 ±1.07	4.81 ± 0.87
5.0	1000	0.66 ± 0.33	4.00 ± 0.57	8.00 ±1.52	12.66 ±1.76	10.66 ±1.36	3.99 ± 0.64

Table 29 (Contd...)

Growth Regulator		No. of tubers(grade)			Total no. of tubers/flask Mean ± S.E.	Total diameter of the tubers (cm) Mean ± S.E.	Total fresh weight of the tubers (gm) Mean ± S.E.
BAP (mgl/l)	CCC (mg/l)	A	B	C			
7.5	100	0.00	1.66 ± 0.33	6.33 ±0.88	8.00 ±1.00	6.60 ±0.90	2.17 ± 0.34
7.5	250	00	1.66 ± 0.33	7.33 ±0.88	9.00 ±1.00	6.80 ±0.70	2.35 ± 0.29
7.5	500	1.33 ±0.33	4.00 ± 0.57	8 00 ±0.57	13.33 ±0.66	11.63 ±0.53	4 89 ± 0 30
7.5	750	1.00 ± 0.57	4.00 ± 0.57	9.66 ±0.33	15.33 ±0.66	11.9 ±0.90	4.97 ± 0.67
7.5	1000	00.00	2.33 ± 0.88	6.00 ±0.57	8.33 ±0.88	6.86 ±1.01	2.22 ± 0.27
10.0	100	00.00 ±0.00	3.66 ± 1.20	5.66 ±1.66	9.33 ±1.20	7.90 ±0.88	2.87 ± 0.37
10.0	250	1 00 ± 0 57	2.33 ± 0.33	5.66 ±0.33	9.00 ±0.57	7.70 ±0.41	3.28 ± 0.48
10.0	500	1.33 ± 0.33	3.66 ± 0.66	4.00 ±0.57	9.00 ±0.57	8.03 ±0.41	3.75 ± 0.28
10.0	750	1.33 ± 0.33	4.00 ± 0.57	5.33 ±0.66	10.66 ±1.20	9.60 ±1.01	4 24 ± 0.59
10.0	1000	0.00	2.0 ± 0.57	3.66 ±1.20	6.66 ±1.45	4.70 ±1.25	1.66 ± 0.48

Values with the same superscript letter are not significantly different at P>0 05 (Duncan's Multiple range test)

Table 30: Effect of various levels of $AgNO_3$ on microtuber production of potato. var. DR (Data recorded after 60 days)

Conc. of $AgNO_3$ (mg/l)	No. of tubers			Total no. of tubers Mean±S.E.*	Average diameter (cm) Mean± S.E.*	Average Fresh Weight (gm) Mean± S.E.*	Total diameter (cm) Mean± S.E.*	Total Fresh Weight (gm) Mean± S.E.*
	A*	B*	C*					
0	$0.25±25_a$	3.00±.00a	$6.50±.64_a$	$9.75±.75_b$	$0.83±.01_a$	$.296±.01_a$	$8.12±.55_{bc}$	$2.89±.26_b$
0.5	$0.25±.25_a$	$3.25±.25_a$	$5.75±.47_a$	$9.25±.25_b$	$0.84±.01_a$	$.303±.01_a$	$7.75±.14_b$	$2.79±.14_b$
1.0	$0.50±.28_a$	$3.25±.25_a$	$5.50±.86_a$	$9.25±.62_b$	$0.86±.01_{ab}$	$.334±.01_{ab}$	$7.97±.41_b$	$3.07±.16_{bc}$
2.5	$0.50±.28_a$	$3.25±.25_a$	$6.25±.25_a$	$10.00±.40_b$	$0.84±.00_a$	$.326±.01_a$	$8.45±.41_{bc}$	$3.27±.28_{bc}$
5.0	$0.75±.25_{ab}$	$3.25±.25_a$	$6.25±.62_a$	$10.25±.25_b$	$0.86±.01_{ab}$	$.339±.02_{ab}$	$8.82±.06_{bcd}$	$3.46±.13_{bc}$
7.5	$1.00±.40_{ab}$	$3.50±.28_a$	$6.00±.70_a$	$10.50±.64_b$	$0.87±.02_{ab}$	$.348±.02_{ab}$	$9.17±.58_{cd}$	$3.65±.33_{cd}$
10.0	$1.50±.28_{bc}$	$3.50±.28_a$	$5.75±.62_a$	$10.75±.47_b$	$0.91±.01_b$	$.387±.02_{bc}$	$9.77±.37_d$	$4.16±.29_d$
15.0	$3.25±.25_d$	$5.75±.47_b$	$5.75±.47_a$	14.75±.25 c	$0.91±.01_b$	$.414±.01_c$	$13.42±.13_e$	$6.10±.11_e$
20.0	$2.00±.00_c$	0.00±.00	0.00±.00	$2.00±.00_a$	$1.21±.03_c$	$.936±.01_d$	$2.42±.06_a$	$1.87±.03_a$

– Explants derived from propagation medium: MS Medium + 3% sucrose + 0.25 mg/l GA_3 + 0.01 mg/l NAA + 2 mg/l Ca-Pantothanate + 1.0 mg/l Thiamine-HCl

– Tuberization medium used: MS Medium + 8% sucrose + 5 mg/l BAP + 500 mg/l CCC + various levels of $AgNO_3$

*Values with the same superscript letter are not significantly different at $P>0.05$ (Duncan's Multiple range test)

Table 31: Effect of various levels of $AgNO_3$ on microtuber production of potato. var. HR (Data recorded after 60 days)

Conc. of $AgNO_3$ (mg/l)	NO. of tubers			Total no. of tubers Mean±S.E.*	Average diameter (cm) Mean± S.E.*	Average Fresh Weight (gm) Mean± S.E.*	Total diameter (cm) Mean± S.E.*	Total Fresh Weight (gm) Mean± S.E.*
	A*	B*	C*					
0	$.25\pm.25_a$	$3.50\pm.a$	$5.25\pm.25_b$	$9.00\pm.40_b$	$.83\pm.00_a$	$.317\pm.01_a$	$7.55\pm.36_b$	$2.87\pm.26_b$
0.5	$.25\pm.25_a$	$3.50\pm.28a$	$6.25\pm.47_c$	$10.00\pm.00_c$	$.83\pm.01_a$	$.307\pm.01_a$	$8.30\pm.17_b$	$3.07\pm.18_b$
1.0	$1.25\pm.25_b$	$4.25\pm.25a_b$	$5.50\pm.28_{bc}$	$11.00\pm.00_d$	$.86\pm.01_{ab}$	$.365\pm.01_{ab}$	$9.55\pm.13_c$	$4.02\pm.15_c$
2.5	$1.25\pm.25_b$	$4.25\pm.25a_b$	$5.50\pm.28_{bc}$	$11.00\pm.00_d$	$.87\pm.01_{ab}$	$.371\pm.01_{abc}$	$9.60\pm.11_c$	$4.08\pm.14_c$
5.0	$1.50\pm.28_{bc}$	$4.25\pm.25_b$	$5.25\pm.25_b$	$11.25\pm.25_d$	$.88\pm.00_{ab}$	$.407\pm.01_{bcd}$	$9.97\pm.24_c$	$4.58\pm.16_{cd}$
7.5	$2.0\pm.00_{bcd}$	$4.75\pm.25b$	$4.75\pm.25_b$	$11.50\pm.28_{de}$	$.88\pm.02_{ab}$	$.433\pm.00_{cd}$	$10.10\pm.24_c$	$4.98\pm.13_{de}$
10.0	$2.25\pm.25_{cd}$	$5.00\pm.40b$	$5.00\pm.00_b$	$12.25\pm.47_{ef}$	$.93\pm.00_{bc}$	$.446\pm.00_d$	$11.37\pm.42_d$	$5.46\pm.19_{ef}$
15.0	$2.75\pm.25_d$	$5.75\pm.25_b$	$4.50\pm.28_{bc}$	$13.00\pm.40_f$	$.93\pm.00_{bc}$	$.482\pm.01_d$	$12.07\pm.42_d$	$6.27\pm.29_f$
20.0	$1.75\pm.47_{bc}$	$00\pm.00$	$2.00\pm.40_a$	$3.50\pm.50_a$	$.98\pm.04_c$	$.532\pm.04_e$	$3.42\pm.44_a$	$1.85\pm.26_a$

– Explants derived from propagation medium: MS Medium + 3% sucrose + 0.25 mg/l GA_3 + 0.05 mg/l NAA + 2 mg/l Ca-Pantothanate + 1.0 mg/l Thiamine-HCl

– Tuberization medium used: MS Medium + 8% sucrose + 7.5 mg/l BAP + 750 mg/l CCC + various levels of $AgNO_3$

*Values with the same superscript letter are not significantly different at P>0 05 (Duncan's Multiple range test)

Table 32: Effect of various levels of $AgNO_3$ on microtuber production of potato var. DR (Data recorded after 60 days)

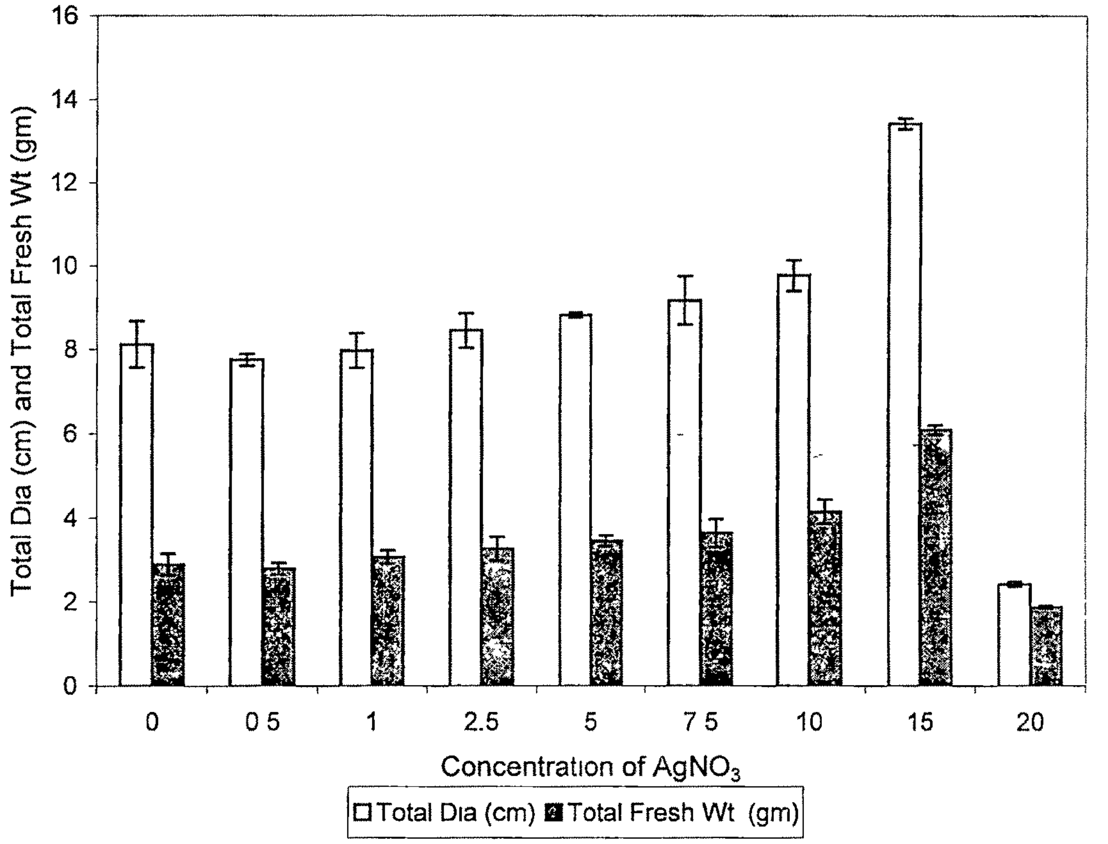
Conc. of $AgNO_3$ (mg/l)	No. of tubers			Total no. of tubers Mean± S.E.*	Average diameter (cm) Mean± S.E.*	Average Fresh Weight (gm) Mean± S.E.*	Total diameter (cm) Mean± S.E.*	Total Fresh Weight (gm) Mean± S.E.*
	A*	B*	C*					
0	$.50 \pm .28_{a}$	$4.00 \pm .40_{b}$	$3.50 \pm .28_{bc}$	$7.75 \pm .47_{b}$	$.92 \pm .02_{ab}$	$.326 \pm .03_{a}$	$7.12 \pm .33_{b}$	$2.51 \pm .24_{a}$
0.5	$.75 \pm .25_{ab}$	$4.75 \pm .47_{b}$	$2.75 \pm .25_{b}$	$8.25 \pm .25_{bc}$	$.97 \pm .05_{ab}$	$.346 \pm .02_{a}$	$8.00 \pm .36_{bc}$	$2.84 \pm .11_{a}$
1.0	$1.50 \pm .28_{bc}$	$5.00 \pm .40_{b}$	$3.00 \pm .40_{bc}$	$9.50 \pm .64_{bcd}$	$.93 \pm .01_{ab}$	$.426 \pm .02_{ab}$	$8.80 \pm .51_{bcd}$	$4.02 \pm .27_{b}$
2.5	$1.50 \pm .28_{bc}$	$4.75 \pm .25_{b}$	$3.25 \pm .25_{bc}$	$9.50 \pm .28_{bcd}$	$.92 \pm .01_{ab}$	$.429 \pm .01_{ab}$	$8.80 \pm .42_{bcd}$	$4.07 \pm .15_{b}$
5.0	$1.50 \pm .28_{bc}$	$4.25 \pm .62_{b}$	$4.25 \pm .47_{bc}$	$10.00 \pm .81_{cd}$	$.90 \pm .01_{a}$	$.443 \pm .02_{ab}$	$9.02 \pm .64_{cd}$	$4.40 \pm .28_{bc}$
7.5	$1.75 \pm .25_{cd}$	$4.75 \pm .47_{b}$	$4.50 \pm .28_{c}$	$11.00 \pm .40_{d}$	$.91 \pm .01_{a}$	$.455 \pm .00_{ab}$	$10.07 \pm .31_{de}$	$5.01 \pm .20_{c}$
10.0	$2.75 \pm .25_{e}$	$4.50 \pm .28_{b}$	$4.00 \pm .91_{bc}$	$11.25 \pm .47_{d}$	$.96 \pm .01_{ab}$	$.524 \pm .02_{bc}$	$10.82 \pm .28_{e}$	$5.87 \pm .19_{d}$
15.0	$5.00 \pm .40_{f}$	$5.25 \pm .47_{b}$	$3.75 \pm .25_{bc}$	$14.00 \pm .40_{e}$	$1.02 \pm .00_{b}$	$.699 \pm .02_{c}$	$14.35 \pm .29_{f}$	$9.37 \pm .24_{e}$
20.0	$2.50 \pm .28_{de}$	$.50 \pm .50_{a}$	$1.00 \pm .70_{a}$	$4.0 \pm 1.3_{a}$	$1.17 \pm .06_{c}$	$.872 \pm .10_{d}$	$4.50 \pm 1.29_{a}$	$3.10 \pm .59_{a}$

– Explants derived from propagation medium: MS Medium + 3% sucrose + 0.25 mg/l GA_3 + 0.05 mg/l NAA + 2 mg/l Ca-Pantothanate + 1.0 mg/l Thiamine-HCl + 0.5 mg/l $AgNO_3$

– Tuberization medium used: MS Medium + 8% sucrose + 5 mg/l BAP + 500 mg/l CCC + various levels of $AgNO_3$

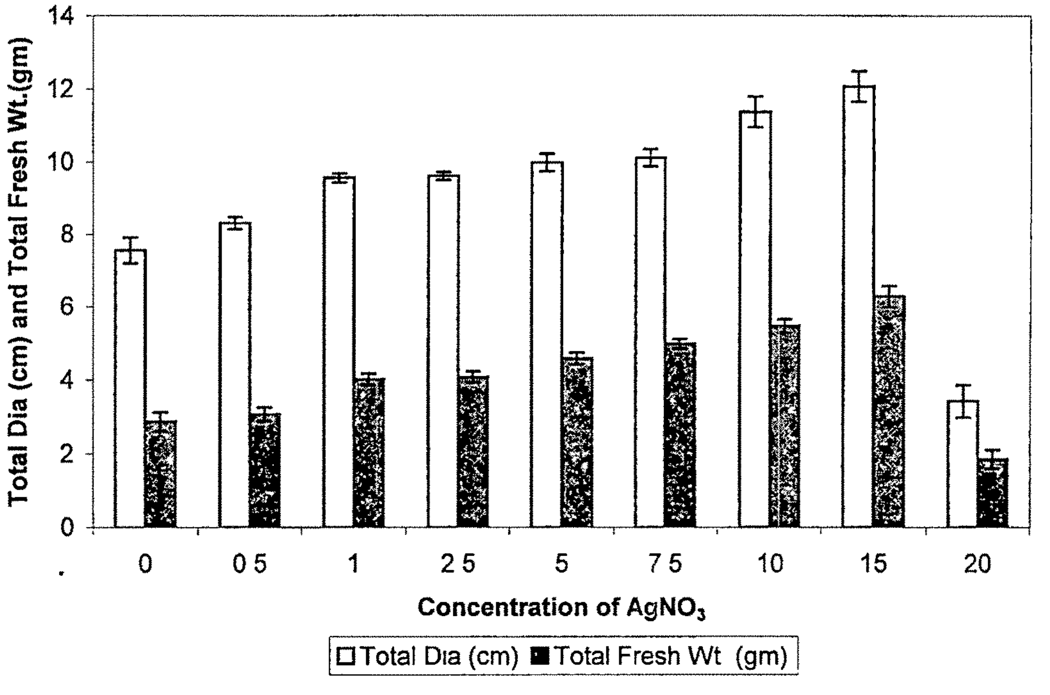
*Values with the same superscript letter are not significantly different at $P>0.05$ (Duncan's Multiple range test)

Chart-1: Effect of various levels of $AgNO_3$ on microtuber production of potato. var DR



- Explants derived from propagation medium without $AgNO_3$
- Tuberization medium with various levels of $AgNO_3$

Chart-2: Effect of various levels of $AgNO_3$ on microtuber production of potato. var HR



- Explants derived from propagation medium without $AgNO_3$
- Tuberization medium with various levels of $AgNO_3$

Plate 28. Potato microtuber production :

(a) to (d) Shoots derived from propagation medium without any $AgNO_3$ used for tuberization and tuberization medium was supplemented with various levels of $AgNO_3$ (2.5, 5, 10, 15 mg/l) var DR.

In all figures, the bar = 1 Cm


a
b
c
d

Plate 29. (a) to (d):Harvested microtubers produced from tuberization medium supplemented with $AgNO_3$ var DR.

In all figures, the bar = 1 Cm


a
b
c
d

Plate 30 Potato microtuber production:

(a) to (d): Shoots derived from propagation medium without any $AgNO_3$ and used for tuberization and tuberization medium was supplemented with various levels of $AgNO_3$ (5, 10, 15, 20 mg/l). var HR.

In all figures, the bar = 1 Cm


a
b
c
d

Plate 31. (a) to (c): Harvested microtubers produced from tuberization medium supplemented with $AgNO_3$ after graded them to A (>0.700 gms), B (>0.400) and C (< 400 gms) categories. var HR.

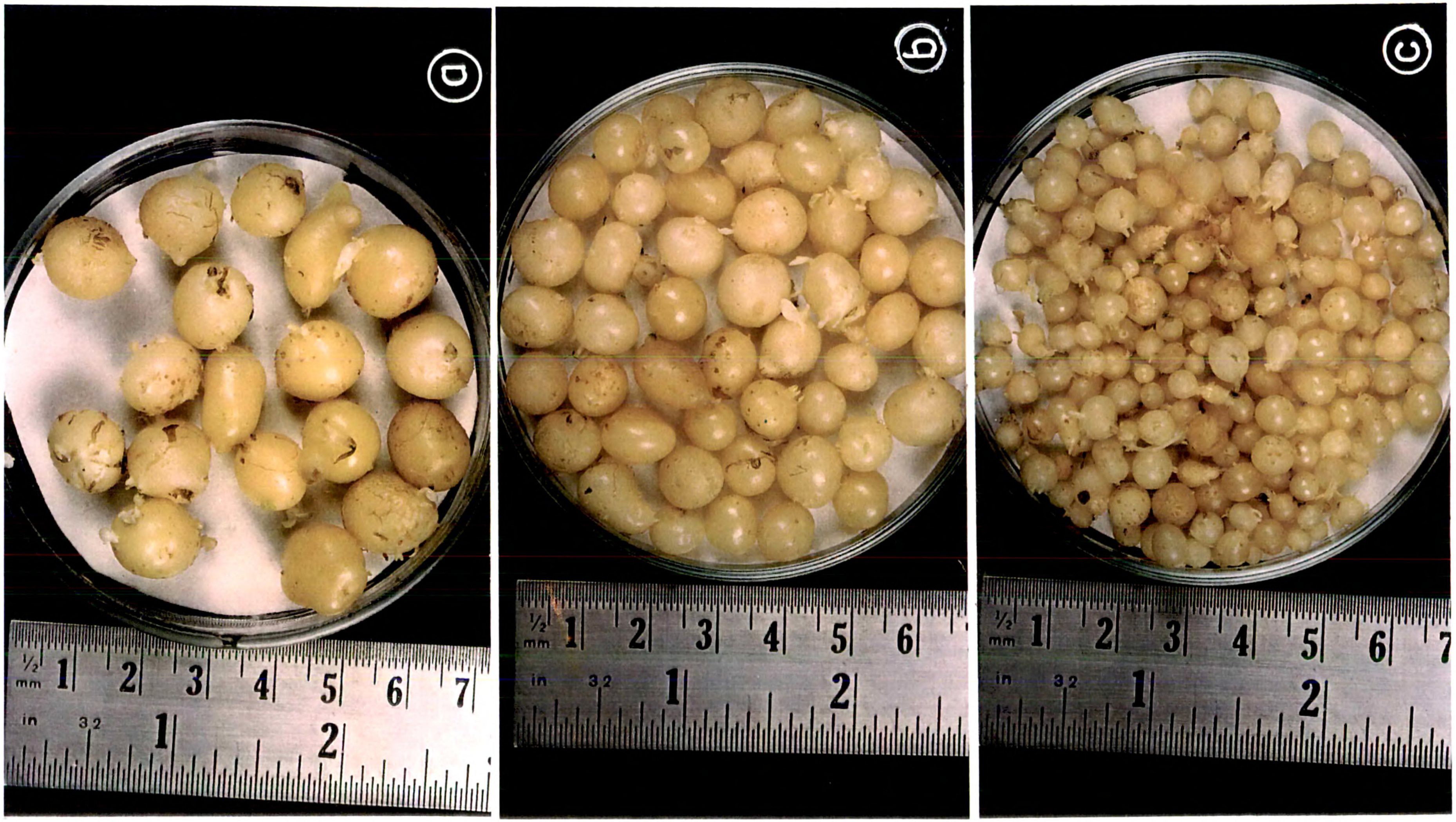

a
b
c

Plate 32. (a) to (d) Shoots derived fiom propagatıon medıum supplemented with 0.5 mg/l $AgNO_3$ used for tuberization and tuberizatıon medium was supplemented with varıous levels of $AgNO_3$ mg/l (5, 10, 15, 20) var DR.

In all figures, the bar = 1 Cm

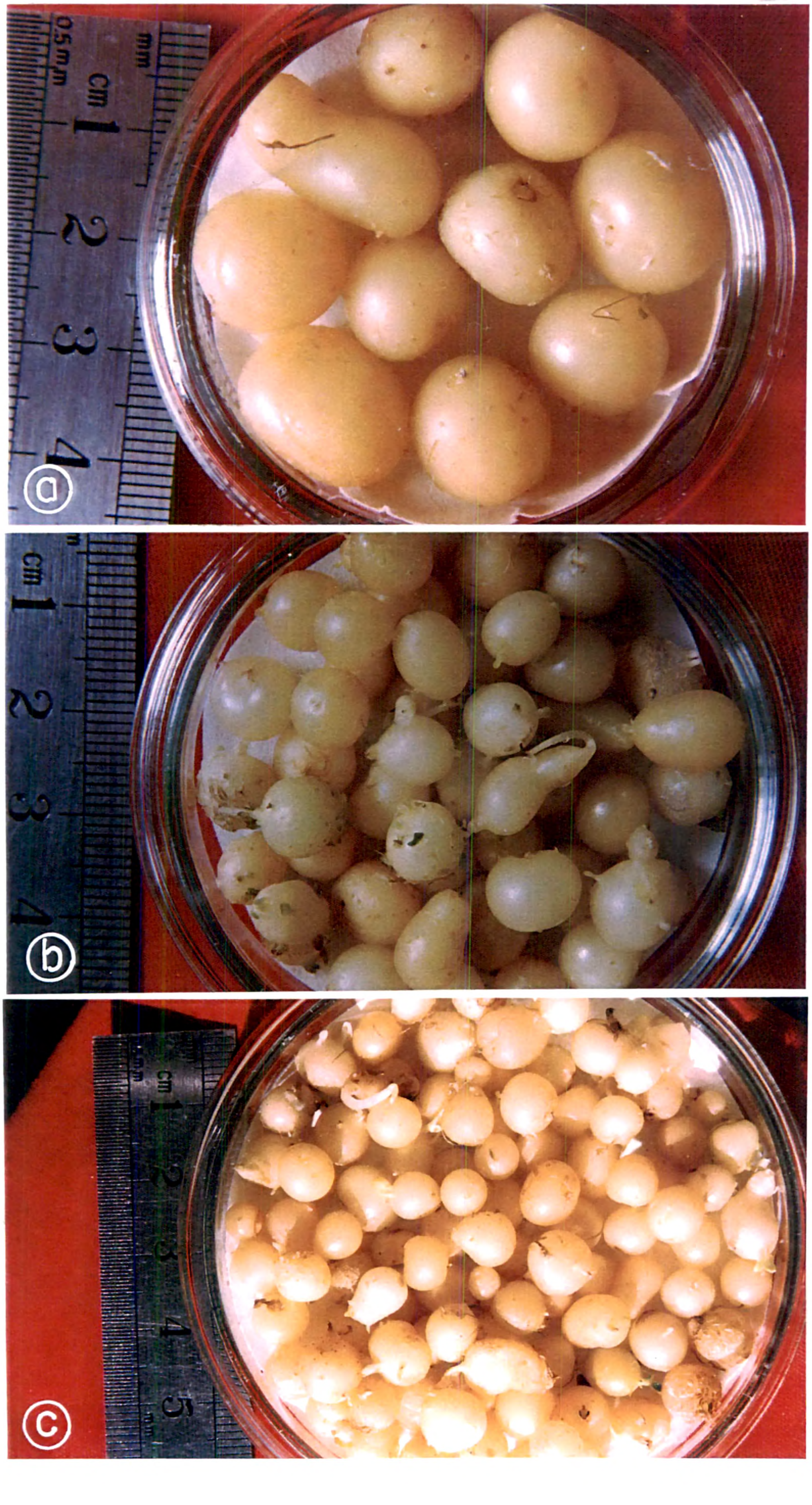

a
b
c
d

Plate 33. Harvested microtubers produced from tuberization medium supplemented with $AgNO_3$ using shoots derived from propagation medium supplemented with 0.5 mg/l $AgNO_3$. var DR.

(a) 'A' grade tuber.

(b) 'B' grade tuber

(c) 'C' grade tuber.



These tubers are white, and with 3-5 eyes. Var HR also showed a response to $AgNO_3$ similar to that of var DR with respect to all the parameters examined (Table:33; Plate: 34 and 35; Chart: 3 and 4).

Shoots used for tuberization, derived from liquid propagation medium supplemented with 5.0 mg/l $AgNO_3$

When the concentration of $AgNO_3$ in the propagation medium was raised to 5.0 mg/l, production of healthy shoots with green leaves, having wider leaf lamina was observed. However, the number of nodes per shoot got significantly reduced (Table:20 and 21). When these shoots were subjected to tuberization medium having increasing concentration of $AgNO_3$, the number of tubers and their size did not show any marked difference as compared to control (Table: 34 and 35; Plate: 36, 37 and 38; Chart : 5 and 6).

Shoots used for tuberization, derived from liquid propagation medium supplemented with 10 mg/l $AgNO_3$ and varying levels of GA_3

Shoots derived from propagation medium supplemented with 10 mg/l $AgNO_3$ and varying levels of GA_3 were used for tuberization. When the concentration of GA_3 was increased from 0.25 mg/l to 10.0 mg/l in the propagation medium it adversely affected the microtuber production in the tuberization medium. Concentration of GA_3 above 4.0 mg/l in the propagation medium completely inhibited tuberization in both the varieties (Plate· 39 and 40). Shoots derived from propagation medium containing 0.25 mg/l GA_3 and 10 mg/l $AgNO_3$ when cultured in tuberization medium supplemented with $AgNO_3$ (10 mg/l) produced the maximum number of tubers in var DR and HR (Table:36 and 37).

Storage and shelf life of potato microtubers

Microtubers of var DR and var HR produced on liquid medium were graded into A (>0.700 gm), B (>0.400 gm) and C (< 0.400 gm) categories (Plate: 41 and 42) and stored at 4° C for 30, 60, 90 120, 150 and 180 days in petridishes sealed with

Table - 33: Effect of various levels of $AgNO_3$ on microtuber production of potato var HR (Data recorded after 60 days)

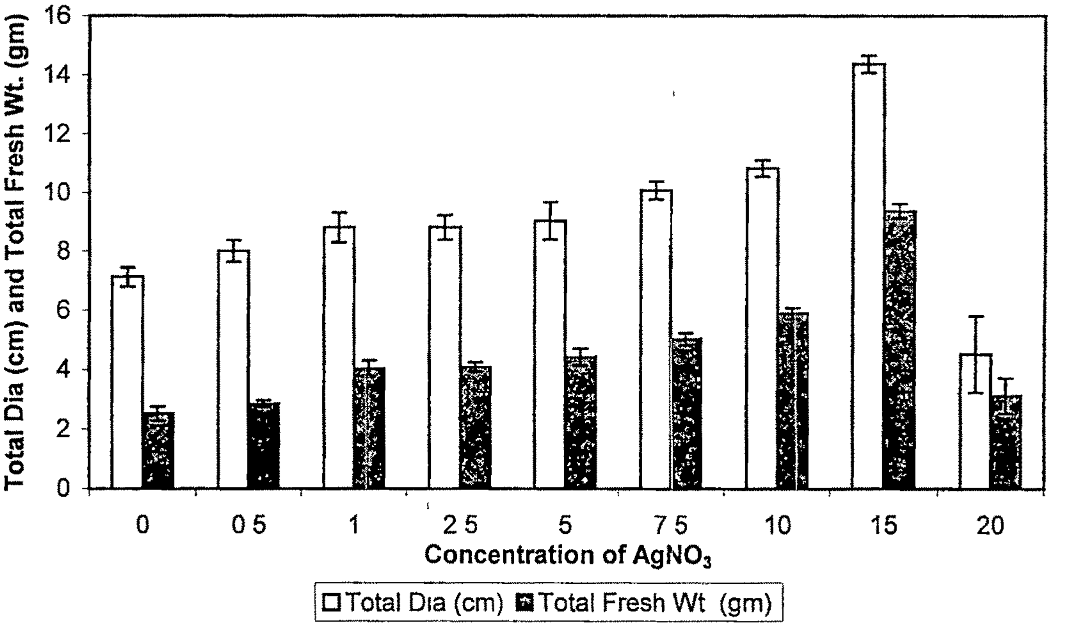
Conc. of $AgNO_3$ (mg/l)	No. of tubers			Total no. of tubers Mean± S.E.*	Average diameter (cm) Mean± S.E.*	Average Fresh Weight (gm) Mean± S.E.*	Total diameter (cm) Mean± S.E.*	Total Fresh Weight (gm) Mean± S.E.*
	A*	B*	C*					
0	$.25\pm.25_a$	$3.25\pm.25_b$	$5.75\pm.25_b$	$9.25\pm.25_{bc}$	$.82\pm.00_a$	$.302\pm.01_a$	$7.57\pm.17_b$	$2.811\pm.24_a$
0.5	$.50\pm.28_{ab}$	$3.50\pm.28_{bc}$	$4.75\pm.47_b$	$8.75\pm.25_b$	$.87\pm.01_a$	$.335\pm.01_{ab}$	$7.57\pm.21_b$	$2.944\pm.21_a$
1.0	$1.25\pm.25_{bc}$	$4.75\pm.47_d$	$4.25\pm.25_b$	$10.25\pm.47_{bcd}$	$.93\pm.01_a$	$.386\pm.01_{abc}$	$9.45\pm.27_c$	$3.956\pm.20_b$
2.5	$1.25\pm.25_{cd}$	$4.50\pm.28_{cd}$	$4.25\pm.75_b$	$10.50\pm.64_{cd}$	$.91\pm.01_a$	$.413\pm.01_{abc}$	$9.55\pm.42_c$	$4.305\pm.09_{bc}$
5.0	$2.25\pm.25_d$	$4.50\pm.28_{cd}$	$4.25\pm.47_b$	$11.00\pm.81_d$	$.94\pm.01_a$	$.452\pm.00_{abcd}$	$10.25\pm.76_{cd}$	$4.972\pm.37_{cd}$
7.5	$2.25\pm.25_d$	$4.50\pm.28_{cd}$	$4.00\pm.40_b$	$10.75\pm.25_{cd}$	$.94\pm.01_a$	$.477\pm.02_{bcd}$	$10.12\pm.28_{cd}$	$5.139\pm.32_d$
10.0	$2.50\pm.28_d$	$4.75\pm.47_d$	$4.50\pm.64_b$	$11.75\pm.25_d$	$.95\pm.01_a$	$.503\pm.02_{cd}$	$11.20\pm.24_d$	$5.900\pm.23_e$
15.0	$6.00\pm.40_e$	$4.75\pm.25_d$	$4.75\pm.85_b$	$15.25\pm.47_e$	$1.02\pm.00_{ab}$	$.720\pm.01_d$	$15.55\pm.46_e$	$10.800\pm.19_f$
20.0	$2.50\pm.28_d$	$.25\pm.25_a$	$.75\pm.47_a$	$3.50\pm.64_a$	$1.21\pm.21_b$	$.878\pm.14_e$	$3.95\pm.55_a$	$2.862\pm.35_a$

– Explants derived from propagation medium: MS Medium + 3% sucrose + 0.25 mg/l GA_3 + 0.05 mg/l NAA + 2 mg/l Ca-Pantothanate + 1.0 mg/l Thiamine-HCl + 0.5 mg/l $AgNO_3$

– Tuberization medium used: MS Medium + 8% sucrose + 7.5 mg/l BAP + 750 mg/l CCC + various levels of $AgNO_3$

*Values with the same superscript letter are not significantly different at P>0 05 (Duncan's Multiple range test)

Chart-3: Effects of various levels of $AgNO_3$ on microtuber production of potato. var DR



- Explants derived from propagation medium with 0 5 mg/l $AgNO_3$
- Tuberization medium with various levels of $AgNO_3$

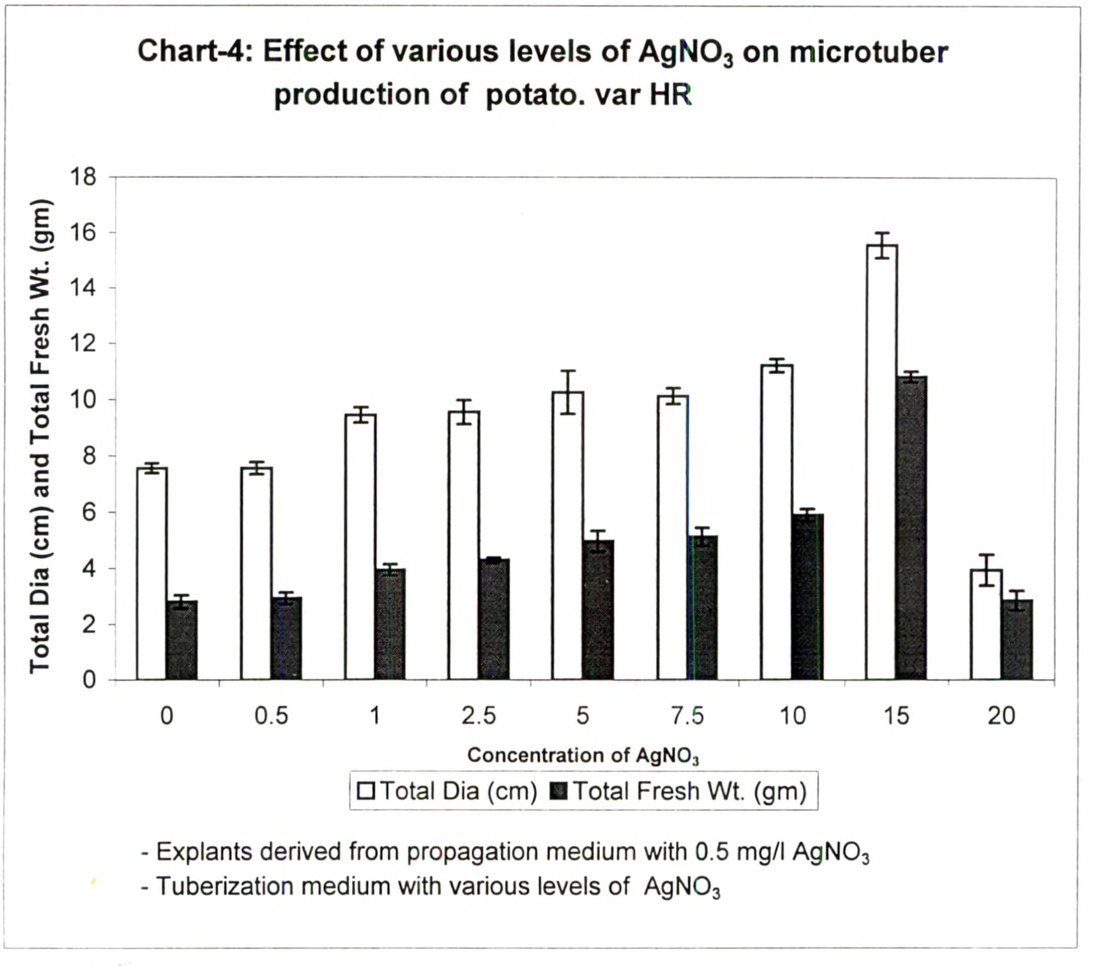


Chart-4: Effect of various levels of AgNO3 on microtuber production of potato. var HR
Total Dia (cm) and Total Fresh Wt. (gm)
18
16
14
12
10
8
6
4
2
0
0
0.5
1
2.5
5
7.5
10
15
20
Concentration of AgNO3
Total Dia (cm)
Total Fresh Wt. (gm)
- Explants derived from propagation medium with 0.5 mg/l AgNO3
- Tuberization medium with various levels of AgNO3

Table 34: Effect of various levels of $AgNO_3$ on microtuber production of potato. var. DR (Data recorded after 60 days)

Conc. of $AgNO_3$ (mg/l)	No. of tubers			Total no. of tubers Mean±S.E.*	Average diameter (cm) Mean± S.E.*	Average Fresh Weight (gm) Mean± S.E.*	Total diameter (cm) Mean± S.E.*	Total Fresh Weight (gm) Mean± S.E.*
	A*	B*	C*					
0	$0.60 \pm 0.24_a$	$4.2 \pm 0.58_a$	$3.4 \pm 0.24_c$	$8.0 \pm 0.63_b$	$0.94 \pm 0.05_a$	$0.413 \pm 0.02_a$	$7.48 \pm 0.43_b$	$3.29 \pm 0.28_b$
0.5	$1.0 \pm 0.00_{ab}$	$4.2 \pm 0.48_a$	$3.0 \pm 0.54_c$	$8.2 \pm 0.80_b$	$0.92 \pm 0.01_a$	$0.432 \pm 0.01_a$	$7.54 \pm 0.68_b$	$3.50 \pm 0.25_b$
1.0	$1.4 \pm 0.24_{bc}$	$3.0 \pm 0.54_a$	$3.6 \pm 0.40_c$	$8.0 \pm 0.31_b$	$0.90 \pm 0.02_a$	$0.426 \pm 0.01_a$	$7.26 \pm 0.37_b$	$3.41 \pm 0.23_b$
2.5	$1.6 \pm 0.24_{bcd}$	$3.2 \pm 0.86_a$	$3.6 \pm 0.40_c$	$8.4 \pm 0.67_b$	$0.90 \pm 0.01_a$	$0.452 \pm 0.02_a$	$7.56 \pm 0.61_b$	$3.74 \pm 0.15_{bc}$
5.0	$1.6 \pm 0.24_{bcd}$	$3.6 \pm 0.24_a$	$2.6 \pm 0.24_{bc}$	$8.6 \pm 0.50_b$	$0.95 \pm 0.00_a$	$0.468 \pm 0.01_a$	$8.24 \pm 0.50_b$	$4.01 \pm 0.20_{bc}$
7.5	$2.0 \pm 0.00_{cd}$	$3.6 \pm 1.02_a$	$2.0 \pm 0.31_{ab}$	$7.6 \pm 0.74_b$	$0.98 \pm 0.00_a$	$0.483 \pm 0.03_a$	$7.46 \pm 0.74_b$	$3.62 \pm 0.33_b$
10.0	$3.0 \pm 0.31_e$	$3.6 \pm 0.50_a$	$1.2 \pm 0.20_a$	$7.8 \pm 0.58_b$	$1.0 \pm 0.00_a$	$0.576 \pm 0.03_b$	$7.84 \pm 0.56_b$	$4.44 \pm 0.32_d$
15.0	$3.6 \pm 0.24_e$	$3.0 \pm 0.31_a$	$1.2 \pm 0.20_a$	$7.8 \pm 0.20_b$	$0.99 \pm 0.00_a$	$0.641 \pm 0.03_b$	$7.78 \pm 0.20_b$	$4.97 \pm 0.17_d$
20.0	$2.2 \pm 0.20_d$	$0.0 \pm 0.00_a$	0.0 ± 0.00	$2.2 \pm 0.20_a$	$1.39 \pm 0.19_b$	$1.04 \pm 0.00_c$	$3.22 \pm 0.82_a$	$2.29 \pm 0.18_a$

– Explants derived from propagation medium: MS Medium + 3% sucrose + 0.25 mg/l GA_3 + 0.01 mg/l NAA + 2 mg/l Ca-Pantothanate + 1.0 mg/l Thiamine-HCl + 5.0 mg/l $AgNO_3$

– Tuberization medium used: MS Medium + 8% sucrose + 5 mg/l BAP + 500 mg/l CCC + various levels of $AgNO_3$

*Values with the same superscript letter are not significantly different at $P>0.05$ (Duncan's Multiple range test)

Table 35: Effect of various levels of $AgNO_3$ on microtuber production of potato. var. HR (Data recorded after 60 days)

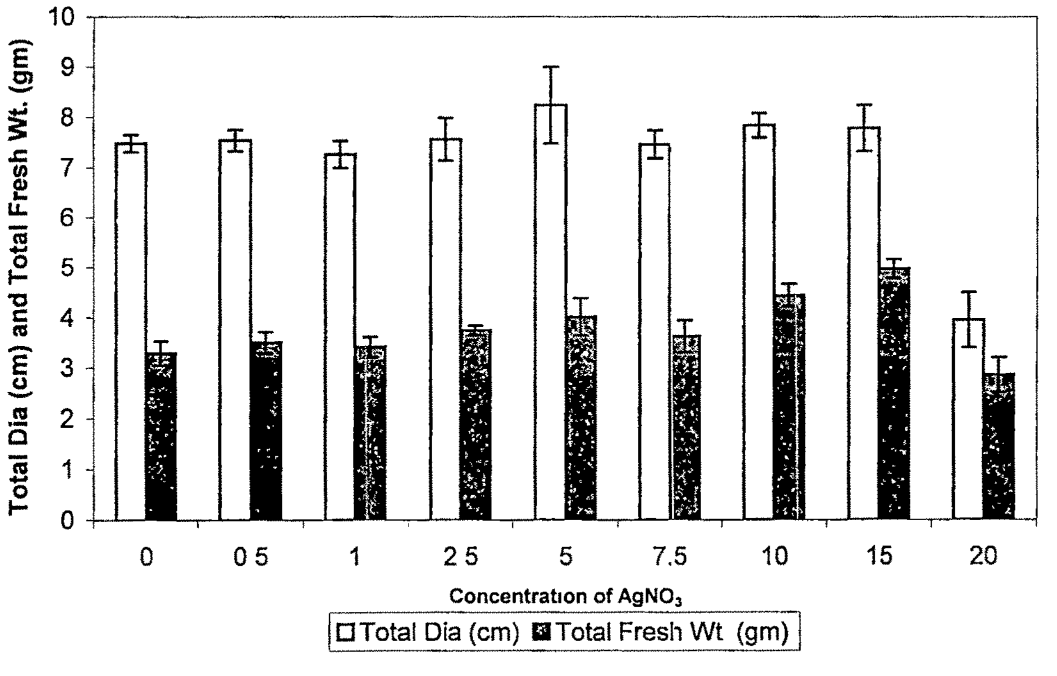
Conc. of $AgNO_3$ (mg/l)	No. of tubers			Total no. of tubers Mean± S.E.*	Average diameter (cm) Mean± S.E.*	Average Fresh Weight (gm) Mean± S.E.*	Total diameter (cm) Mean± S.E.*	Total Fresh Weight (gm) Mean± S.E.*
	A*	B*	C*					
0	$0.66 \pm 0.33_{a}$	$2.0 \pm 0.00_{ab}$	$3.66 \pm 0.88_{bc}$	$6.33 \pm 1.2_{b}$	$0.85 \pm 0\ 01_{a}$	$0.39 \pm 0.02_{a}$	$5.36 \pm 0.95_{ab}$	$2.47 \pm 0.46_{ab}$
0.5	$1.33 \pm 0.33_{abc}$	$2.33 \pm 0.33_{ab}$	$6.0 \pm 1.15_{c}$	$9.66 \pm 1.33_{c}$	$0.84 \pm 0\ 01_{a}$	$0.37 \pm 0.11_{a}$	$8.16 \pm 1.11_{c}$	$3.55 \pm 0.26_{bcd}$
1.0	$1.0 \pm 0.00_{ab}$	$3.33 \pm 0.88_{bcd}$	$2.0 \pm 0.57_{ab}$	$6.33 \pm 0.33_{b}$	$0.97 \pm 0.03_{bc}$	$0.49 \pm 0.36_{ab}$	$6.16 \pm 0.47_{bc}$	$3.12 \pm 0.15_{abcd}$
2.5	$1.66 \pm 0.33_{abc}$	$4.33 \pm 2.08_{cd}$	$3.33 \pm 0.88_{bc}$	$9.33 \pm 0.33_{bc}$	$0.94 \pm 0.21_{bc}$	$0.52 \pm 0.10_{bc}$	$8.80 \pm 0.34_{cd}$	$4.87 \pm 0.28_{e}$
5.0	$1.33 \pm 0.33_{abc}$	$3.0 \pm 0.57_{abcd}$	$4.0 \pm 1.0_{bc}$	$8.33 \pm 0.33_{bc}$	$0.91 \pm 0.19_{ab}$	$0.47 \pm 0.02_{ab}$	$7.66 \pm 0.26_{bcd}$	$3.91 \pm 0.35_{cd}$
7.5	$2.0 \pm 0.57_{bc}$	$2.33 \pm 0.33_{ab}$	$5.0 \pm 0.57_{bc}$	$9.33 \pm 1.33_{bc}$	$0.89 \pm 0.01_{ab}$	$0.47 \pm 0.11_{ab}$	$8.33 \pm 1.19_{c}$	$4.40 \pm 0.78_{cd}$
10.0	$2.0 \pm 0.57_{bc}$	$4.66 \pm 0.33_{d}$	$2.66 \pm 1.2_{bc}$	$9.33 \pm 1.45_{c}$	$0.07 \pm 0.02_{c}$	$0.61 \pm 0.06_{bc}$	$10.0 \pm 1.21_{d}$	$5.73 \pm 0.64_{e}$
15.0	$2.33 \pm 0.33_{c}$	$2.66 \pm 0.33_{abc}$	$4.33 \pm 0.33_{bc}$	$9.33 \pm 0.33_{bc}$	$0.94 \pm 0.26_{bc}$	$0.50 \pm 0.01_{bc}$	$8.80 \pm 0.20_{cd}$	$4.71 \pm 0.16_{e}$
20.0	$2.66 \pm 0.33_{c}$	$1.33 \pm 0.33_{a}$	–	$4.0 \pm 0.00_{a}$	$1.09 \pm 0.32_{c}$	$0.80 \pm 0.35_{c}$	$4.36 \pm 0.14_{a}$	$3.23 \pm 0.09_{a}$

– Explants derived from propagation medium: MS Medium + 3% sucrose + 0.25 mg/l GA_3 + 0.05 mg/l NAA + 2 mg/l Ca-Pantothanate + 1.0 mg/l Thiamine-HCl + 5.0 mg/l $AgNO_3$

– Tuberization medium used: MS Medium + 8% sucrose + 7.5 mg/l BAP + 750 mg/l CCC + various levels of $AgNO_3$

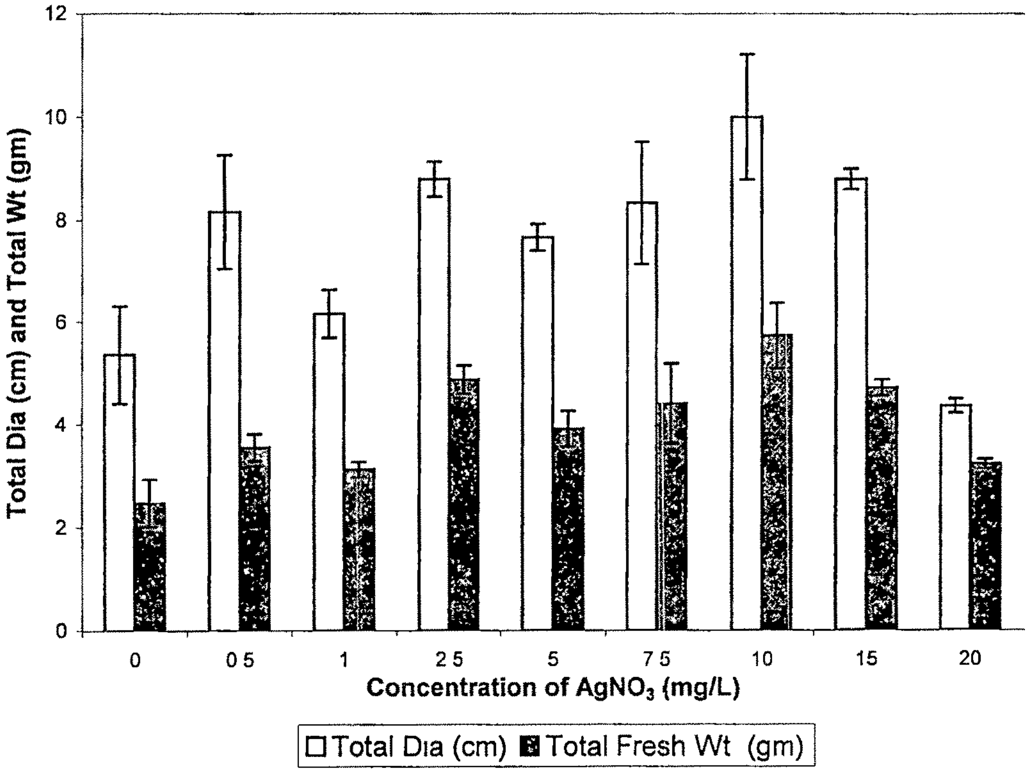
*Values with the same superscript letter are not significantly different at P>0 05 (Duncan's Multiple range test)

Chart-5: Effect of various levels of $AgNO_3$ on microtuber production of potato. var DR



- Explants derived from propagation medium with 5 0 mg/l $AgNO_3$
- Tuberization medium with various levels of $AgNO_3$

Chart-6: Effect of various levels of $AgNO_3$ on microtuber production of potato. var HR



- Explants derived from propagation medium with 5 0 mg/l $AgNO_3$
- Tuberization medium with various levels of $AgNO_3$

Table- 36: Effect of various levels of $AgNO_3$ on microtuber production of potato var DR (Data recorded after 60 days)

Conc. Of GA_3 in propagation medium (mg/l)	Conc.of $AgNO_3$ in tuberization medium (mg/l)	No. of tubers			Total no. of tubers Mean± S.E.*	Average diameter (cm) Mean± S.E.*	Average fresh weight (gm) Mean± S.E.*	Total diameter (cm) Mean± S.E.*	Total fresh weight (gm) Mean± S.E.*
		A*	B*	C*					
0	0	-	-	$3.33\pm.33_{bc}$	$3.33\pm.33_{abc}$	$0.67\pm..01_{a}$	$.185\pm..01_{abc}$	$2.26\pm.21_{abc}$	$.608\pm.01_{a}$
0	0.5	-	-	$3.66\pm0.33_{cd}$	$3.66\pm{}_{.abc}$	$0.7\pm..00_{a}$	$0.174\pm..01_{a}$	$2.56\pm.23_{abc}$	$.630\pm.01_{a}$
0	10	-	-	$4.0\pm..57_{cde}$	$4.0\pm.57_{bc}$	$0.68\pm..01_{a}$	$0.176\pm.01_{ab}$	$2.73\pm.40_{abc}$	$.696\pm.08_{a}$
0.25	0	$2.33\pm..33_{a}$	$2.0\pm0.57_{a}$	$4.33\pm..33_{cde}$	$8.66\pm1.2_{d}$	$0.84\pm..01_{b}$	$0.408\pm.02_{f}$	$7.36\pm1.1_{d}$	$3.59\pm.71_{b}$
0.25	0.5	$4.00\pm0.57_{b}$	$2.66\pm..33_{b}$	$2.33\pm..66_{ab}$	$9.00\pm1.5_{d}$	$0.92\pm.01_{c}$	$0.478\pm.01_{g}$	$8.33\pm1.3_{d}$	$4.35\pm.87_{b}$
0.25	10	$5.33\pm..33_{c}$	$3.00\pm..00_{b}$	–	$8.33\pm..33_{d}$	$1.00\pm..01_{d}$	$0.826\pm.07_{h}$	$8.36\pm.27_{d}$	$6.21\pm.18_{c}$
2	0	-	-	$5.00\pm..57_{de}$	$4.33\pm..33_{c}$	$0.7\pm..02_{a}$	$0.253\pm.00_{e}$	$3.5\pm.36_{bc}$	$1.26\pm.11_{a}$
2	0.5	-	-	$5.33\pm.33_{e}$	$5.33\pm.33_{c}$	$0.68\pm0.1_{a}$	$0.219\pm..00_{cde}$	$3.63\pm.24_{c}$	$1.17\pm.01_{a}$
2	10	-	-	$5.00\pm.57_{de}$	$5.0\pm..00_{c}$	$0.65\pm..02_{a}$	$0.215\pm.00_{cde}$	$3.26\pm.14_{bc}$	$1.08\pm.01_{a}$
4	0	-	-	$2.33\pm..33_{ab}$	$2.33\pm..33_{ab}$	$0.7\pm.03_{a}$	$0.206\pm.00_{abcd}$	$1.66\pm.31_{ab}$	$.481\pm.06_{a}$
4	0.5	-	-	$2.0\pm.57_{a}$	$2.0\pm.57_{ab}$	$0.68\pm.01_{a}$	$0.226\pm.01_{de}$	$1.36\pm.40_{a}$	$.438\pm.10_{a}$
4	10	-	-	$1.66\pm0.33_{a}$	$1.66\pm..33_{a}$	$0.70\pm.00_{a}$	$0.214\pm..01_{dcde}$	$1.16\pm.23_{a}$	$.360\pm.07_{a}$
8	0	-	-	-	-	-	-	-	-
8	0.5	-	-	-	-	-	-	-	-
8	10	-	-	-	-	-	-	-	-
10	0	-	-	-	-	-	-	-	-
10	0.5	-	-	-	-	-	-	-	-
10	10	-	-	-	-	-	-	-	-

– Explants derived from propagation medium: MS Medium + 3% sucrose + 0.01 mg/L NAA + 2 mg/L Ca-Pantothanate + 1.0 mg/L Thiamine-HCl + various levels of GA_3 + 10 mg/L $AgNO_3$

– Tuberization medium used: MS Medium + 8% sucrose + 5 mg/L BAP + 500 mg/L CCC + various levels of $AgNO_3$

*Values with the same superscript letter are not significantly different at P>0.05 (Duncan's Multiple range test)

Table- 37: Effect of various levels of $AgNO_3$ on microtuber production of potato var HR (Data recorded after 45 days)

Conc. of GA_3 in propagation medium (mg/l)	Conc.of $AgNO_3$ in tuberization medium (mg/l)	No.of tubers			Total no. of tubers Mean± S.E.	Average diameter (cm) Mean± S.E.	Average fresh weight (gm) Mean± S.E.	Total diameter (cm) Mean± S.E.	Total fresh weight (gm) Mean± S.E.
		A	B	C					
0	0	-	$1.33±.3_b$	$1.33±.33_a$	$2.66±0.33_{bc}$	$.70±.00_a$	$.276±.00_{abc}$	$1.8±.05_{abc}$	$.734±.01_{ab}$
0	0.5	-	$1.33±.3_b$	$1.33±.33_a$	$2.66± 33_{bc}$	$.80± 00_{bcd}$	$.266±.00_{abc}$	$2.2±.05_{bc}$	$.714±.00_{ab}$
0	10	-	$.66±.33_a$	$1 33±.33_a$	$2.0± 57_{ab}$	$.80±.00_{bcd}$	$.330±.14_{abc}$	$1.7± 05_{abc}$	$.70±.03_{ab}$
0.25	0	$2.0± 00_a$	$2.66± 3_{bc}$	$2.66±.3_{bc}$	$7.32±.33_e$	$89± 01_e$	$.380±.01_{bc}$	$6.6±.08_h$	$2.69±.18_d$
0.25	0.5	$2.66±.33_a$	$3.33±.33_c$	–	$5.99±.33_d$	$.90±.01_{ef}$	$.407±.2_c$	$5.4±.4_g$	$2.63±.109_d$
0.25	10	$4.33±.3_b$	–	–	$4.33±.33_{cd}$	$96±.02_f$	.840±.02 d	$4 1±.04_{ef}$	$3.66±.06_e$
2	0	-	$1.33±.3_b$	$2.66±.3_{bc}$	$4.0±.00_{cd}$	$.85±.02_{cde}$	$.294±.01_{abc}$	$3.40±.10_{de}$	$1.17±.04_{bc}$
2	0.5	-	$2.0±.00_b$	$3.33±.33_c$	$5.33±.33_d$	$82±.05_{bcd}$	$.308±.01_{abc}$	$4.36±.03_{ef}$	$1.63±.02_c$
2	10	-	$2.0±.00_b$	$3.33± 33_c$	$5.33± 33_d$	$.86±.00_{de}$	$.291±.01_{abc}$	$4.60±.25_f$	$1.54±.03_c$
4	0	-	-	$1.66±.66_{ab}$	$1.66±.33_a$	$78±.01_b$	$.233±.00_{ab}$	$1.30±.25_{ab}$	$.38±.06_a$
4	0.5	-	-	$3.0±.57_c$	$3.00±.57_{bc}$	$.78±.00_b$	$.208±.00_a$	$2.33±.43_{bc}$	$.625±.11_a$
4	10	-	-	$3.33±.33_c$	$3.33±.33_c$	$.79±.01_{bc}$	$.225±.00_a$	$2.63±.28_{cb}$	$.747±.06_{ab}$
8	0	-	-	-	-	-	-	-	-
8	0.5	-	-	-	-	-	-	-	-
8	10	-	-	-	-	-	-	-	-
10	0	-	-	-	-	-	-	-	-
10	0.5	-	-	-	-	-	-	-	-
10	10	-	-	-	-	-	-	-	-

– Explants derived from propagation medium: MS Medium + 3% sucrose + 0.05 mg/L NAA + 2 mg/L Ca-Pantothanate + 1.0 mg/L Thiamine-HCl + various levels of GA_3 + 10 mg/L $AgNO_3$

– Tuberization medium used: MS Medium + 8% sucrose + 7.5 mg/L BAP + 750 mg/L CCC + various levels of $AgNO_3$

*Values with the same superscript letter are not significantly different at P>0.05 (Duncan's Multiple range test)

Plate 34. (a) to (d): Shoots derived from propagation medium supplemented with 0.5 mg/l $AgNO_3$ used for tuberization and tuberization medium was supplemented with various levels of $AgNO_3$ mg/l (5, 10, 15, 20) var HR.

In all figures, the bar = 1 Cm

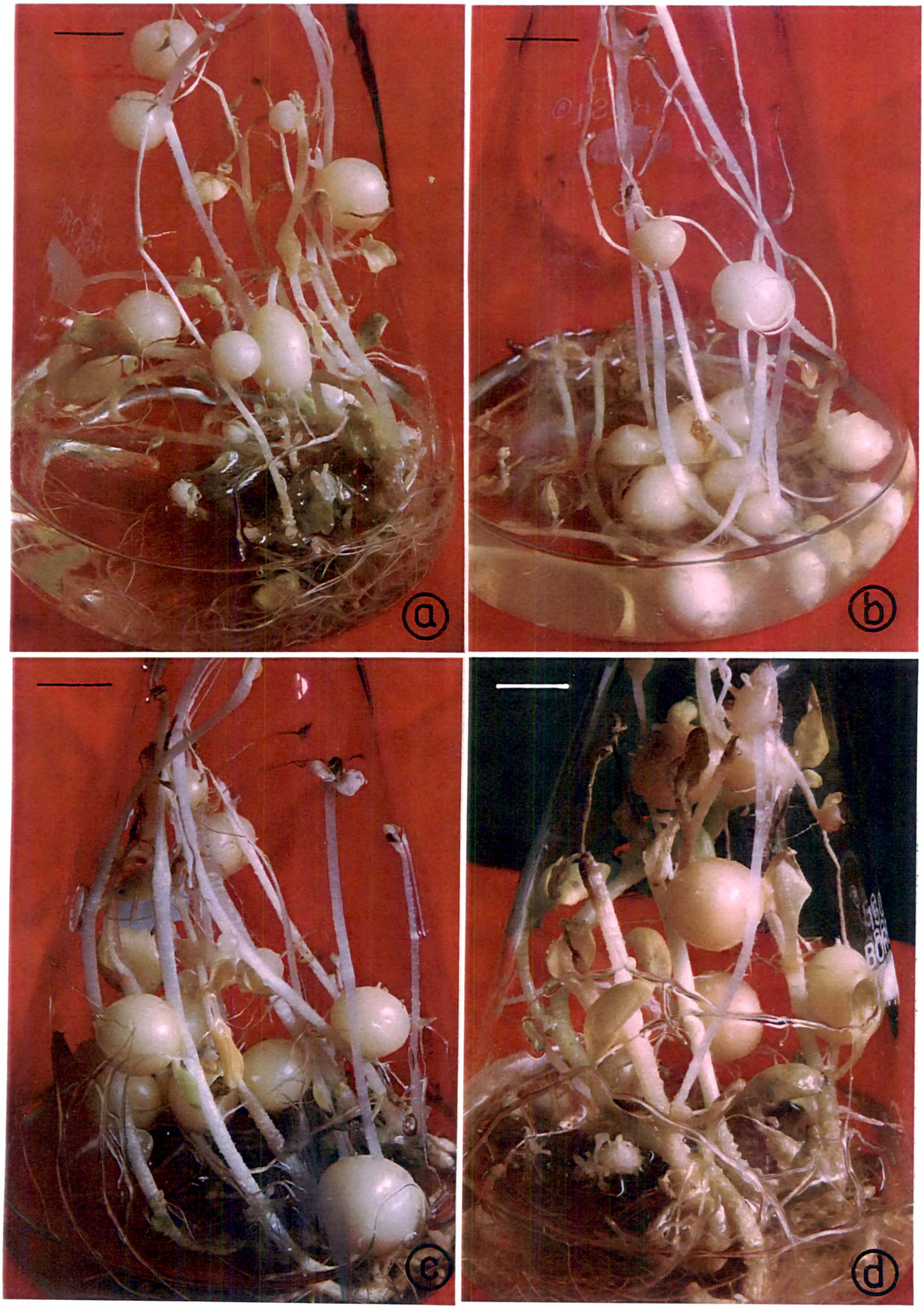

a
b
c
d

Plate 35. Harvested microtubers produced from tuberization medium supplemented with $AgNO_3$ using shoots derived from propagation medium supplemented with 0.5 mg/l $AgNO_3$ var HR.

(a) 'A' grade tuber.

(b) 'B' grade tuber

(c) 'C' grade tuber.

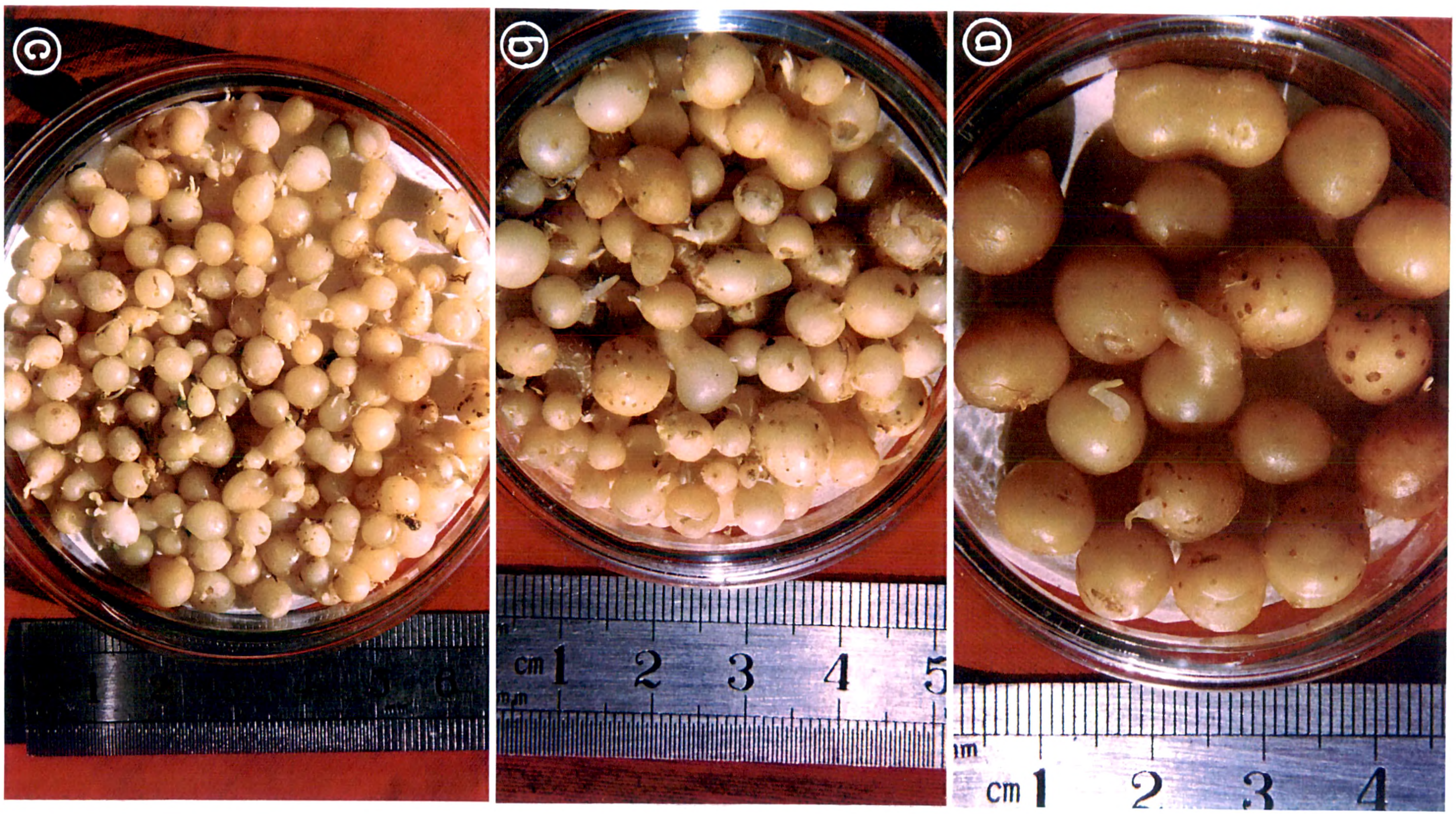


Plate 36. (a) to (d): Stunt shoots derived from propagation medium supplemented with 5 mg/l $AgNO_3$ used for tuberization medium and tuberization medium was supplemented with various levels of $AgNO_3$ mg/l (5, 10, 15, 20) var DR.

In all figures, the bar = 1 Cm

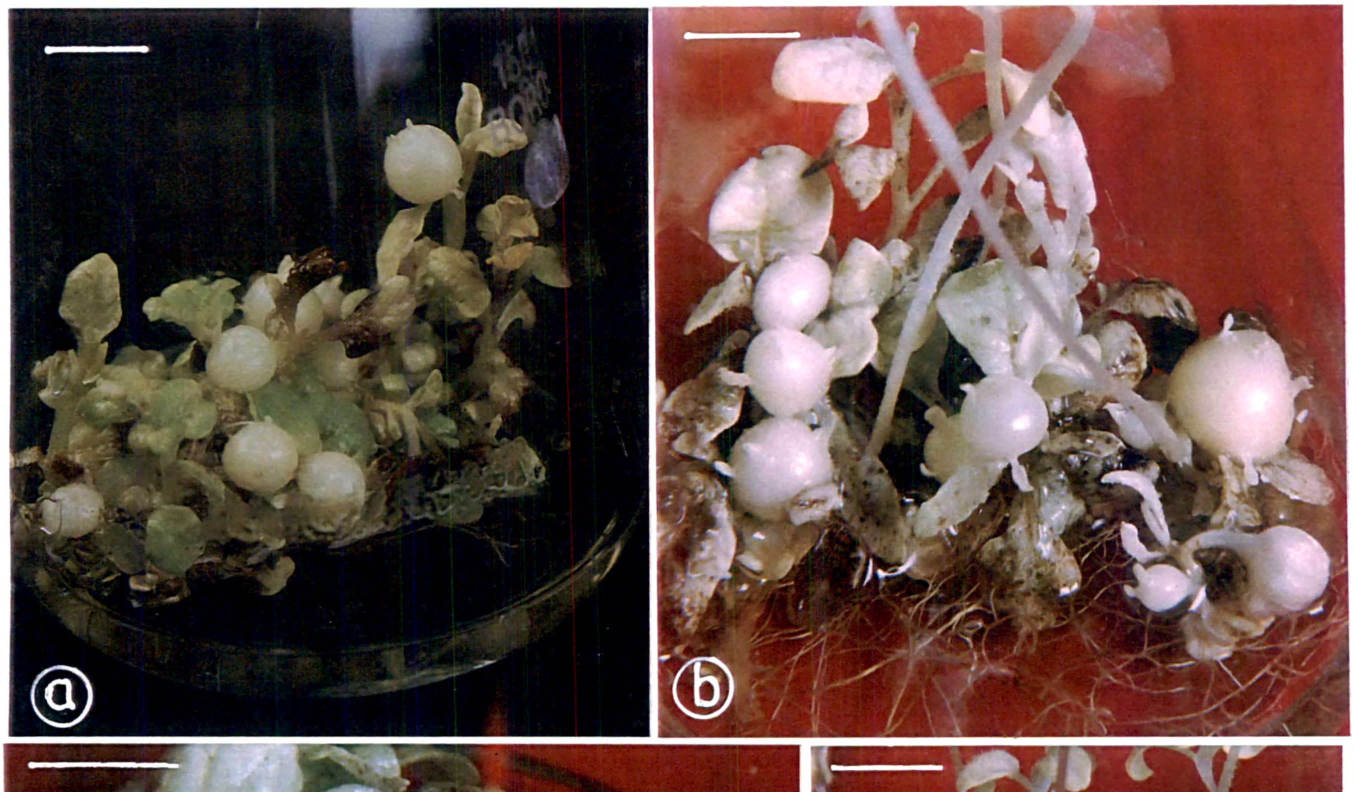

a
b
c
d

Plate 37. (a) to (d): Stunt shoots derived from propagation medium supplemented with 5 mg/l $AgNO_3$ used for tuberization medium and tuberization medium was supplemented with various levels of $AgNO_3$ mg/l (5, 10, 15, 20) var HR.

In all figures, the bar = 1 Cm

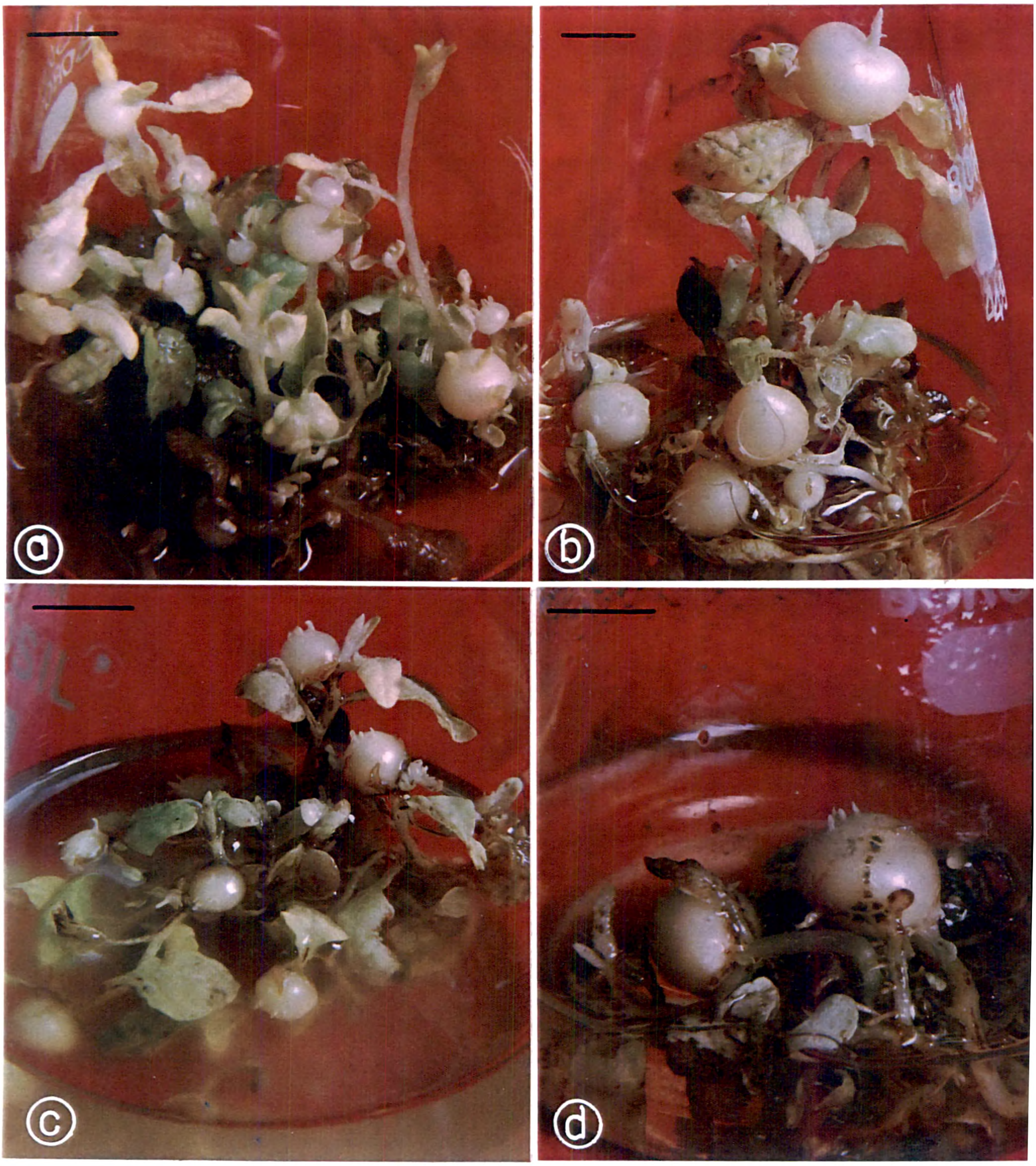


Plate 38. Harvested microtubers derived from high concentration of $AgNO_3$ (20 mg/l) using shoots derived from propagation medium containing high concentration of $AgNO_3$ (5 mg/l).

(a) var HR

(b) var DR

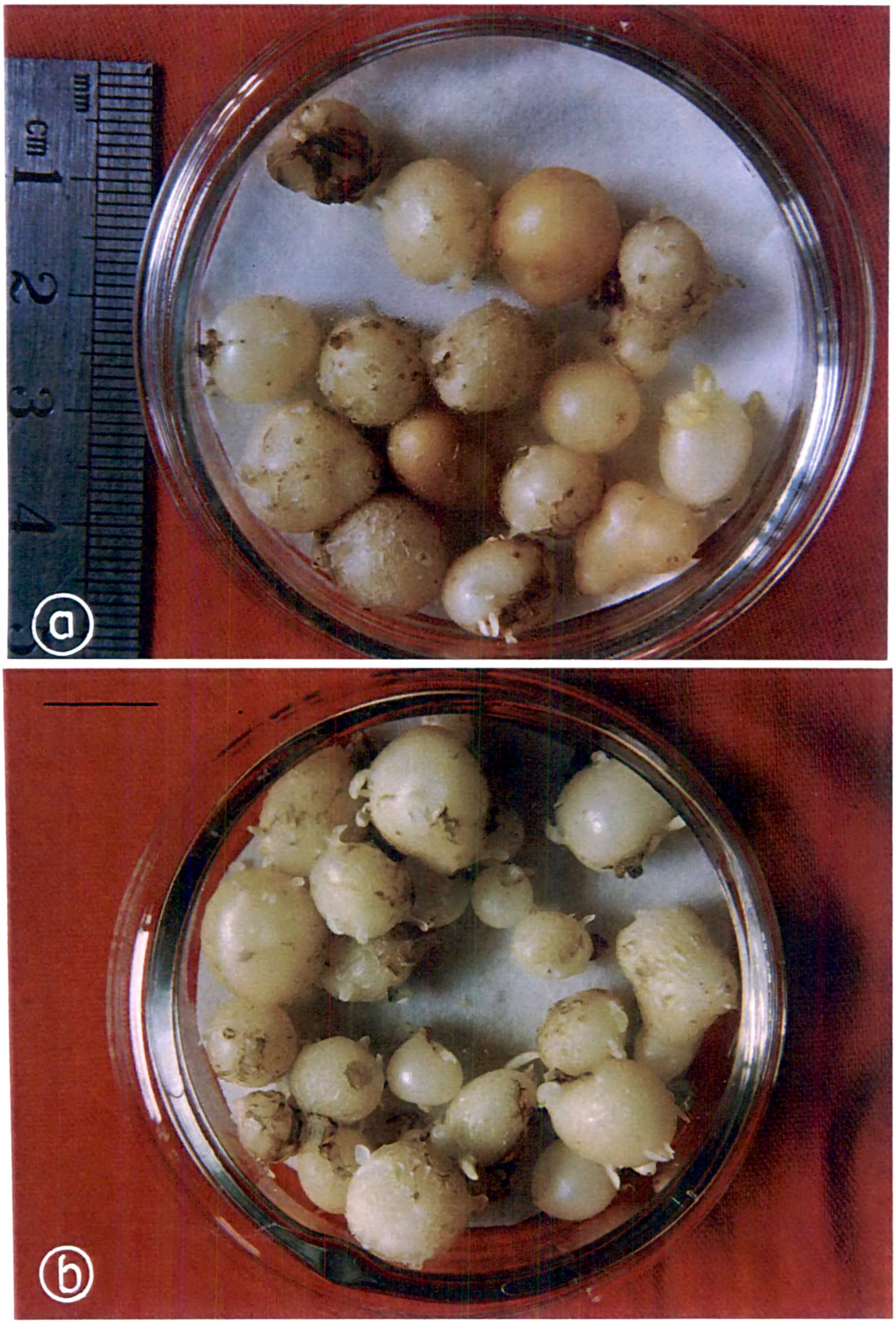

mm
cm 1
2
3
4
a
b

Plate 39. Potato microtuber production:

(a) Shoots derived from propagation medium containing 0.25 mg/l GA_3 and 10 mg/l $AgNO_3$ and tuberization medium was supplemented with 10 mg/l $AgNO_3$.

(b) Shoots derived from propagation medium containing 8 mg/l GA_3 and 10 mg/l $AgNO_3$ and tuberization medium was supplemented with 10 mg/l $AgNO_3$.

– var DR


a
b

Plate 40. Potato microtuber production

(a) Shoots derived from propagation medium containing 0.25 mg/l GA_3 and 10 mg/l $AgNO_3$ and tuberization medium was supplemented with 10 mg/l $AgNO_3$.

(b) Shoots derived from propagation medium containing 8 mg/l GA_3 and 10 mg/l $AgNO_3$ and tuberization medium was supplemented with 10 mg/l $AgNO_3$.

– var HR

In all figures, the bar = 1 Cm

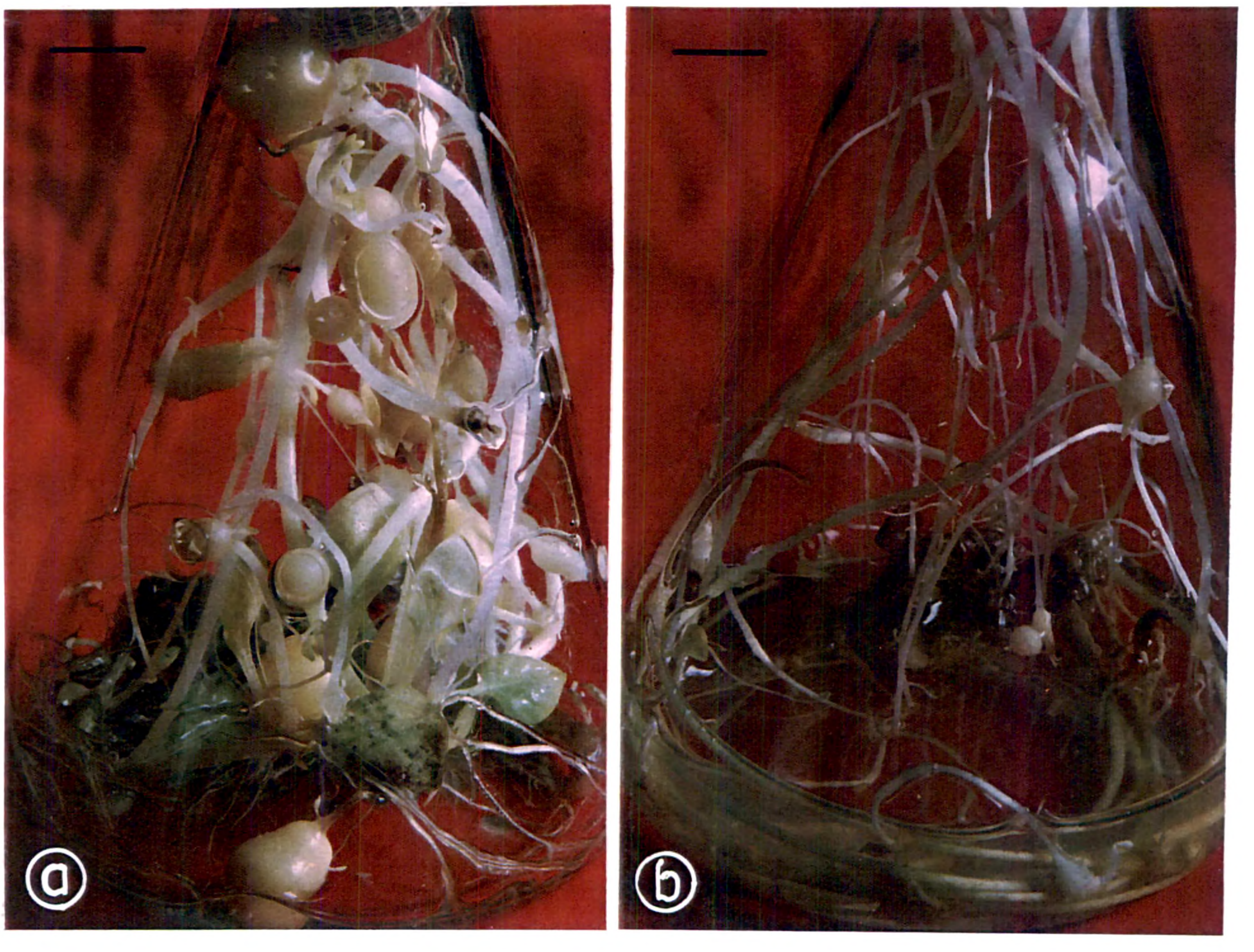

a
b

Plate 41. Different grades of microtubers of var DR showing their size.

(a) Grade A

(b) Grade B

(c and d)Grade C

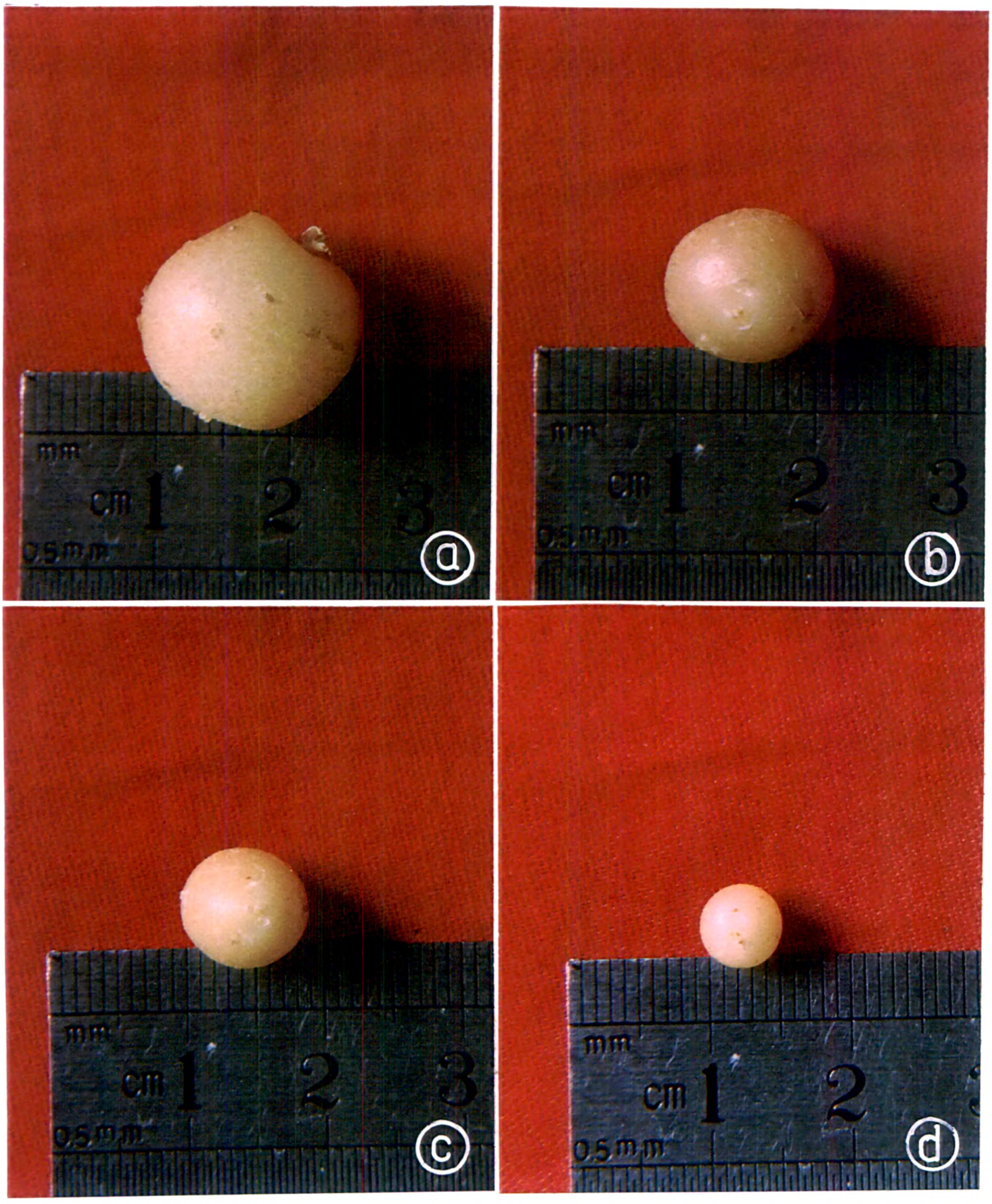
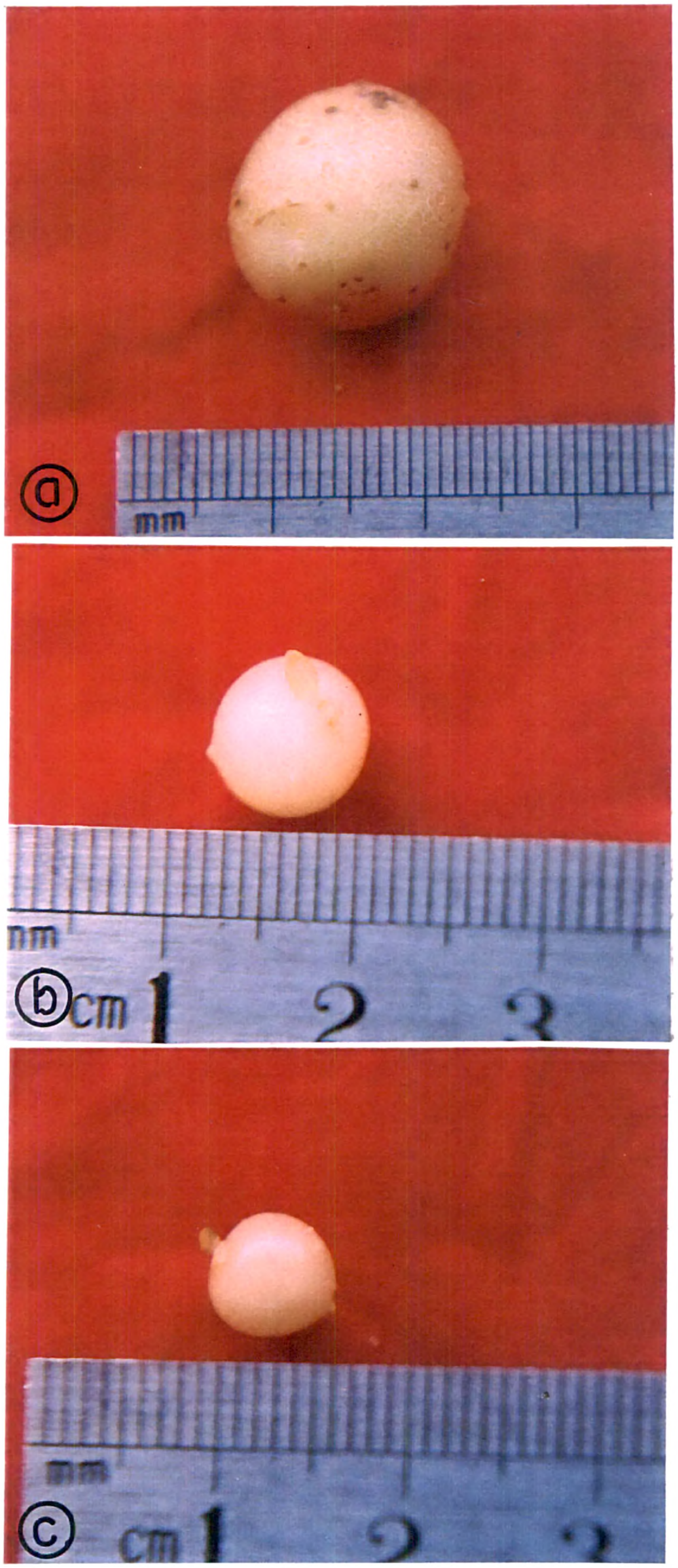

a
b
c
d

Plate 42. Different grades of microtubers of var HR showing their size.

(a) Grade A

(b) Grade B

(c) Grade C


a
mm
b
mm
cm 1 2 3
c
mm
cm 1 2 3

parafilm (Plate: 43). Storability and germinability of these microtubers were tested. Percentage germination of microtubers of A and B grades of var DR increased with the duration of storage and it was highest at the end of 4 months storage (120 days) (Table:38). In case of var HR, A grade tubers gave 100% germination after 120 days while B grade tubers gave cent percent germination after 90 days of storage (Table:39). Both the varieties showed decrease in percentage of germination from 5^{th} month onwards. Microtubers of C grade belonging to both varieties did not exhibit much dormancy. These tubers during their storage sprouted resulting in their shrinkage and failed to establish when planted in the soil (Plate: 44). Tubers of C grades belonging to both the varieties exhibited highest percentage of germination at the end of 2^{nd} and 3^{rd} month of storage respectively (Table: 38 and 39).

Field performance of microtubers

One week before planting microtubers were removed from the refrigerator to diffuse light at room temperature to harden the sprouts. Sixteen tubers (16) each belonging to A, B and C grades of var DR have been employed in the field trials. Tubers belonging to A and B grades germinated within 8-10 days from the date of planting. However, 93% C grade tubers germinated within 5-6 days and showed 87.5% survival rate. In the case of A and B grades of tubers, 100% germination as well as 100% survival rate have been recorded (Table:40). All the three grades of microtubers of var HR also exhibited a performance similar to that of var DR under field conditions (Table:40). Plants emerged from A, B and C grade microtubers had only a single shoot. Among the three grades of microtubers, only A and B grade tubers gave rise to plants with healthy stem and large green leaves (Plate: 45, 46 and 47).

Conversion of microtubers into minitubers

Plants raised from all three grades of microtubers of both varieties produced minitubers within a period of 100 days. Among the three grades tried, best results were obtained with A and B grades in case of both varieties (Table:41). But the total yield per plant emerged from B grade tubers were only about 50 % of A grade. Thus the

Table -38: Storage and germination capacity of different grades (A, B, C) of microtubers. var DR (Harvested in August 2000)

Month of Observation	Germination % / Dormancy Break		
	Grade - A	Grade - B	Grade - C
September 2000	58.00 ± 1.88	66.00 ± 2.44	90.00 ± 3.16
October 2000	78.00 ± 2.00	82.00 ± 2.00	93.00 ± 2.44
November 2000	88.00 ± 3.74	88.00 ± 3.74	93.00 ± 2.44*
December 2000	100.0 ± 0.00	100.0 ± 0.00*	93.00 ± 2.44*•
January 2001	98.00 ± 2.00*	80.00 ± 0.00*	60.00 ± 3.16*•
February 2001	76.00 ± 4.00*	68.00 ± 2.00*	58.00 ± 3.74*•

* Germinated in the storage condition

• Shrinkage of the tuber in storage condition.

Table - 39: Storage and germination capacity of different grades (A, B, C) of microtubers. var HR (Harvested in August 2000)

Month of Observation	Germination % / Dormancy Break		
	Grade - A	Grade - B	Grade - C
September 2000	50.00 ± 5.70	70.00 ± 5.70	93.33 ± 3.30
October 2000	80.00 ± 5.70	90.00 ± 5.70	93.33 ±3.30
November 2000	96.33 ± 3.30	100.0 ± 0.00	100.0 ± 0.00*
December 2000	100.0 ± 0.00	100.0 ± 0.00*	83.33 ± 3.30*
January 2001	86.66 ± 3.30*	76.66 ± 3.30*	60.00 ± 0.00*•
February 2001	60.00 ± 0.00*	56.66 ± 3.30*	40.00 ± 5.70*•

* Germinated in the storage condition

• Shrinkage of the tuber in storage condition.

Table-40: Performance of plants raised from microtubers of var DR and var HR in nursery beds (Data recorded after 60 days)

Variety	Grade of the tubers	No. of the tubers planted	No. of tubers germinated	% of germination	Time taken for germination (Days)	Survival rate till harvest %	Length of the plants(cm) Mean ± S.E.*	No. of nodes Mean ± S.E.*
DR	A	16	16	100	10	100	$16.73 \pm 2.47_b$	$10.80 \pm 0.97_b$
	B	16	16	100	8	100	$9.80 \pm 0.69_a$	$9.80 \pm 0.69_b$
	C	16	15	93.75	5	87.5	$9.13 \pm 1.29_a$	$6.40 \pm 0.69_a$
HR	A	12	12	100	11	100	$13.41 \pm 1.43_c$	$8.0 \pm 0.79_b$
	B	12	12	100	9	100	$9.41 \pm 0.72_b$	$6.66 \pm 0.51_b$
	C	12	12	100	6	83.33	$4.75 \pm 0.52_a$	$3.33 \pm 0.30_a$

*Values with the same superscript letter are not significantly different at P>0.05 (Duncan's Multiple range test)

Table-41: Production of plants raised from microtubers in nursery beds. var DR and var HR
(Data recorded after 100 days)

Variety	Grade of the tubers	Total no. of minitubers Mean± S.E.	Average diameter of minitubers Mean ± S.E.	Average fresh weight of the minitubers Mean ± S.E.	Total yield per plant (gms) Mean ± SE
DR	A	5.66± 0.63	1.58± 0.05	2.05± 0.13	11.63 ± 0.08
	B	3.80± 0.26	1.17± 0.04	1.80± 0.09	6.84 ± 0.00
	C	1.76± 0.20	1.13± 0.02	0.91± 0.05	1.64 ± 0.05
HR	A	3.58± 0.31	1.29± 0.02	1.97± 0.05	7.10 ± 0.03
	B	2.75± 0.27	1.17± 0.04	1.33± 0.07	3.67 ± 0.07
	C	1.58± 0.14	1.04± 0.03	0.96± 0.07	1.53 ± 0.05

Table - 42: Storage and germination studies of encapsulated potato nodal segments. var. DR

Days after Storage	MS Medium with 3% sucrose	Soil : Peat - 1:1
0 Day	73.33	50.00
10 Days	60.00	40.00
20 Days	56.66	36.00
30 Days	50.00	10.00

Table - 43: Storage and germination studies of encapsulated potato nodal segments. var. HR

Days after Storage	MS Medium with 3% sucrose	Soil : Peat - 1:1
0 Day	66.66	13.33
10 Days	41.66	16.66
20 Days	41.66	-
30 Days	33.33	-

Plate 43. Different grades of microtubers after one month of storage –

(a) Grade A

(b) Grade B

(c) Grade C

In all figures, the bar = 1 Cm

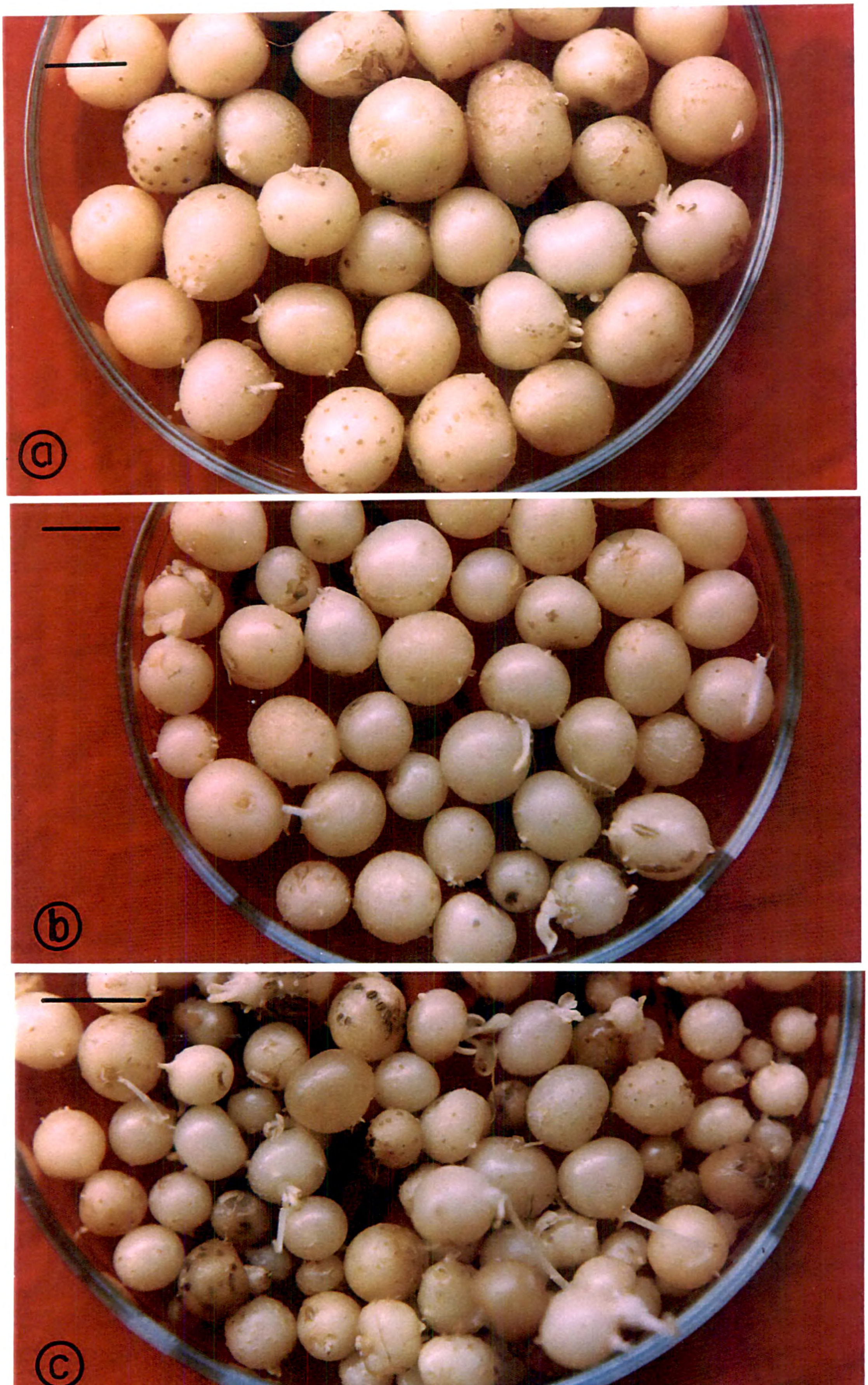

a
b
c

Plate 44. Different grades of microtubers in storage condition at 4°C showing germination.

(a) A grade microtubers after 6 months of storage

(b) B grade microtubers after 6 months of storage

(c) B grade microtubers after 5 months of storage

(d) C grade microtubers after 5 months of storage

In all figures, the bar = 1 Cm

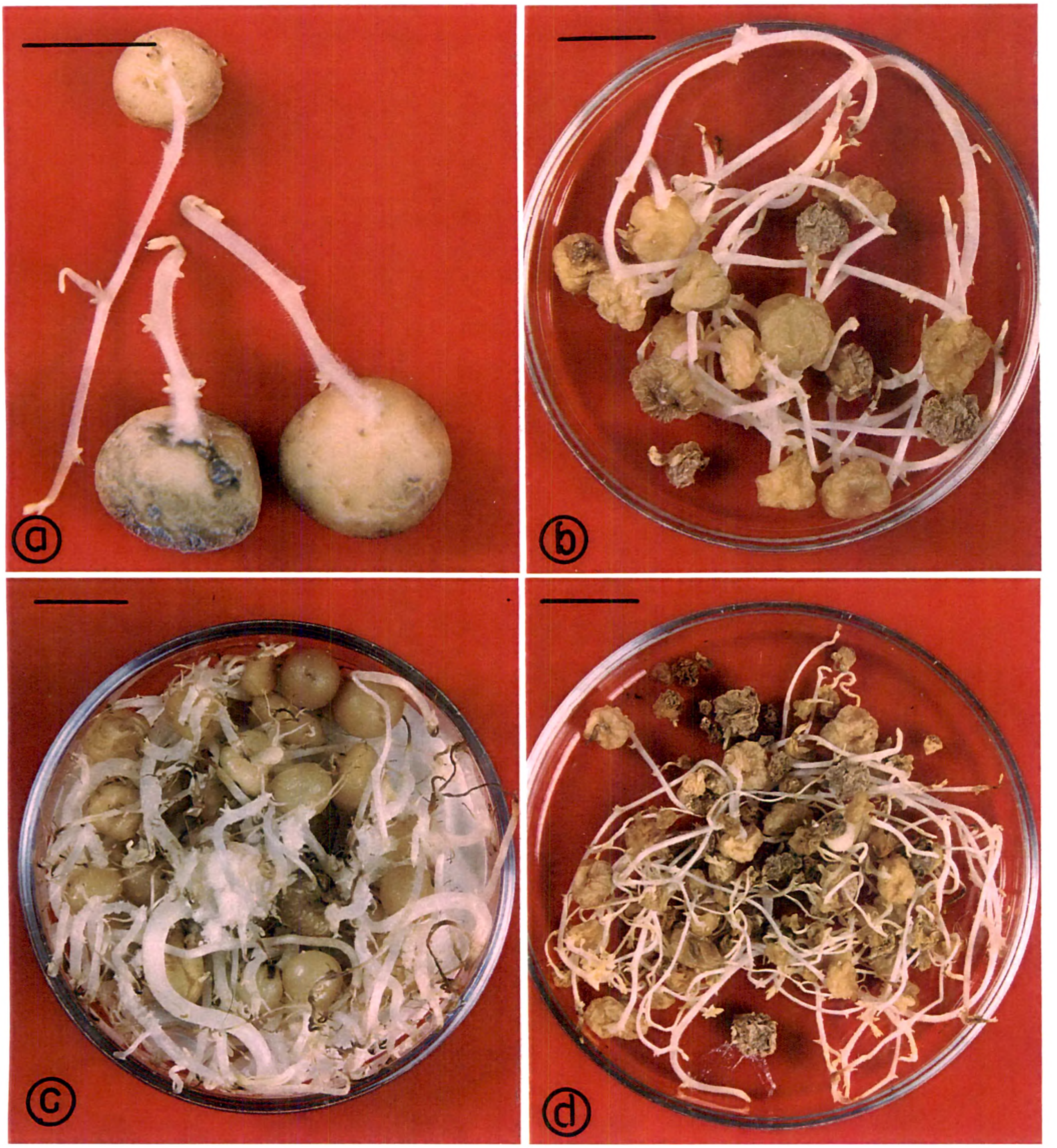

a
b
c
d

Plate 45. Plants of potato var DR raised from –

(a) B Grade microtubers

(b) A Grade microtubers

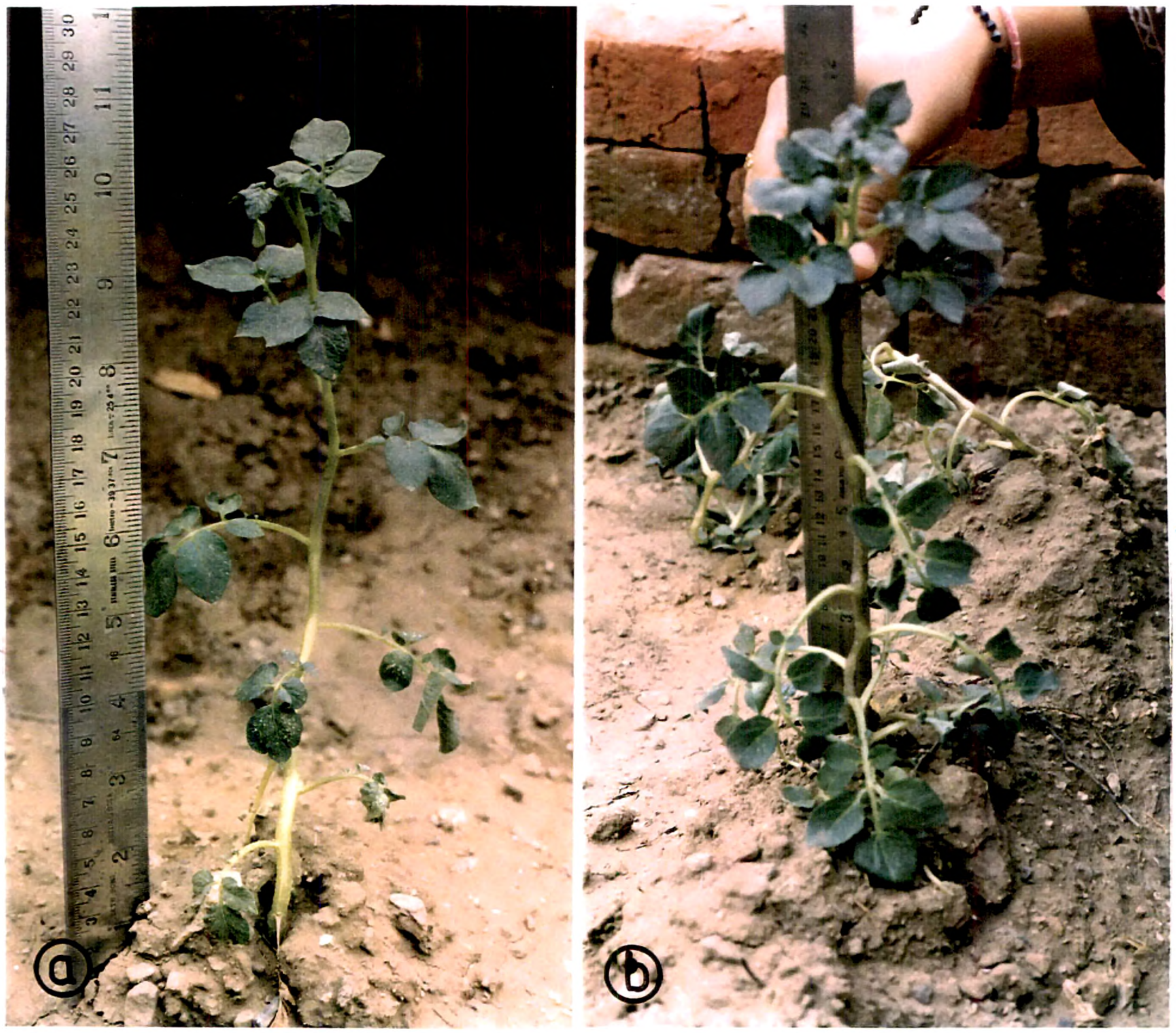

a
b

Plate 46. Plants of potato var HR raised from –

(a) A Grade microtubers

(b) B Grade microtubers


a
b

Plate 47. Plants raised from C grade microtubers.

(a) var HR

(b) var DR

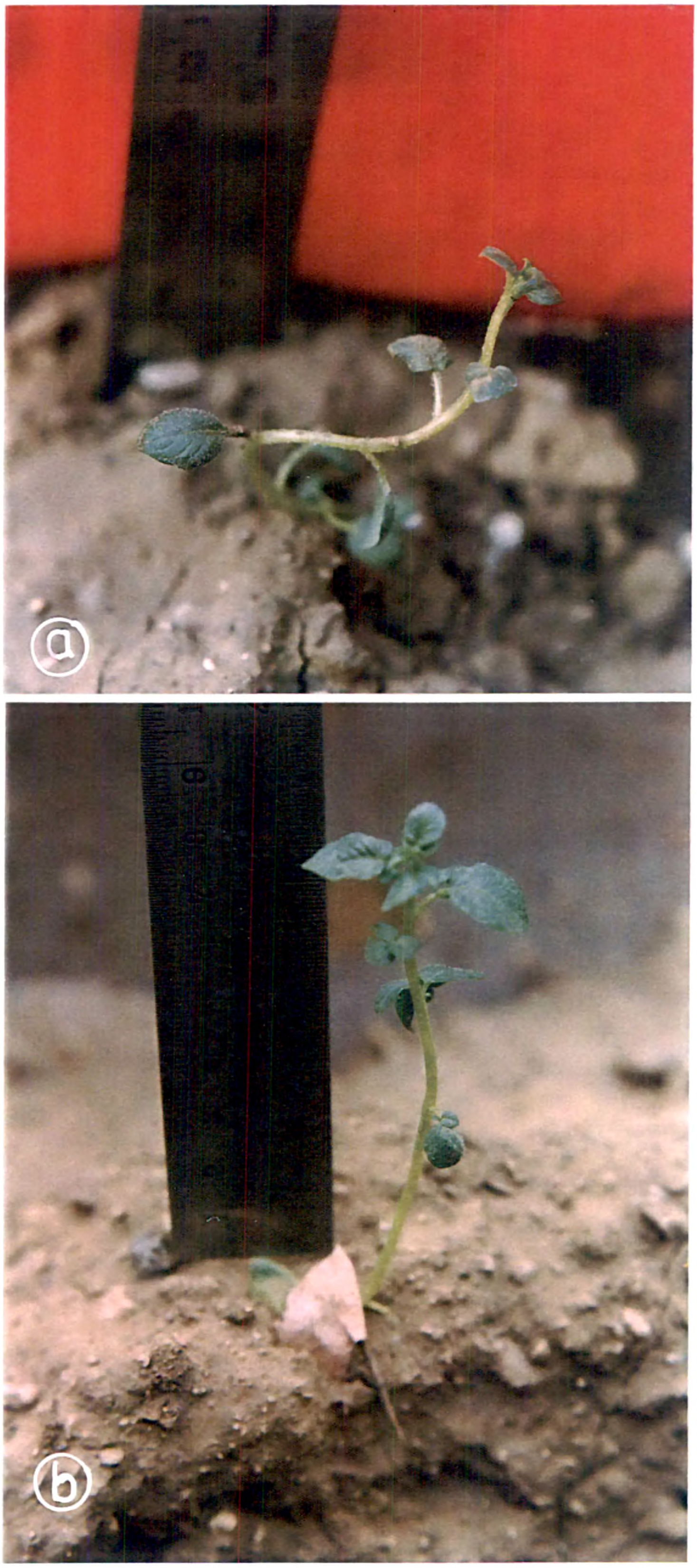

a
b

Plate 48. Minitubers of var DR produced from microtubers of

(a) A Grade

(b) B Grade

(c) C Grade

(d) Harvested minitubers

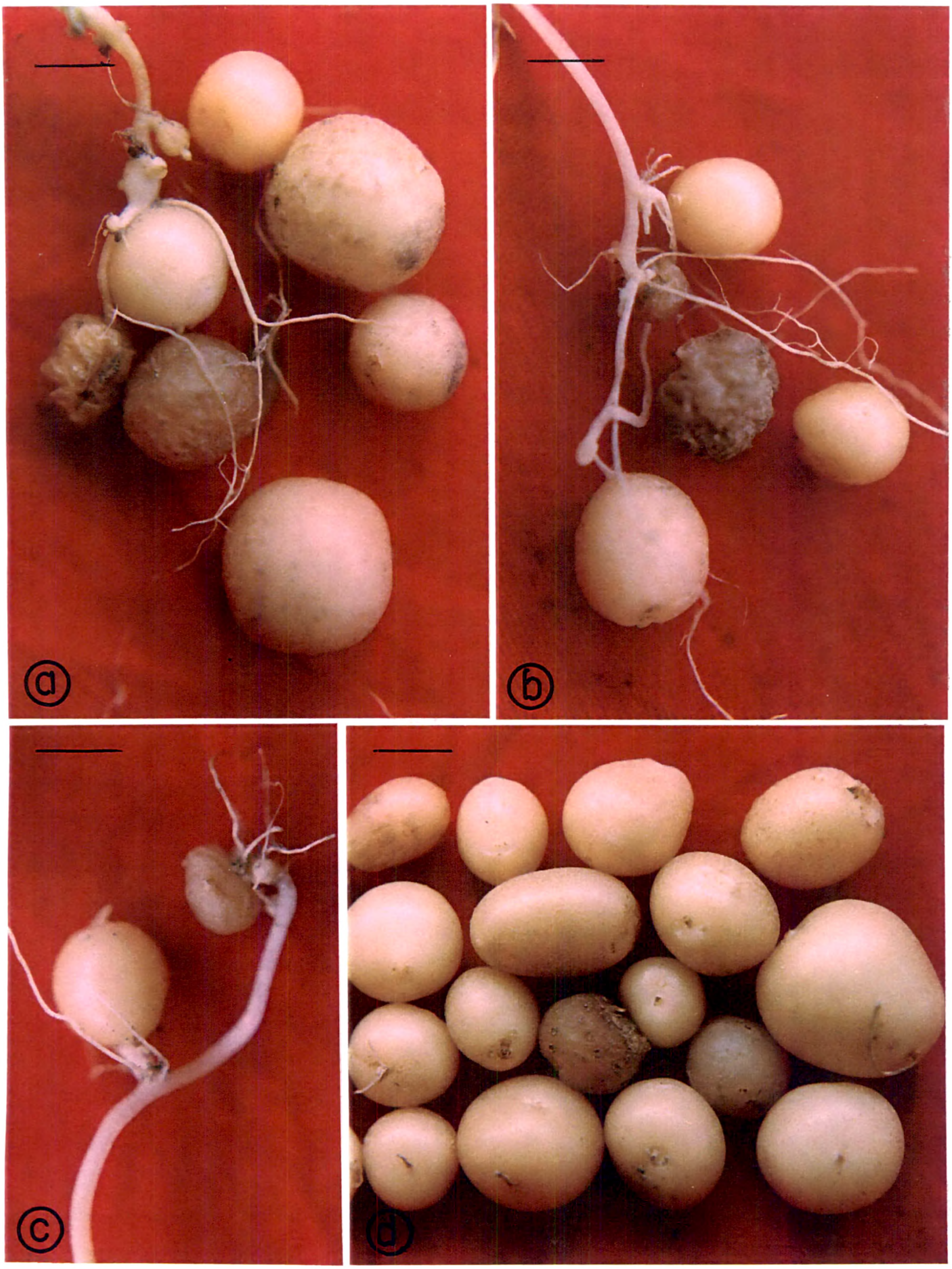

a
b
c
d

Plate 49. Minitubers of var HR produced from microtubers of

(a) A Grade

(b) B Grade

(c) C Grade

(d) Harvested minitubers

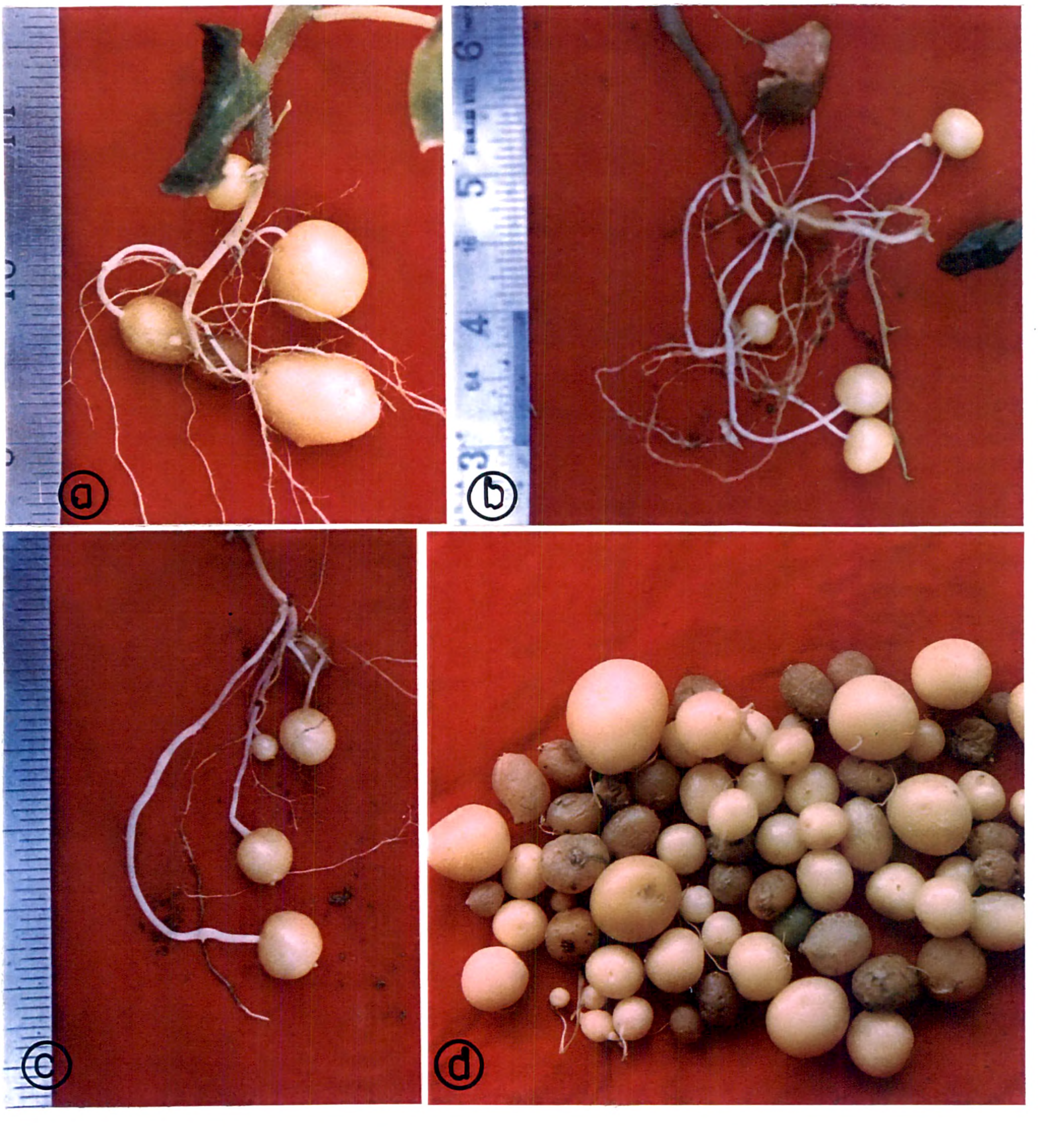
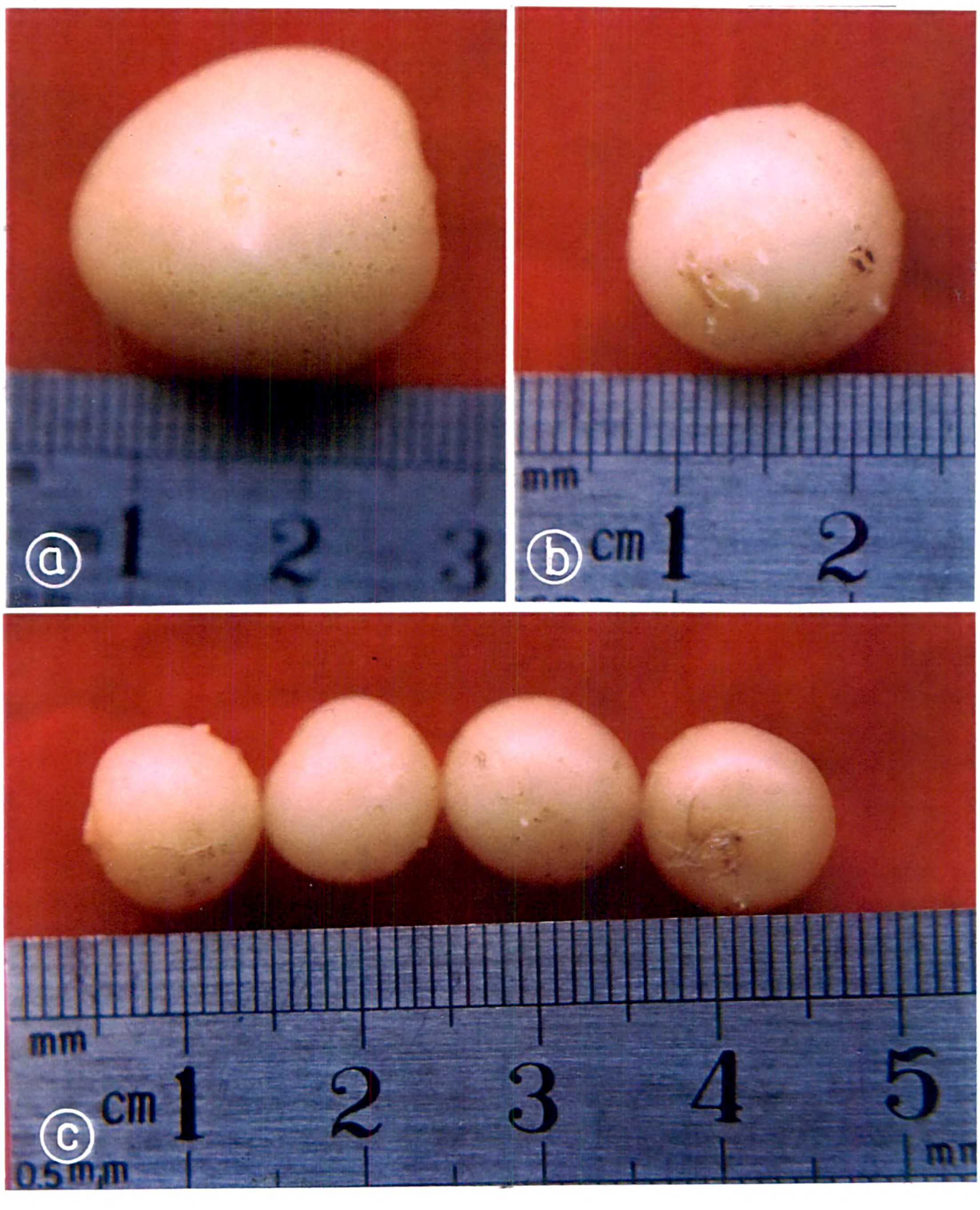

a
b
c
d

Plate 50. Minitubers showing their size.


a
1 2 3
b
mm
cm 1 2
c
mm
cm 1 2 3 4 5
0.5mm

highest yield in case of both varieties was obtained from plants raised from A grade microtubers (Plate: 48, 49 and 50).

Encapsulation of potato nodal segments

Nodal segments of shoots cultured on MS medium were encapsulated using 3% (w/v) sodium alginate. These alginate coated nodes were small, shiny and whitish beads with an average diameter of 4-6 mm (Plate: 51). These beads were stored in small petridishes sealed with parafilm at 4° C in a desiccator for a period of 30 days. The ability of encapsulated nodes was tested at the end of various intervals of storage. Encapsulated nodal segments exhibited 73 % and 66% of sprouting in var DR and var HR respectively on day one on MS basal medium (Plate: 52). However, with increase in the duration of storage, the percentage of sprouting by the encapsulated nodes decreased sharply and reached 50% and 33% in case of var DR and var HR respectively (Table: 42,43). The performance of these beads in a mixture of soil and peat (1:1) was poor (Plate: 53) compared to that on the MS medium in case of var DR. However, on MS medium as well as in soil peat mixture the same trend, i.e. decrease in percentage of sprouting with increase in the duration of storage was observed. In case of var HR the performance in the soil-peat mixture was very poor (Table: 43).

Developmental studies of potato microtubers

The development of potato tubers under *in vivo* condition is controlled by a number of factors such as photoperiod, nutrition, temperature and moisture. Under *in vitro* condition microtubers were formed from the axillary buds of shoots. Here the microtuberization process was strongly influenced by medium composition as well as by photoperiod and temperature. In the present study, developmental stages of microtubers were examined to understand the initial anatomical changes during tuberization. Cell divisions, cell enlargement and starch deposition have been considered as the earliest changes that occur in the *in vitro* shoot bud during microtuberization.

The *in vitro* shoots inoculated on control medium (propagation medium) do not show starch accumulation in any of the axillary buds. But when propagation medium

Plate 51. (a) Encapsulated potato nodal segments. var DR

(b) Encapsulated potato nodal segments. var HR

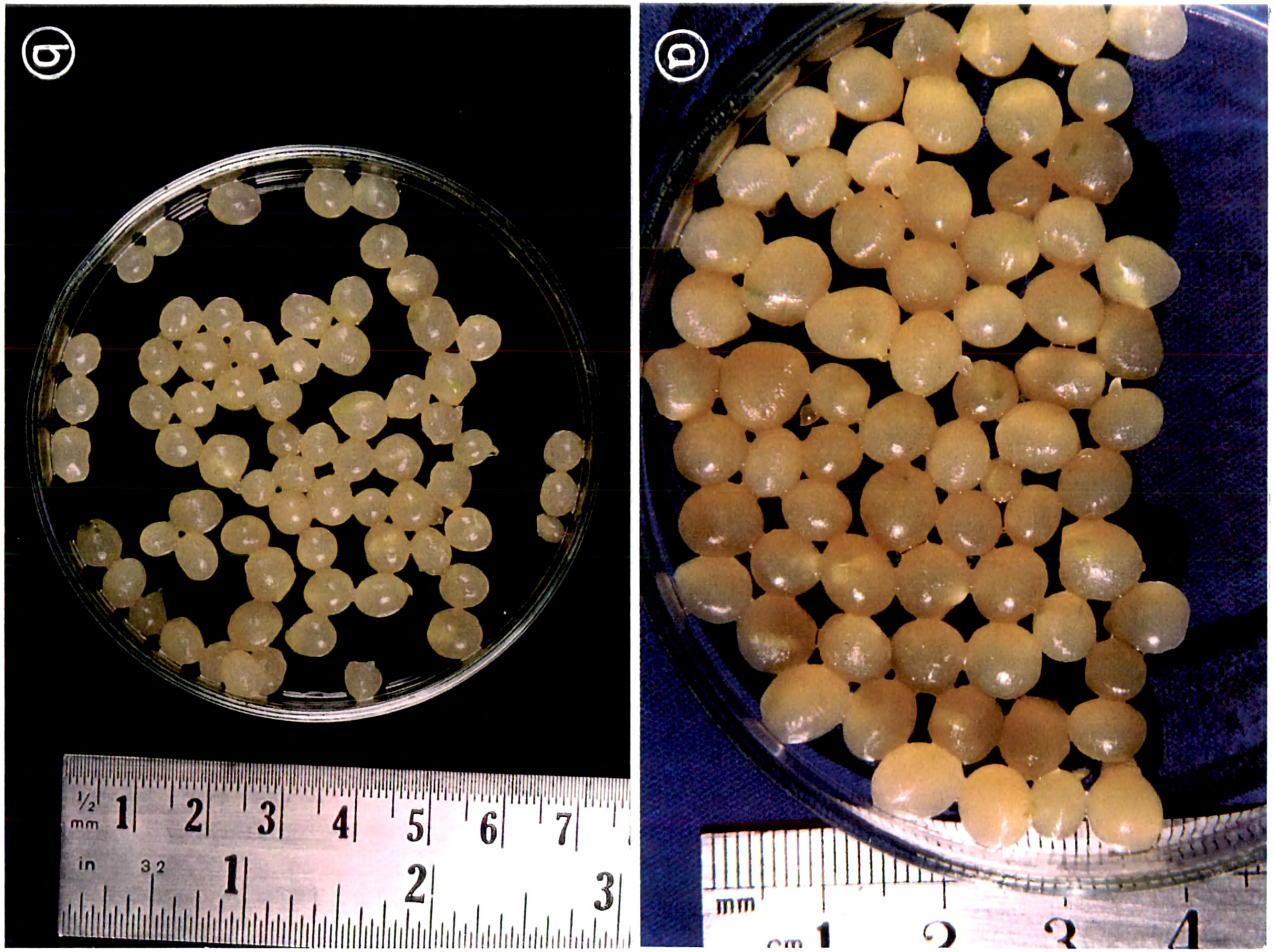

a
b

Plate 52. (a) to (c). Germination of Encapsulated nodal segments of potato in MS semisolid medium.

In all figures, the bar = 1 Cm

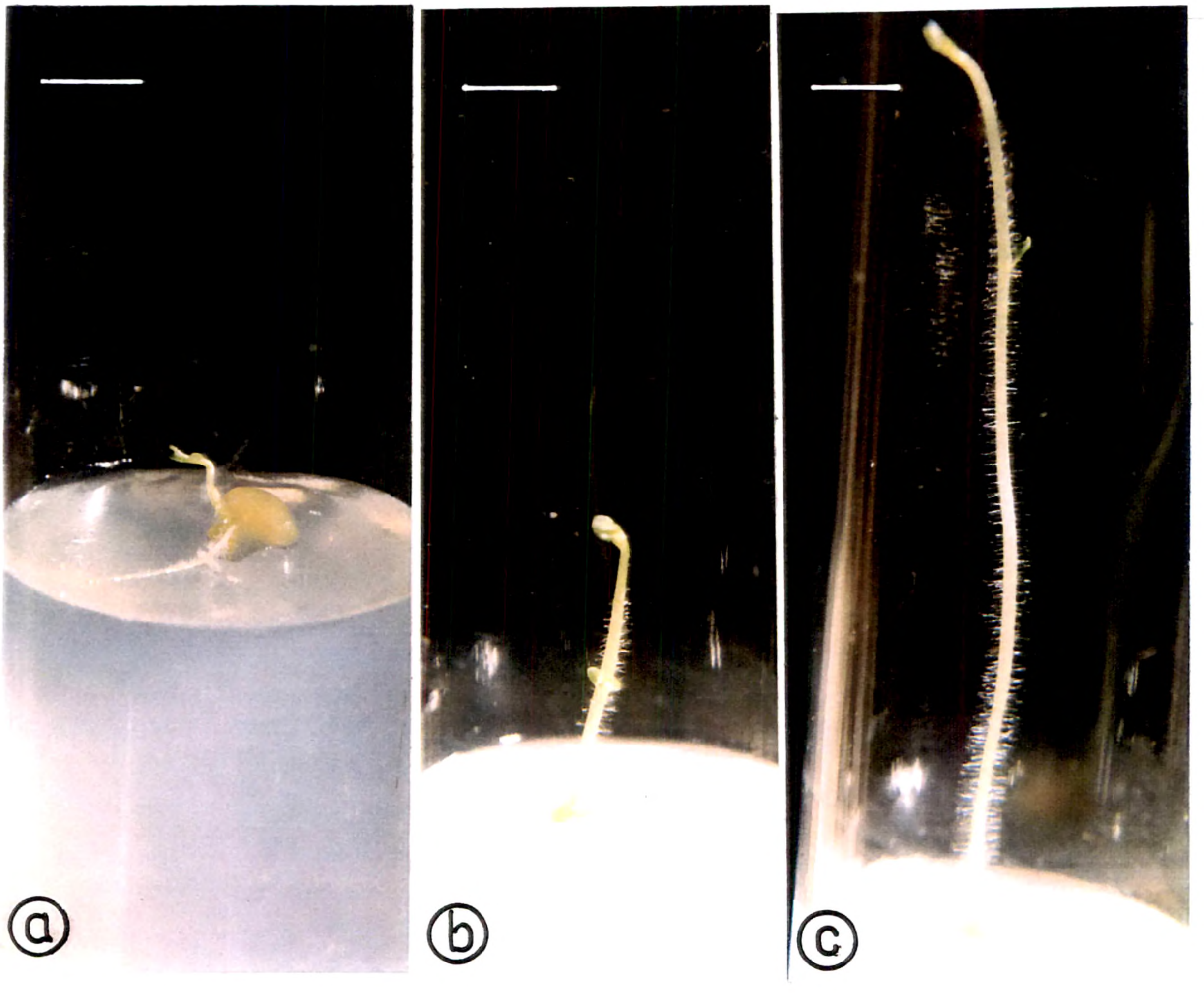
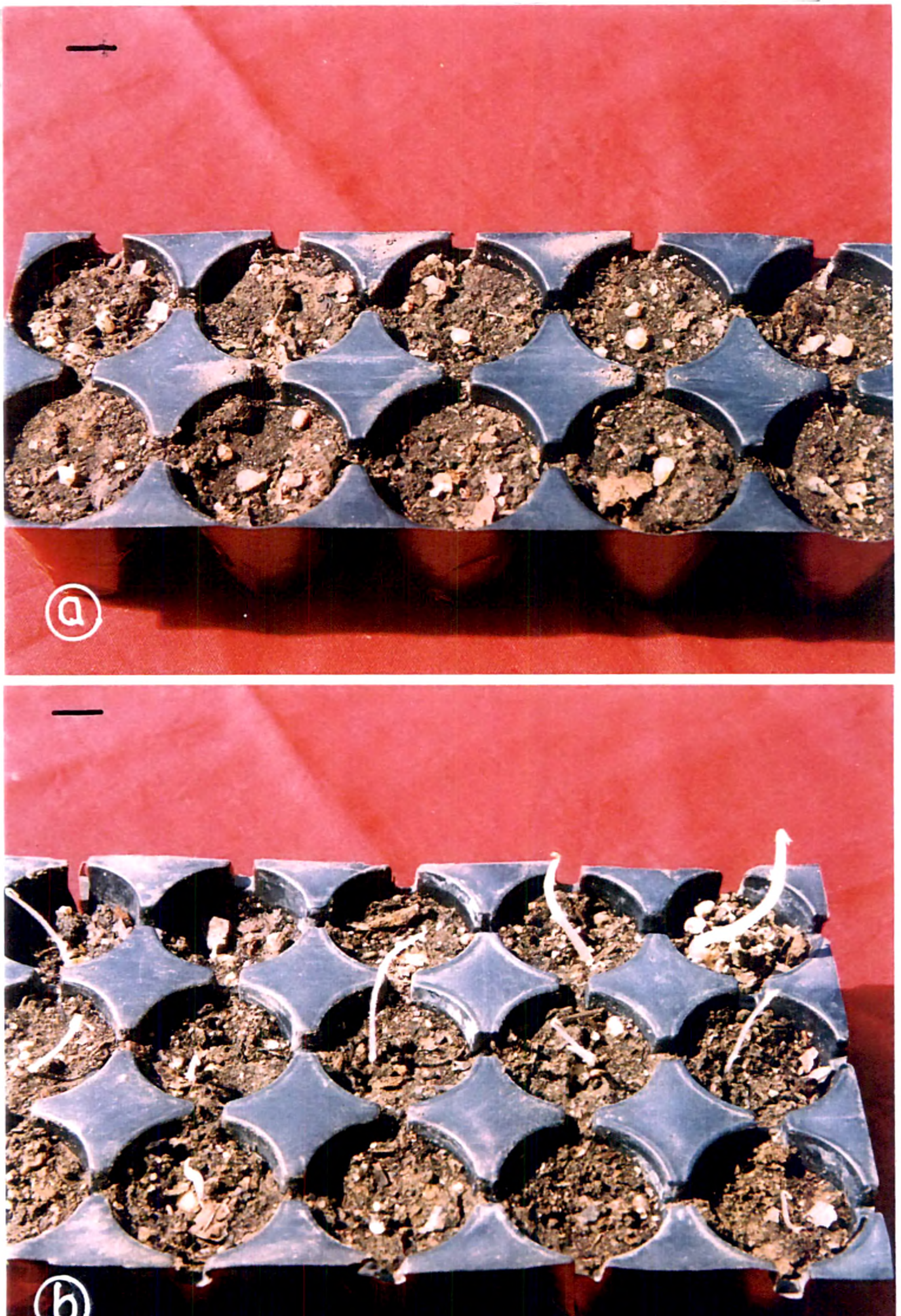

a
b
c

Plate 53. (a) to (b). Germination of Encapsulated nodal segments of potato in soil-peat mixture (1:1).

In all figures, the bar = 1 Cm


a
b

was replaced by liquid tuberization medium, starch deposition began right on the first day itself in the meristem of axillary bud.

During the study of the ontogeny of the axillary bud meristems into tubers, four developmental stages have been observed in the present investigation viz. bud initiation, bud growth, cessation of bud/axillary shoot growth and tuber initiation.

(A) Bud Initiation

The apex of an *in vitro* potato shoot was convex with a single layer of tunica enclosing corpus. Anticlinal divisions were predominant in the tunica layer while the cells of corpus divide in various planes. The meristem of the bud is derived from the peripheral meristem of the shoot apex (Plate: 54a). It differentiates as a detached meristem with an axillary position, lying in the axil of the subtending leaf. Hence, the bud meristem that develops into a tuber was seen to be lateral in position, having oblique orientation and consisting of 6-7 layers deep densely stained cells (Plate: 54b).

The axillary bud meristem was gradually blocked out from the peripheral meristem of the shoot apex. Elongation of the peripheral meristem of shoot apex adjacent to bud meristem cells resulted in an arcuate zone of cells delimiting the bud from the shoot apex and other surrounding cells. It brought about an axillary position for the bud. One or two rows of cells adjacent and adaxial to the bud meristem vacuolated, parenchymatized and divided anticlinally to form the arcuate zone of cells. These paranchymatized cells which are of a sector of peripheral meristem of shoot apex ultimately delimit the small mass of densely stained cells, 'the bud meristem' near the axil of a leaf from the surrounding cells up to the base of the subtending leaf.

The earliest bud meristem was not connected with the vasculature of the axil or the axillant leaf. Before the first leaf initiation in the early axillary bud, cortical and pith cells, just below the apical meristem, showed very early signs of parenchymatization with the accumulation of starch grains.

(B) Bud Growth

The bud meristem gradually developed a convex bulge protruding it. The bud tip remained meristematic and was densely stained with conspicuous large nucleus and prominent nucleolus (Plate: 54 c and d). The bud further protruded because of

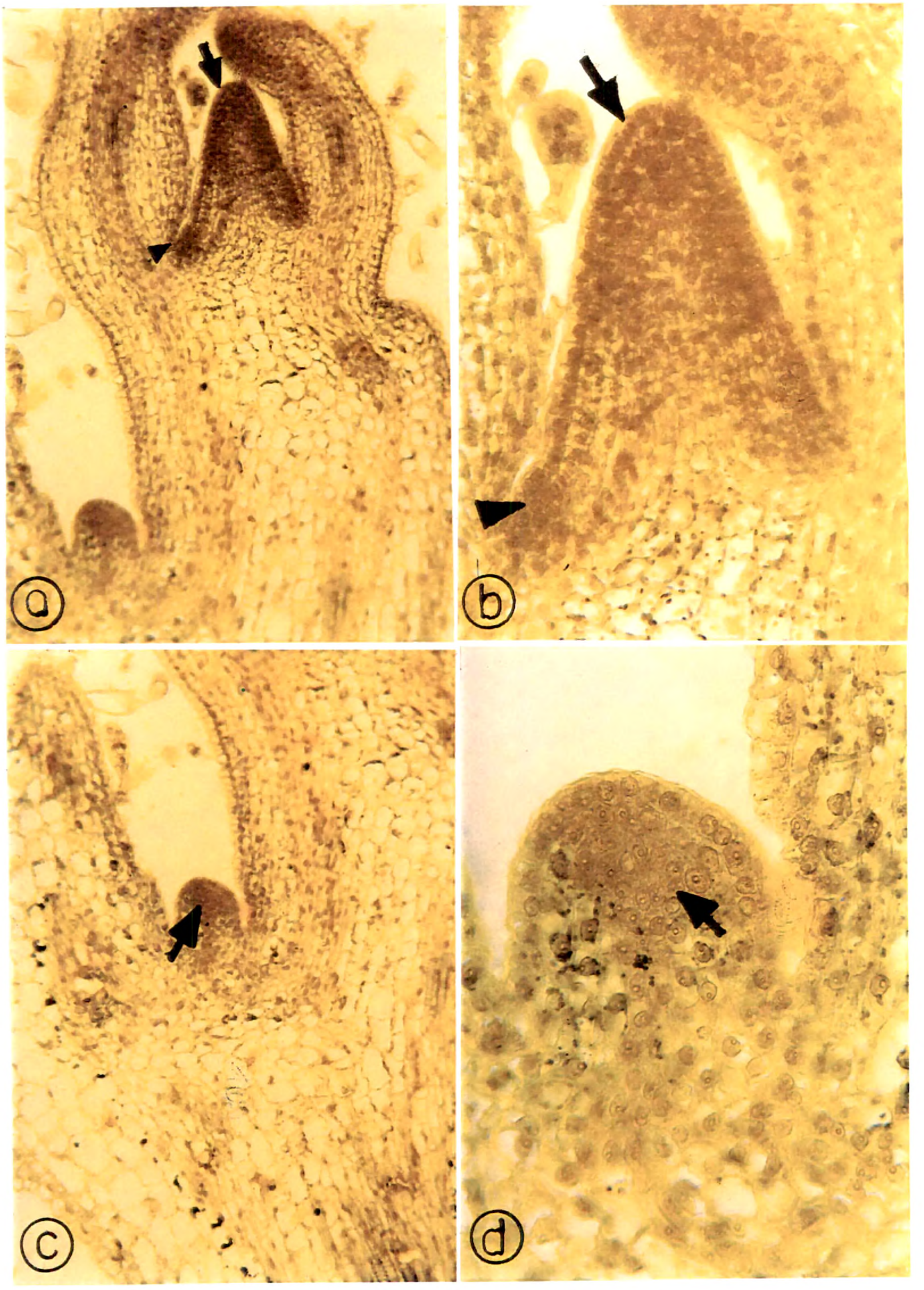
Plate 54. L.S. of shoot apex - initial stage of potato microtuber induction

(a) The meristem of the bud derived from the peripheral meristem of shoot apex. (arrow shows apical meristem, arrow head shows axil of the subtending leaf with 6-7 layers densely stained cells). 285 X

(b) Magnified view of (a). 712.5 X

(c) Meristematic zone with conspicuous large nucleus and prominent nucleus. 285 X

(d) Magnified view of (c) 1140 X


a
b
c
d

anticlinal and oblique periclinal divisions in the inner cells of the bud meristem. Sometime the bud showed presence of 2 layers of tunica. Anticlinal divisions were apparently noticed in the tunica layers (Plate: 55a and b).

The apical or terminal region of the bud still consisted of densely stained eumeristemetic cells with conspicuous nucleus and nucleolus, and a deeply vacuolated pith and cortex region with heavy deposition of starch (Plate: 55c and d). The axillary bud now appeared cylindrical and much elongated. Procambial cells were present between the outer ground meristem and vacuolated pith cells. The procambial cells were densely stained, narrow and elongated. They extended to the meristematic zone at the tip. Files of young pith and ground meristem cells were not highly developed (Plate: 56 a to d).

With further elongation of the bud an early development of the first leaf primordia occurred. The pith cells and cortical cells were fully differentiated and demarcated.

(C) Cessation of bud / axillary shoot growth

The tuber originated from an axillary bud, which elongated and formed stolons before any circumferential enlargement occurs. The stolon tip exhibited the general morphological features of the apical bud of an areial stem having leaf primordia. The leaf primordia remained suppressed till the entire development of the tuber.

(D) Tuber Initiation

During the process of stolon elongation, transverse cell divisions were noted in the apical region and later they elongated. When the subapical region began to swell transverse cell division in the apical region stopped and the cells of the sub-apical region enlarged, divided longitudinally and thus the radial growth began. The cells in the pith and cortex divided longitudinally, enlarged at the sub-apical region, resulting in the swelling (Plate: 57a). Initially the elongated stolon had more or less the same diameter, but the heavy deposition of starch indicated it to be the tuber primordium (Plate: 57b).

Plate 55. L.S. of elongated bud destined to form microtuber

(a) Anticlinal and periclinal division in the inner cells of the bud meristem. (arrow shows tunica layer of the bud meristem). 285X

(b) Magnified view of (a) 1140 X

(c) Bud meristem showing starch deposition. (arrow shows procambial cells and arrowhead shows densely stained eumeristematic cells with conspicuous nucleus and nucleolus). 285 X

(d) Magnified view of (c) 1140 X

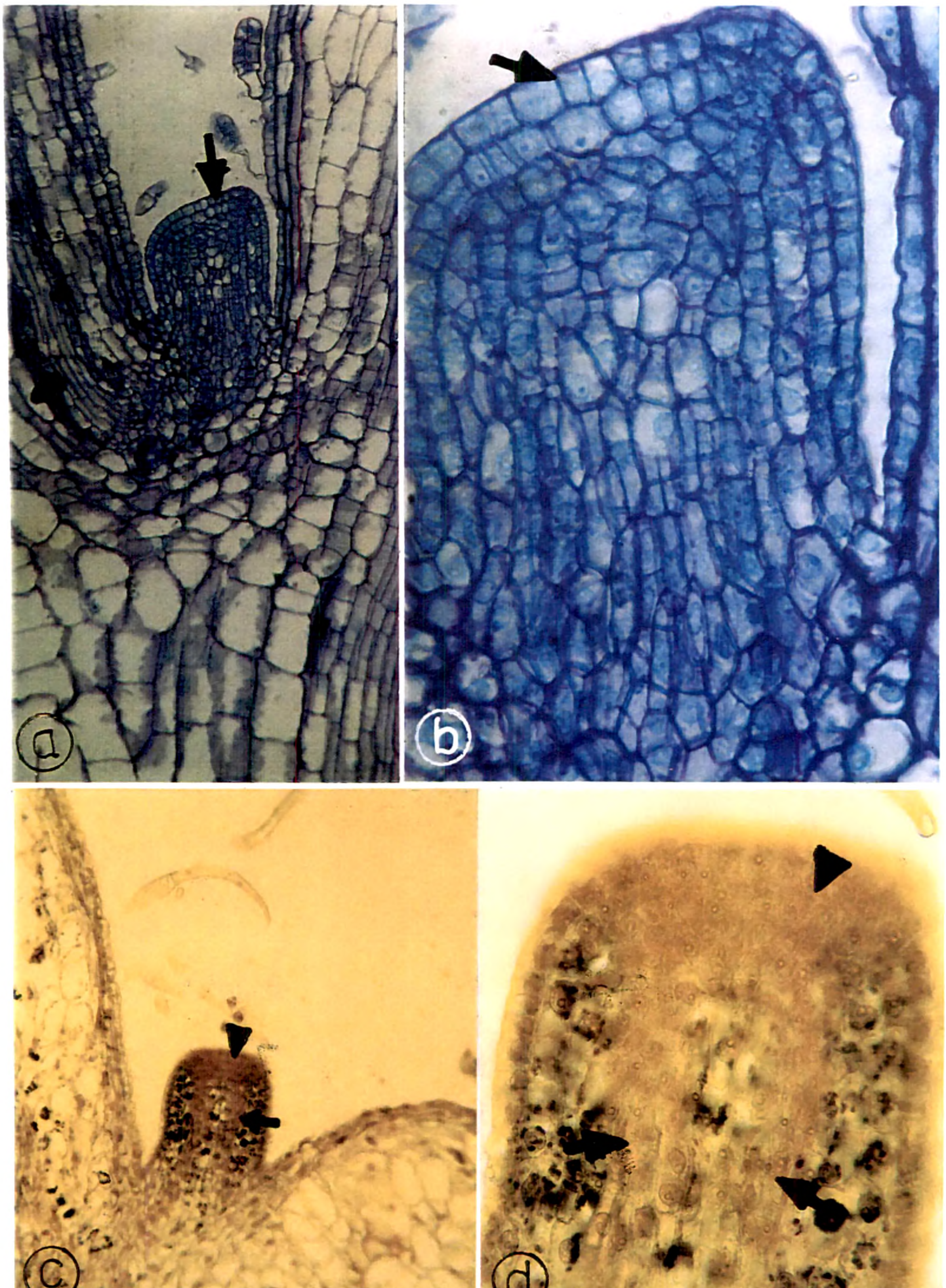

a
b
c
d

Plate 56. Microtuber development -

(a) Early development of leaf primordia (arrow head) 285 X

(b) Magnified view of (a). 712.5 X

(c) and (d). Cessation of bud. 285 X

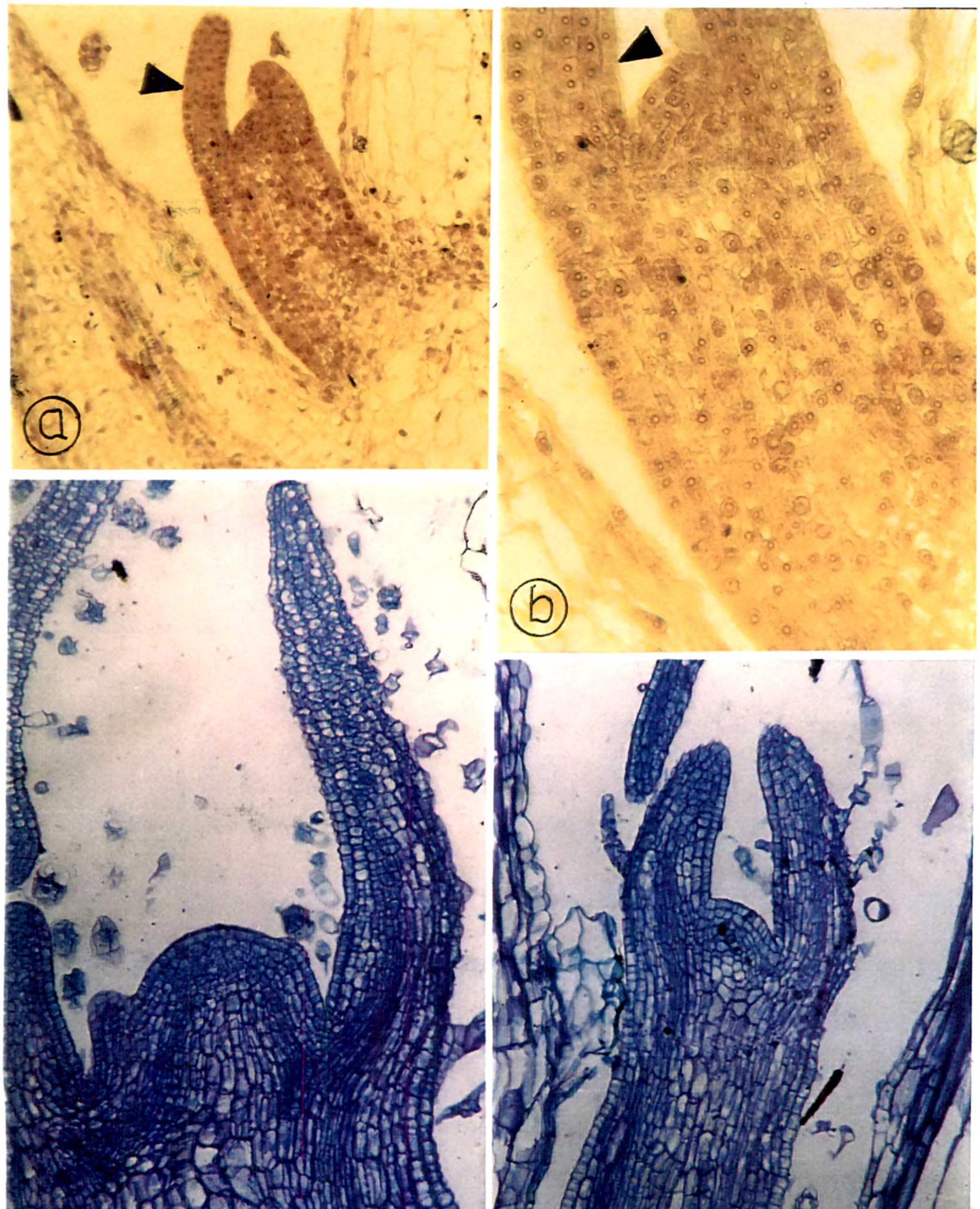

a
b
c
d

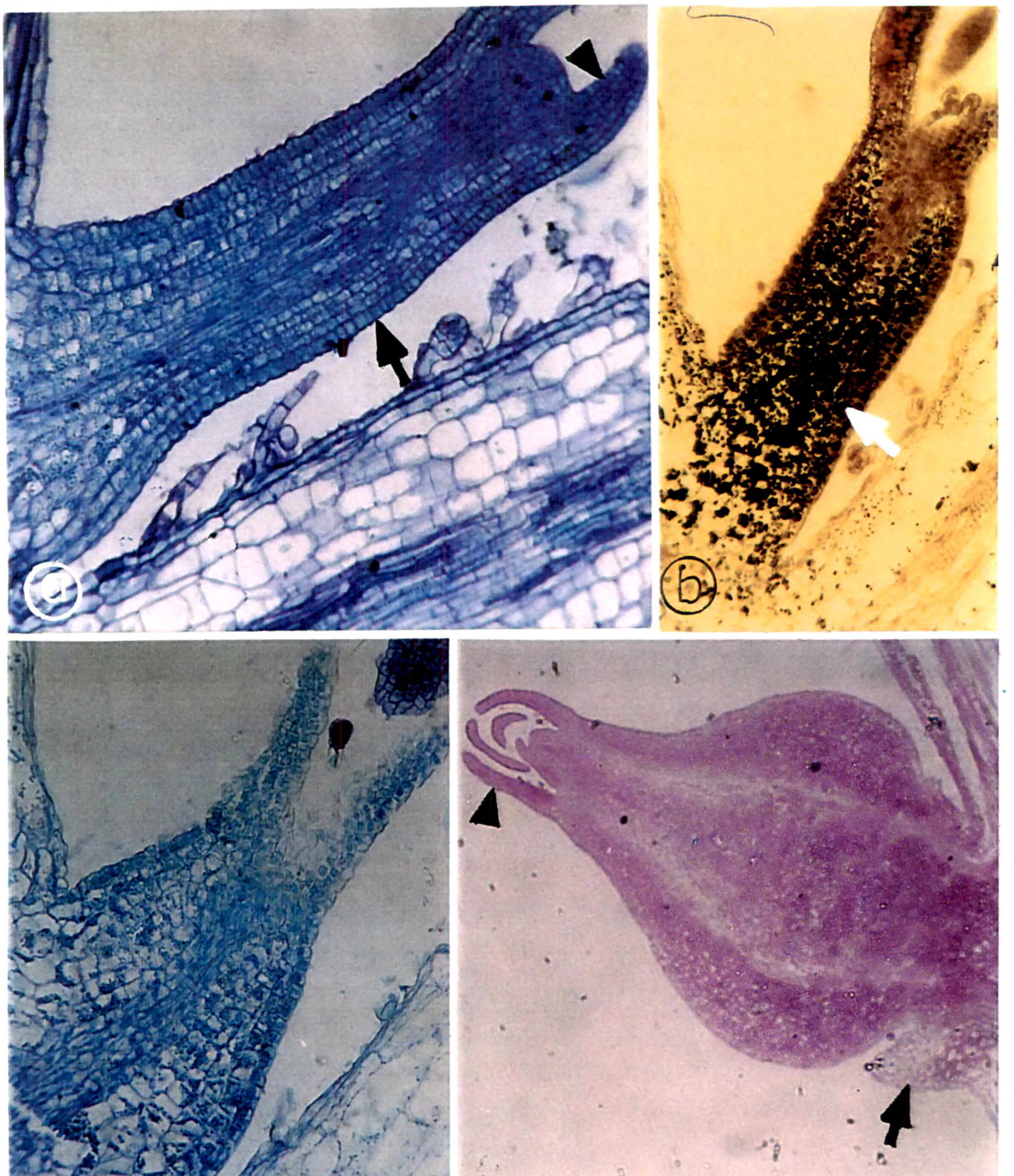
Plate 57. Microtuber development -

(a) Cells in pith and cortex started dividing longitudinally, resulting in the initiation of tuber. (arrow shows swelling in the subapical region, arrowhead shows leaf primordia). 285 X

(b) Heavy deposition of starch (arrow). 285 X

(c) Cell division and cell enlargement in the mid section tissues. 285X

(d) Bulging of the midsection and formation of a microtuber (arrow shows scale leaf and arrowhead shows leaf primordia). 142.5 X



a
b
c
d

Both cell division and cell expansion were involved in tuber development. The tuber underwent pronounced circumferential expansion which was most pronounced at midsection. Tissues formed in the bud end of a young tuber soon became mid section tissues. Mid section tissues became relatively closer to stem end tissues as the radial growth of the tuber increase (Plate: 57 c and d). Cell division was most frequent at the mid section than at bud end or stem end.

Another important event in tuber development was the heavy deposition of starch grain in cortical region. The size and number of the starch grains increased with the increase in size of microtubers (Plate: 58). During the initial stages of the microtuber development, starch grains were aggregated more in the cortical region than that of the parenchymatous pith region. The peripheral row of cortical cells adjacent to the periderm (skin) often showed less number of starch grains than deeper cortical cells. Formation of xylem elements was observed during the tuber maturation phase. Xylem elements have been observed mainly in the cortical region (Plate: 59).

Adventitious root formation was observed to arise endogenously from the stem at extra-axillary position (either side of axillary bud) of tuber primordia as well as most of the dormant buds (Plate: 60, 61 and 62). The cells forming the root primordium were mostly derived from interfascicular parenchyma, medullary rays or vascular rays. The root tip showed densely stained meristematic cells. The tip of root primordium showed heavy deposition of starch especially in the root cap region (Plate:63).

Skin formation

During the development of a stolon tip into a microtuber, periderm was gradually formed on the outside of the tuber. Initially it consisted of one layer of suberized cells till 15 days when visually the swelling in stolon could be seen (Plate:64). Initiation of periderm occurred by division was both epidermal and sub-epidermal cells. When microtubers reached to 0.1 cm in diameter, periderm is clear and distinct. It consisted of 3-4 files of cells formed by continuous cell divisions. The epidermal cells underwent anticlinal division, sub-epidermal cells (below the epidermis) immediately showed periclinal and anticlinal divisions when the tuber was 0.5 cm. This dividing cell layer formed the phellogen. The newly formed cells

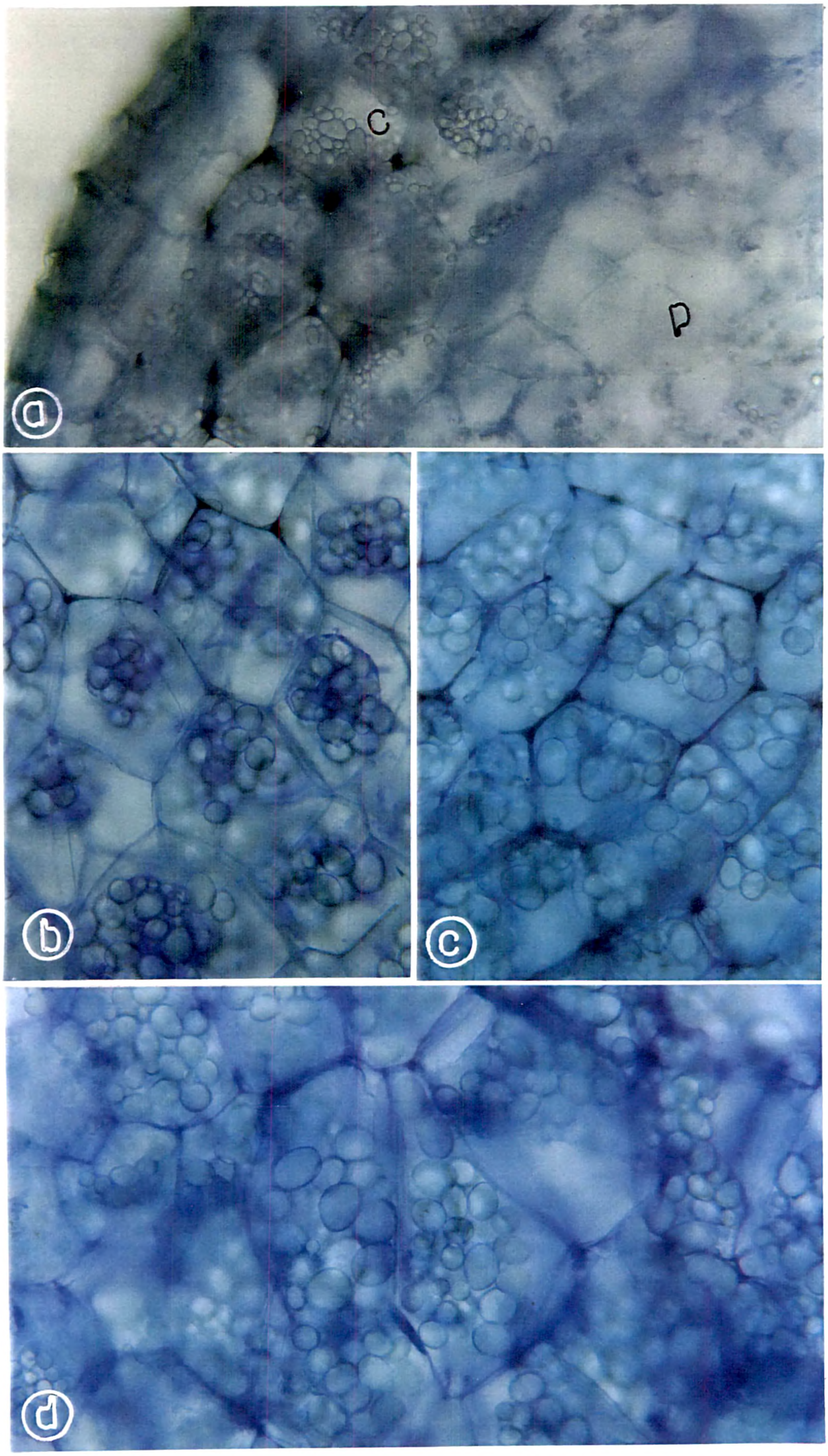
Plate 58. Microtuber development -

(a) Starch grains of microtubers with a diameter of 0.1 cm (P - Pith cell, C - Cortex). 712.5 X

(b) Starch grains of microtubers (0.2 cm in diameter) 712.5 X

(c) Starch grains of microtubers (0.5 cm in diameter) 712.5 X

(d) Starch grains of microtubers (0.8 cm in diameter) 712.5 X



a
c
p
b
c
d

Plate 59. Microtuber development -

(a) to (d) Xylem elements in the cortical region
(arrow and X – Xylem, C – Cortical region) a - 285 X, b -712.5 X, c - 285 X and d – 712.5 X

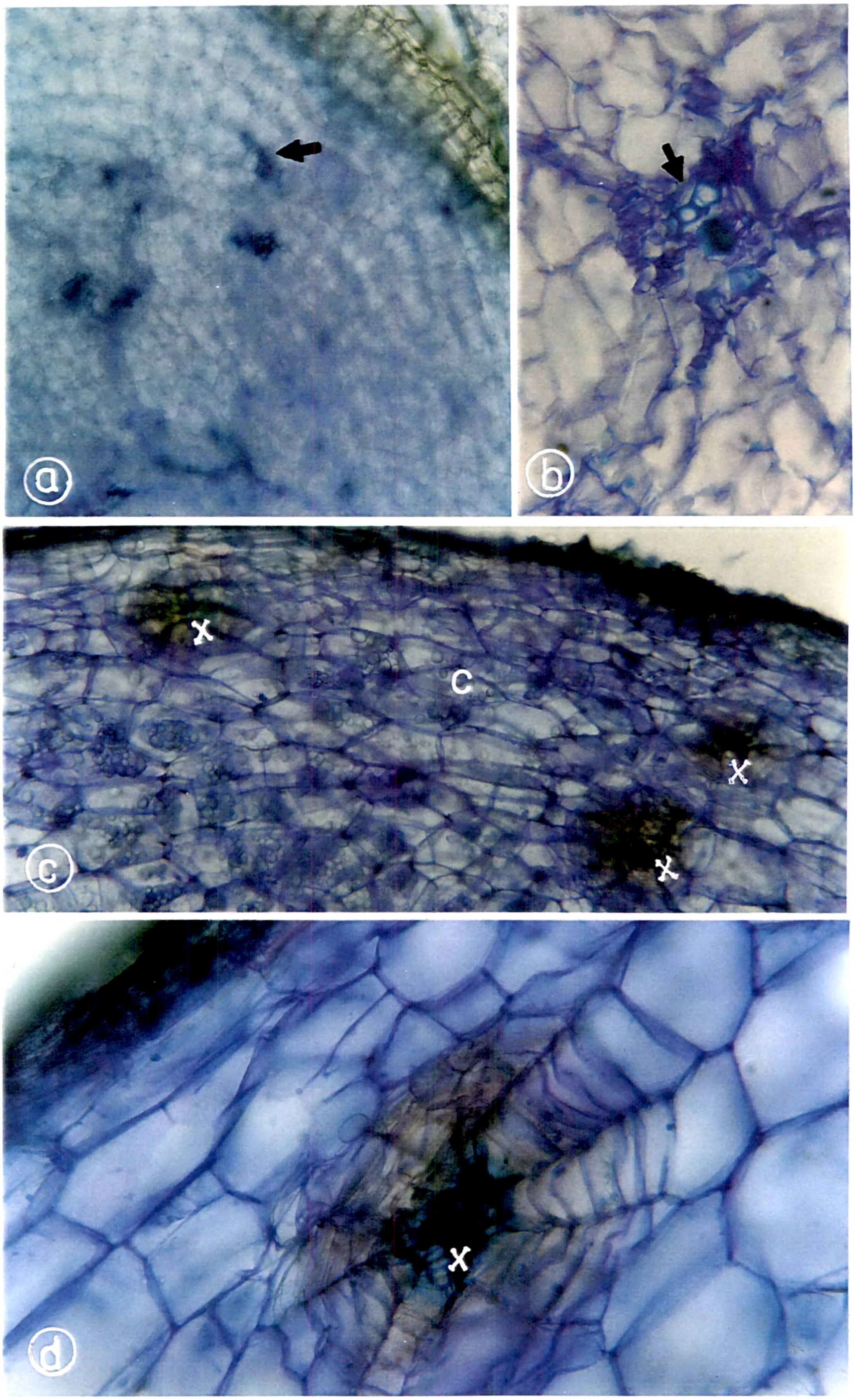

a
b
x
c
x
x
c
x
d

Plate 60. Microtuber development -

(a) to (d). Initiation of adventitious root formation on extra axially position. a - 285 X, b – 712.5 X, c – 285 X, d – 285 X

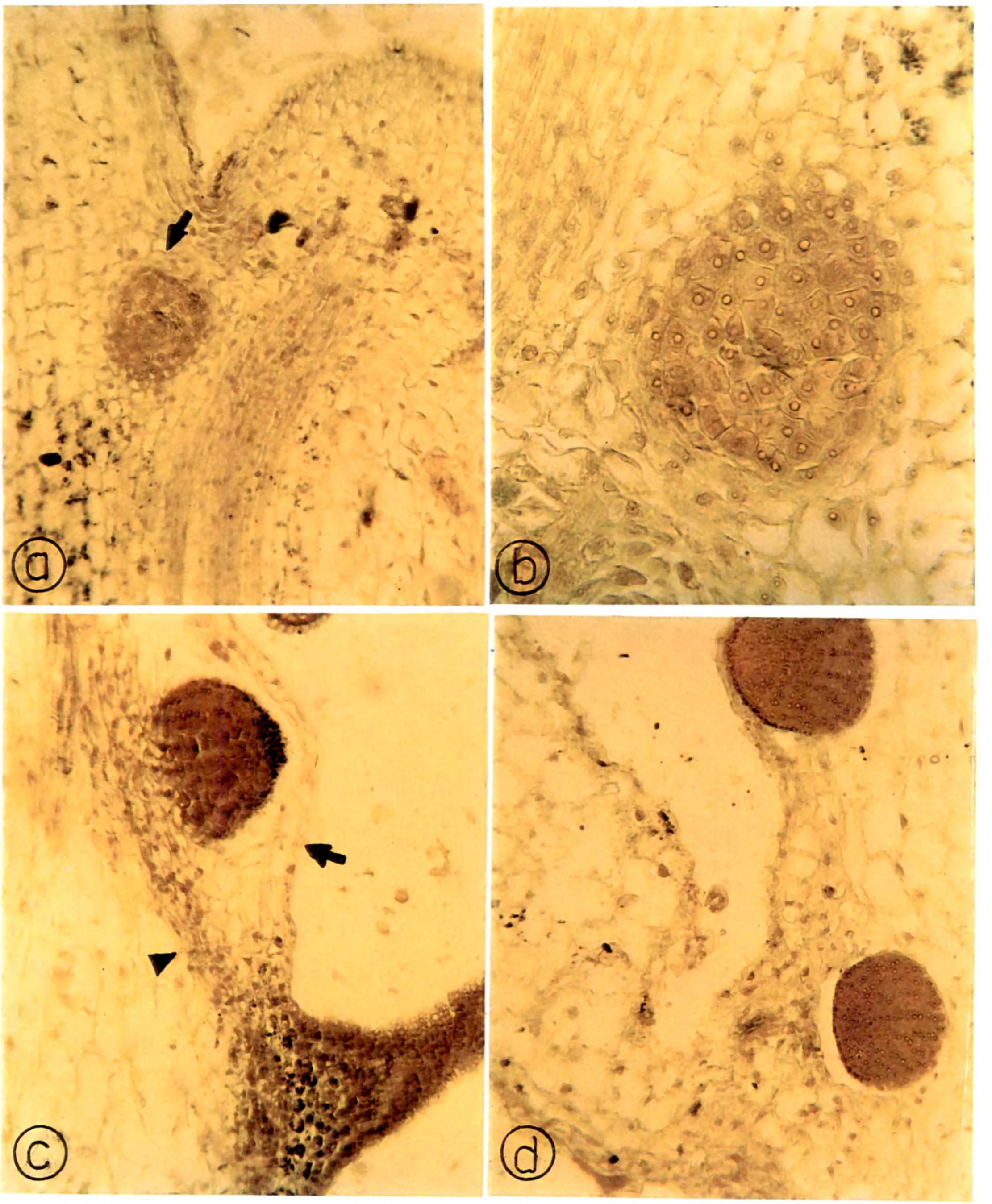

a
b
c
d

Plate 61. (a) to (b). Magnified view of a adventitious root.
a – 285 X, b – 1140 X

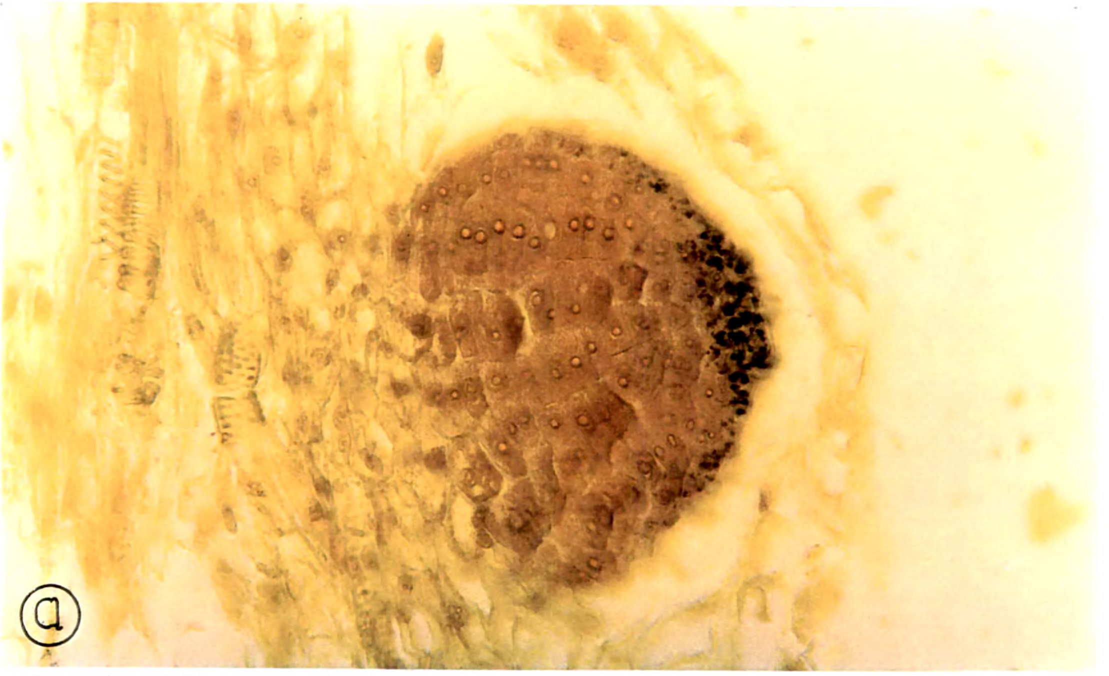

a
b

Plate 62. (a) to (b). Magnified view of a adventitious root.
a – 712.5 X, b – 285 X

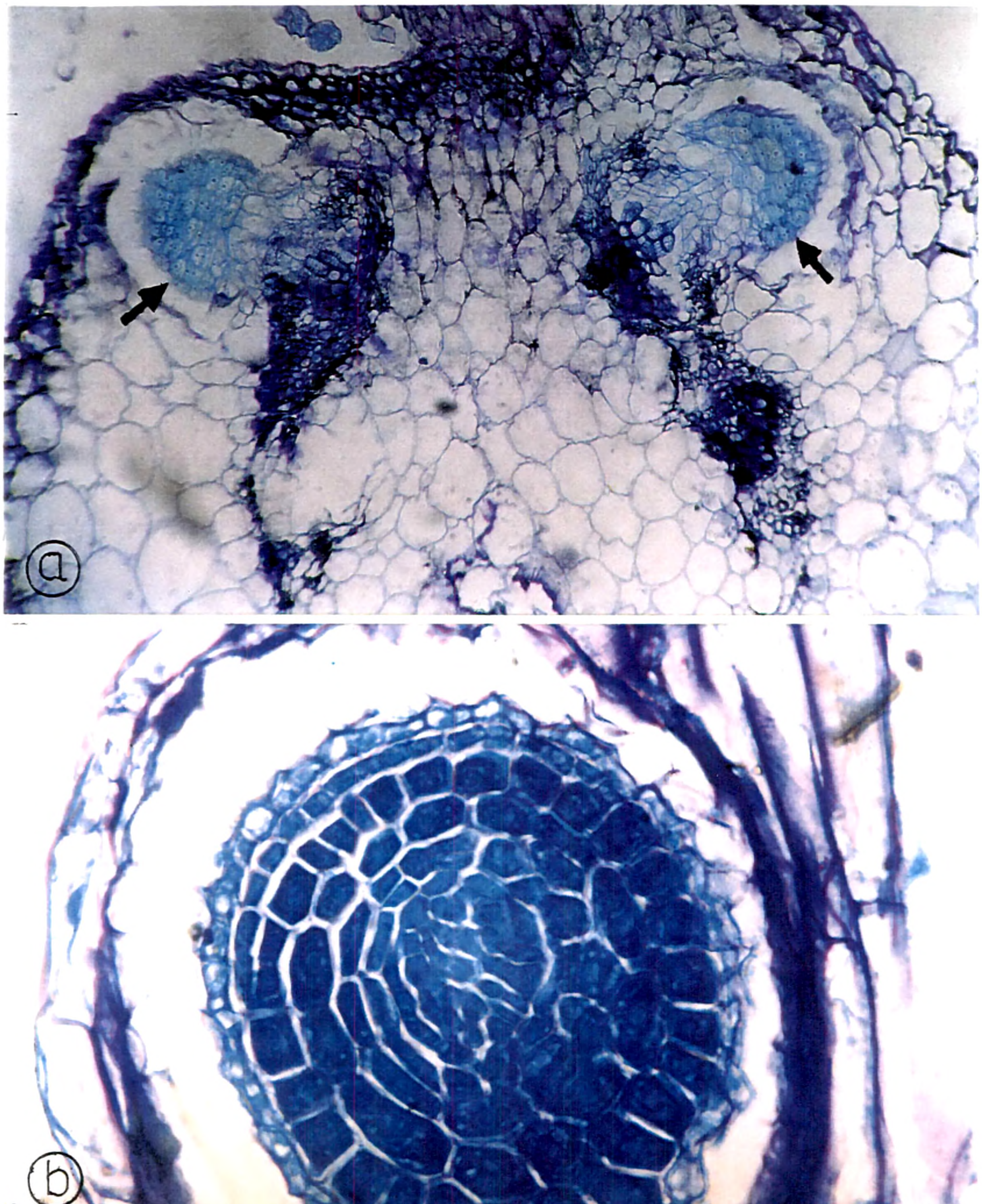

a
b

Plate 63. (a) and (b). Adventitious roots coming out by piercing the tissues (arrow) a - 285 X, b – 285 X

(c) Starch deposition on the root cap. 1140 X

(d) Section of buds with adventitious roots. 142.5 X

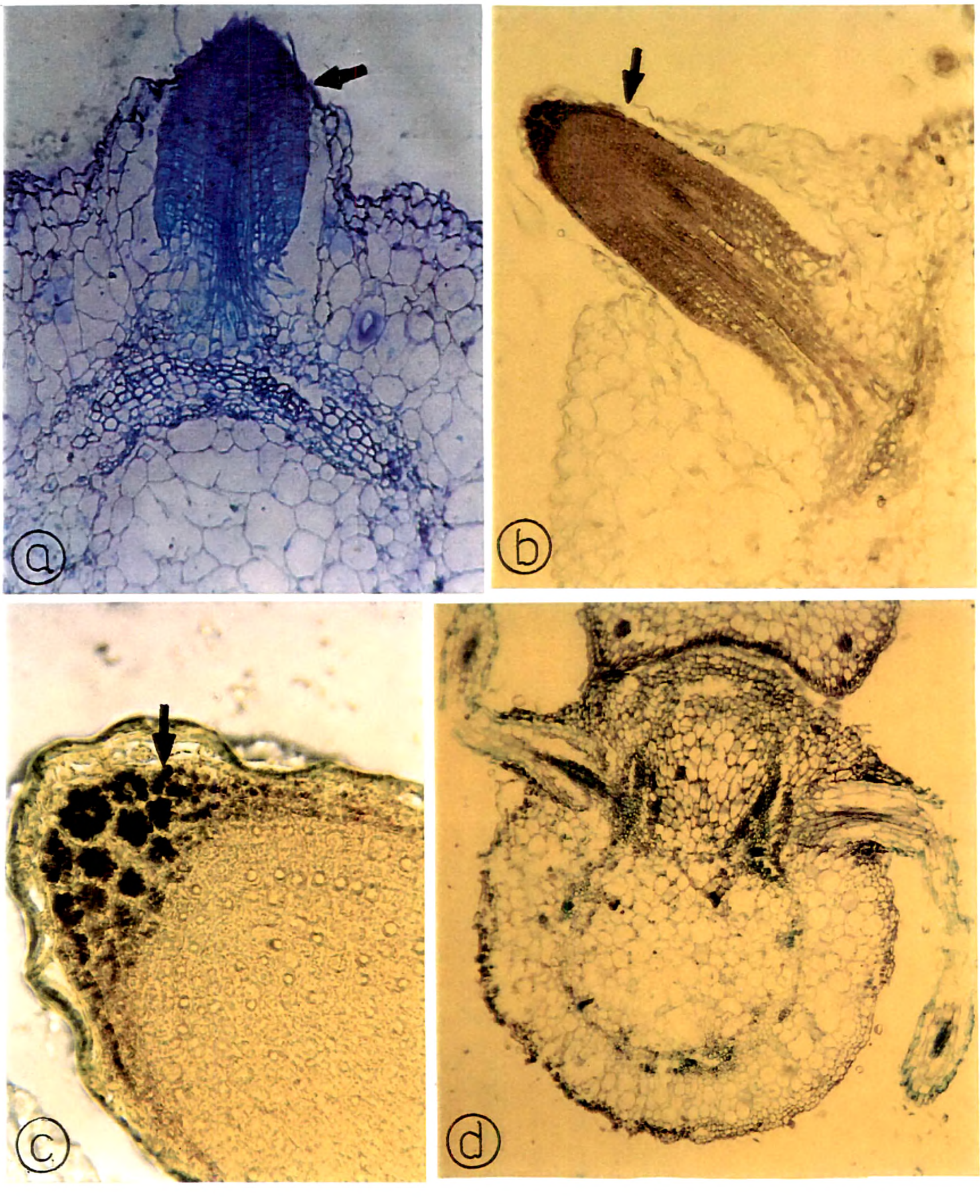
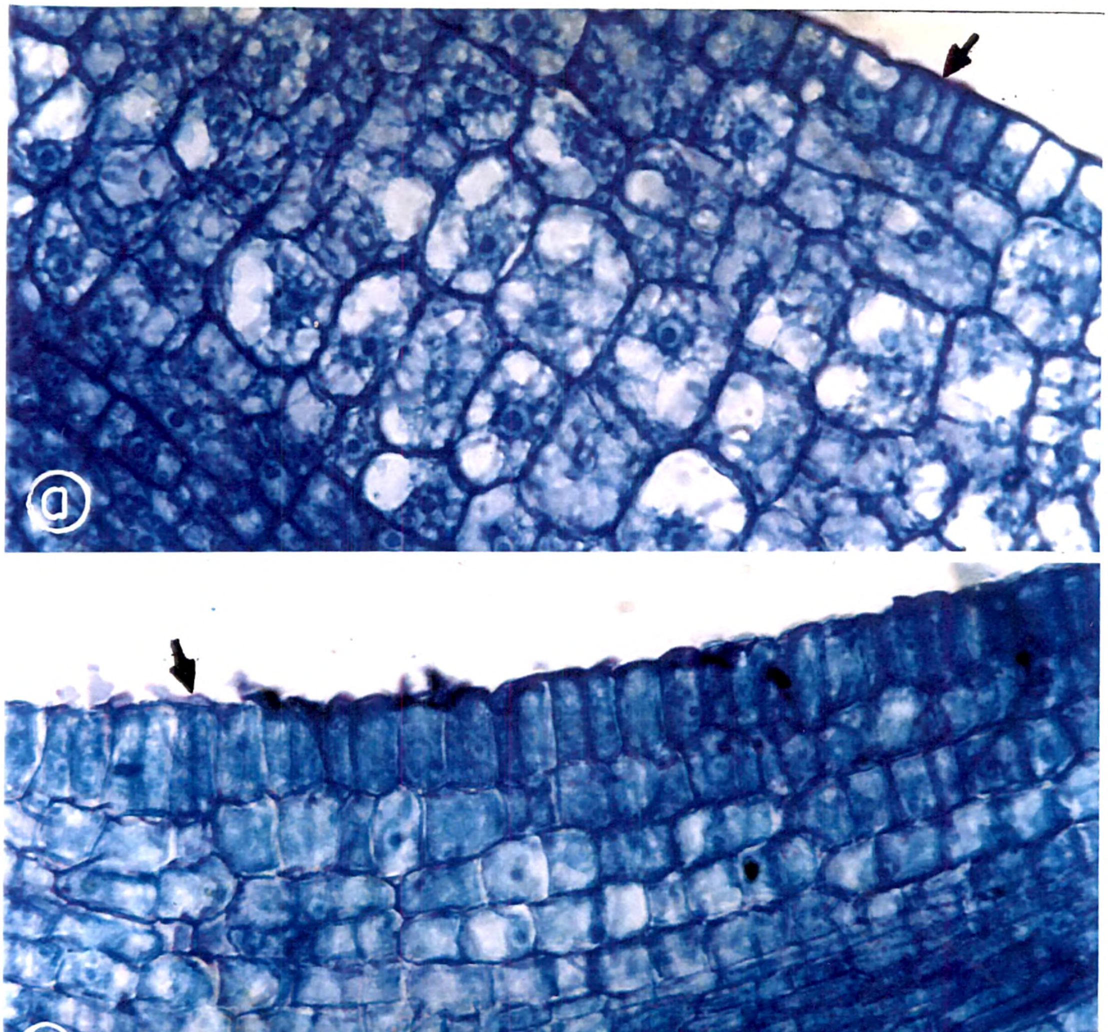

a
b
c
d

Plate 64. Section of 15 days old microtubers

(a) and (b). One layer of tunica cells showing anticlinal and periclinal division. a – 1140 X, b – 1140 X


a
b

(Phellem) became the layers of periderm and later got suberized. At a later stage periderm reached to a 9-10 files of cells which formed the skin of the microtubers (Plate: 67a and b). Coinciding with the development of periderm, lenticels were also formed in the skin. Lenticels were observed as small white dots on the well-developed epidermis. These lenticels had considerable volume of filling tissue and also had an opening to the external environment (Plate: 67c). Eyes or buds were formed in the axils of the small scale leaves of microtubers. They had the same anatomical structure of a bud on stem (Plate: 67d).

Plate 65. Section of microtubers -

(a) Periderm files of diameter 0.1 cm (25 days old) 712.5 X

(b) Periderm files of diameter 0.2 cm (30 days old) 712.5 X

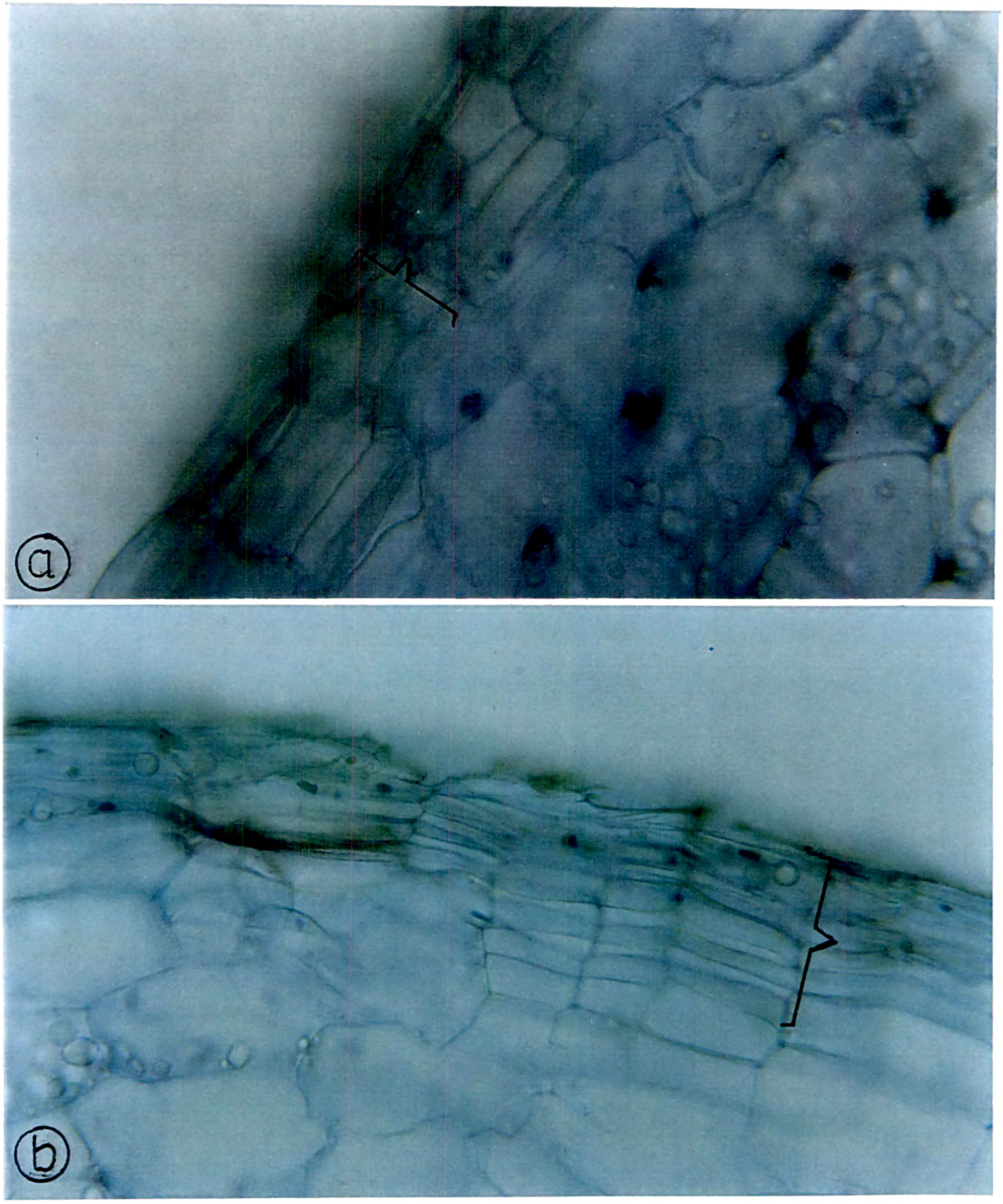

a
b

Plate 66. Section of microtubers -

(a) Periderm files of diameter 0.5 cm (45 days old) 712.5 X

(b) Periderm files of diameter 0.8 cm (60 days old) 712.5 X

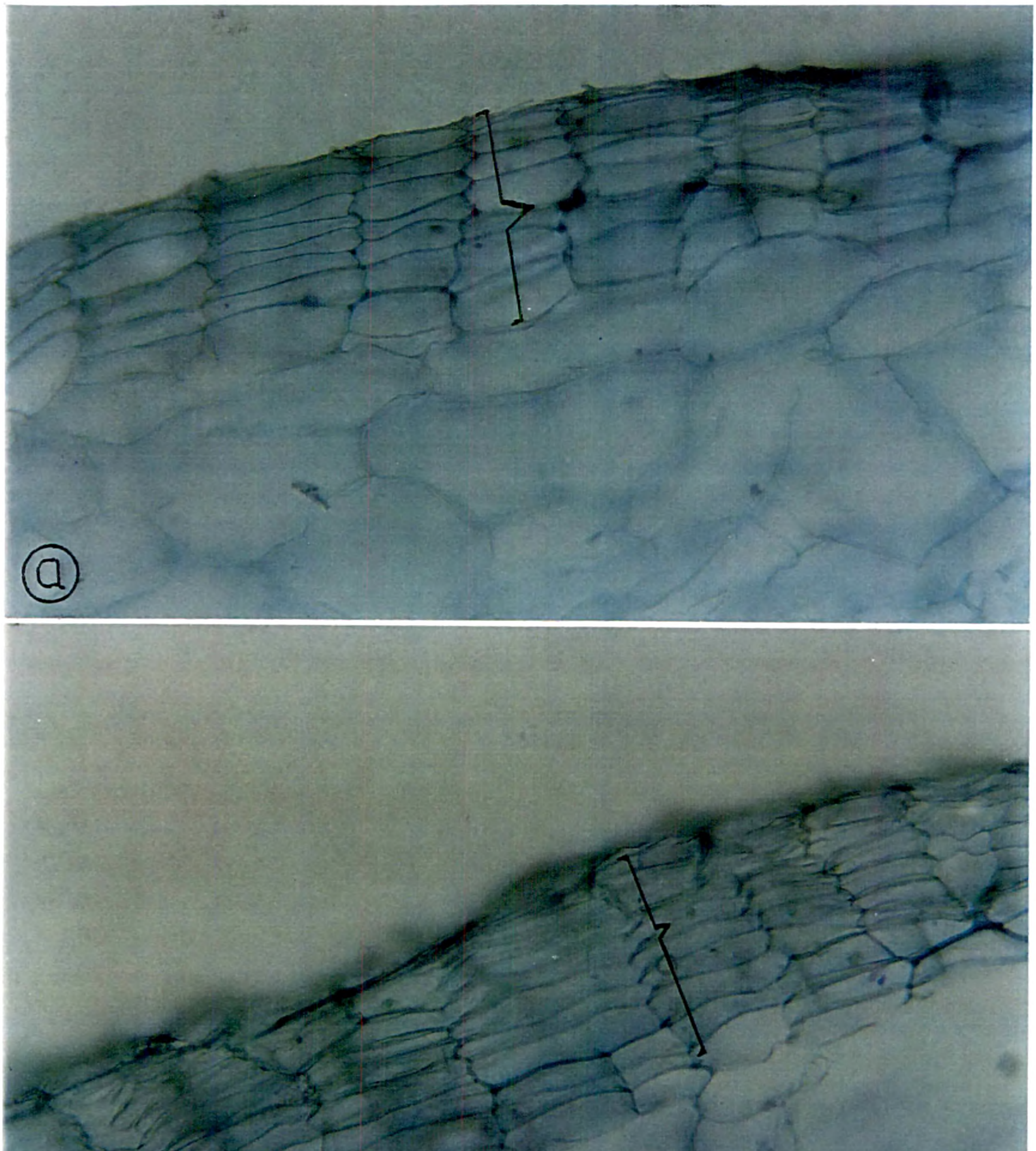
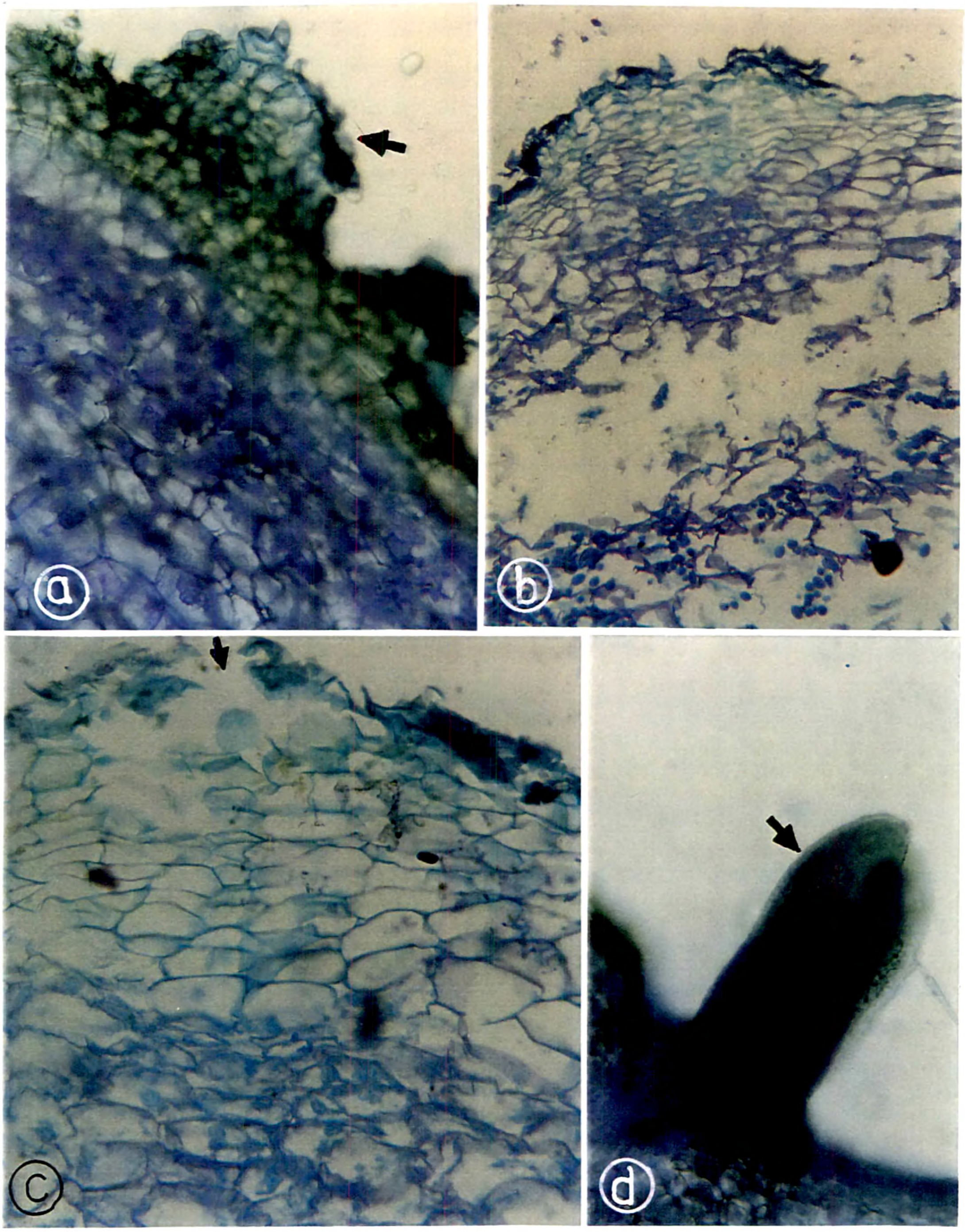

a
b

Plate 67. (a) Initiation of lenticels on the periderm. 285 x

(b) and (c) Lenticels showing an opening to the external environment (arrow) 285 x , 712·5 x

(d) Eye formation in the skin of a microtuber. 285 x


a
b
c
d