Aim..

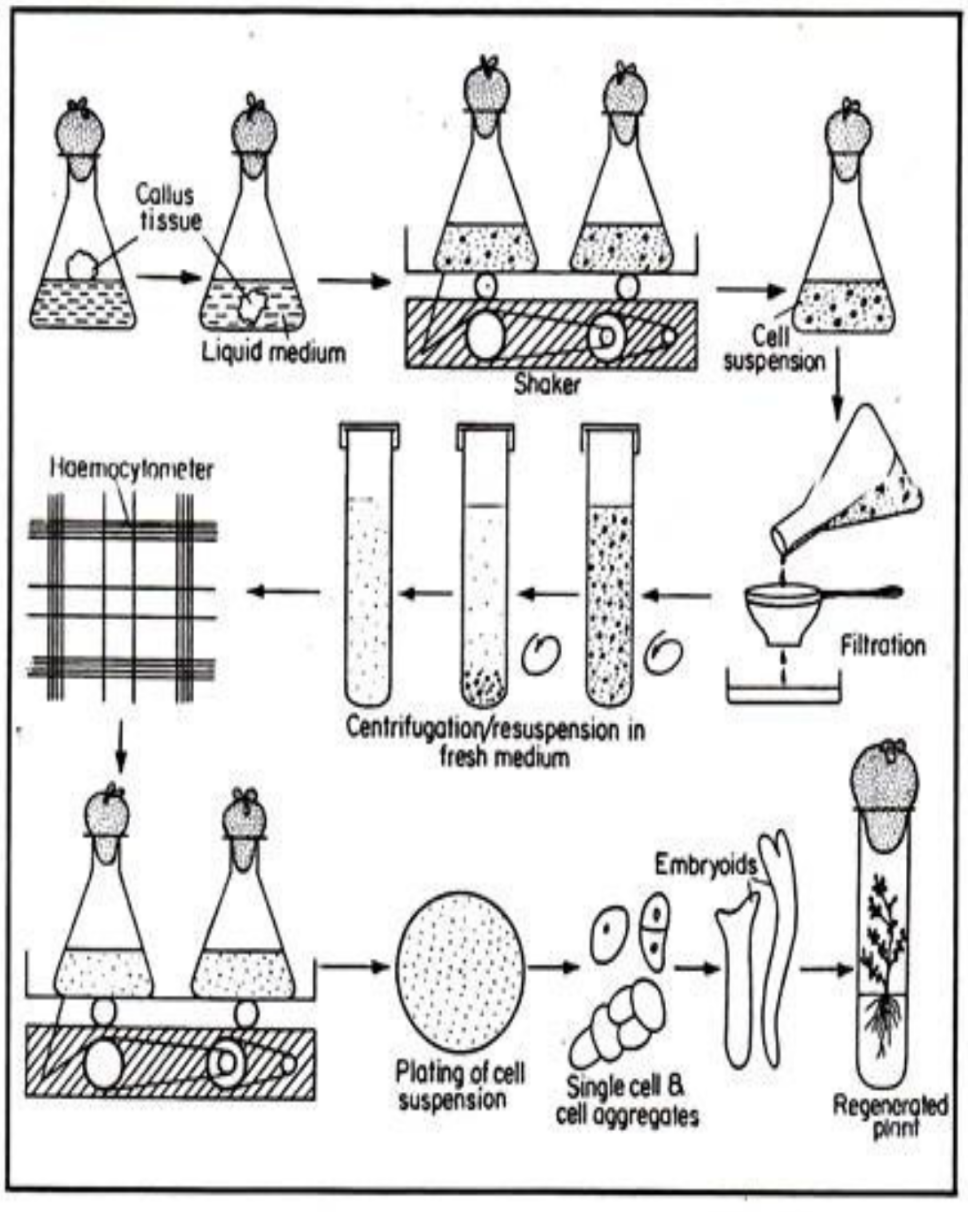
To establish cell suspension culture

Principle:

Callus proliferates as an unorganised mass of cells. So it is very difficult to follow many cellular events during its growth and develop mental phases. To overcome such limitations of callus culture, the cultivation of free cells as well as small cell aggregates in a chemically defined liquid medium as a suspension was initiated to study the morphological and biochemical changes during their growth and developmental pha9ises.

Procedure...

1. Take 150/250 ml conical flask



❒ Fig 4.1

Flow diagram illustrating the method of cell suspension culture and regeneration of plant through embryogenesis

containing autoclaved 40/60 ml liquid medium (Fig 4.1).

Transfer 3-4 pieces of pre-established callus tissue (approx. wt. 1 gm. each) from the culture tube using the spoon headed spat ula to conical flasks.

3. Flame the neck of conical flask, close the mouth of the flask with a piece of alluminium foil or a cotton plug. Cover the closure with a piece of brown paper.

4. Place the flasks within the clamps of a rotary shaker moving at the 80-120 rpm (revolution per minute)

5. After 7 days, pour the contents of

each flask through the sterilized sieve pore diameter -60µ- 100µ and collect the filtrate in a big sterilized container. The filtrate contains only free cells and cell aggregates.

6. Allow the filtrate to settle for 10-15 min. or centrifuge the filtrate at 500 to 1,000 rpm and finally pour off the supernatant.

7. Re-suspend the residue cells in a requisite volume of fresh liquid medium and dispense the cell suspension equally in several ster ilized flasks (150/250 ml). Place the flasks on shaker and allow the free cells and cell aggregates to grow.

8. At the next subculture, repeat the previous steps but take only one-fifth of the residual cells as the inoculum and dispense equally in flasks and again place them on shaker.

9. After 3-4 subcultures, transfer 10 ml of cell suspension from each flask into new flask containing 30 ml fresh liquid medium.

Result

Cell suspension culture is prepared successfully