Indian Agricultural Research Institut

LABORATORY

No. _____ of 19

Subject PROTOPLASTS etc.

Name S. K. Mukherjee

लगे रहो -
लगे रहो,
मिलेगा - मिलेगा

Requirements

For Protoplast Isolation

(1) Enzyme (Filter Sterilized)
(2) Washing Medium (Mannitol) } Auto-clave
(3) " " (Sucrose) }
(4) Culture Medium (Filter Sterilized)
(5) A pair of 100 ml beakers with nylon
(6) 12 petri plates (Small)
(7) A pair of 100 ml flasks OR (for enzy treatm
(8) A pair of 100 ml beakers
(8) 12 Capped Cfg tubes
(9) 12 5ml pipettes / pasteur pipettes, with cotton stuffed at sucking end.
(10) 1 test tube stand.
(12) 2 medium sized Petri plates for chopping and pre-plasmolysis
(13) Para film

12/2/82 24

Nglauca leaves pc at 20-24°C for 3 days.

2 min 6000 rpm
- 7½ hours enzyme (9M) at 25°C
- Light fraction (that which float in enzyme mixture. Heavy fraction (that which sinks in enzyme mixture)
- 2 min centrifugation in 16 S
- 3 times.

Plating: at 0 hrs 13/2

Heavy
- ① KM - 1 flask
- ② ON - 1 flask

Light
- ① ON - 1 flask
- ② KM - 1 " (lid turned) dammit!

Light (cen?) KM - 5 petri plates

ॐ श्री गणेशाय नमः

2/3/82 26

10 hr isolation in 9MEn

(a) SR1 leaves (very fresh & young)

(b) NG 8PC

- NG floating fraction in 9M
- SR1 floating " in En (i)
  & Sunk " " " (ii)

1ml NG floating fr. + 1ml SR1 floating (1:1)
+ 1ml PEG → RM tube → stand
for 15 mins. Add 2.5ml 10pH, shake
& stand for 15 mins; Add
5ml 16S and centrifuge for 2 mins
Wash floating fraction in 16S
twice and plate in KM.

Took wrong tube & discarded the right one

~~illegible~~

. ½ml NG + 1ml SR1 Sunk (1:2)
+ ½ml PEG → RM tube → stand
for 10 mins. Add 2ml 10pH,
shake and stand for 10 mins
Add 6ml 16S and centrifuge

→ Too much of aggregation. Did not
form a band. Take more population
(higher density in future).
Use fresh 10PH. Better luck next time

(4) Sunk fraction & ~~floated~~ tried to be floated with .16% Sucrose → absolutely no band. Something wrong with 16S? → <u>No Nothing's wrong</u>
∴ Henceforth shift to <u>10 M Em and 16 S sucrose</u> for <u>N.t.</u> (SR1)? (May not be necessary if only floating fraction is required)

[Some photographs of representation of floating fraction protoplasts of N.t (SR1) were taken – don't know how they will come out!]

↓ lousy ∴ <u>thrown away</u>

<u>15.4.82:</u>

<u>N. glauca</u> leaves (12 nos.) chopped (1mm). 9M mannitol for 1 hr. Then 10 hrs in 9M Em after evacuation in Em. Digestion fairly good.

[In future <u>don't</u> shake the digested material <u>vigorously</u>]

(RUSKS EATEN at this point)

Muslin cloth filtrate had innumerable protoplasts of different sizes and chloroplast contents – very encouraging at this point.

<u>PLATING DONE at 0hrs 16/4/82</u>

(ff) Floating fraction → Plated (8 Plates)

2 KM , 2 KM (LD)

2 MSM , 2 MSG

[[illegible] After plating observation revealed 50% dead in MSM & MSG and Majority dead in KM].

P.T.O.