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FACULTY OF ENGINEERING &
TECHNOLOGY

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LECTURE 7

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PROTOPLAST FUSION

- It is the technique in which two or more protoplast are fused into a single cell protoplast.
- The protoplast fusion allows us to bring an desirable plant traits in combination that are not possible by sexual means.
- May occur between same or different plant
- Mutinucleate protoplasm obtained after fusion
- Protoplast fusion can be used to make crosses within species (intraspecific), between species (interspecific), within genera (intrageneric) and between genera (intergeneric).

The protoplasts fusion may be of three types:

1. Spontaneous fusion
2. Mechanical fusion
3. Induced fusion

Spontaneous Fusion

In spontaneous fusion, the adjacent protoplasts in enzyme mixture have tendency to fuse together to form homokaryons (having same type of nucleus).

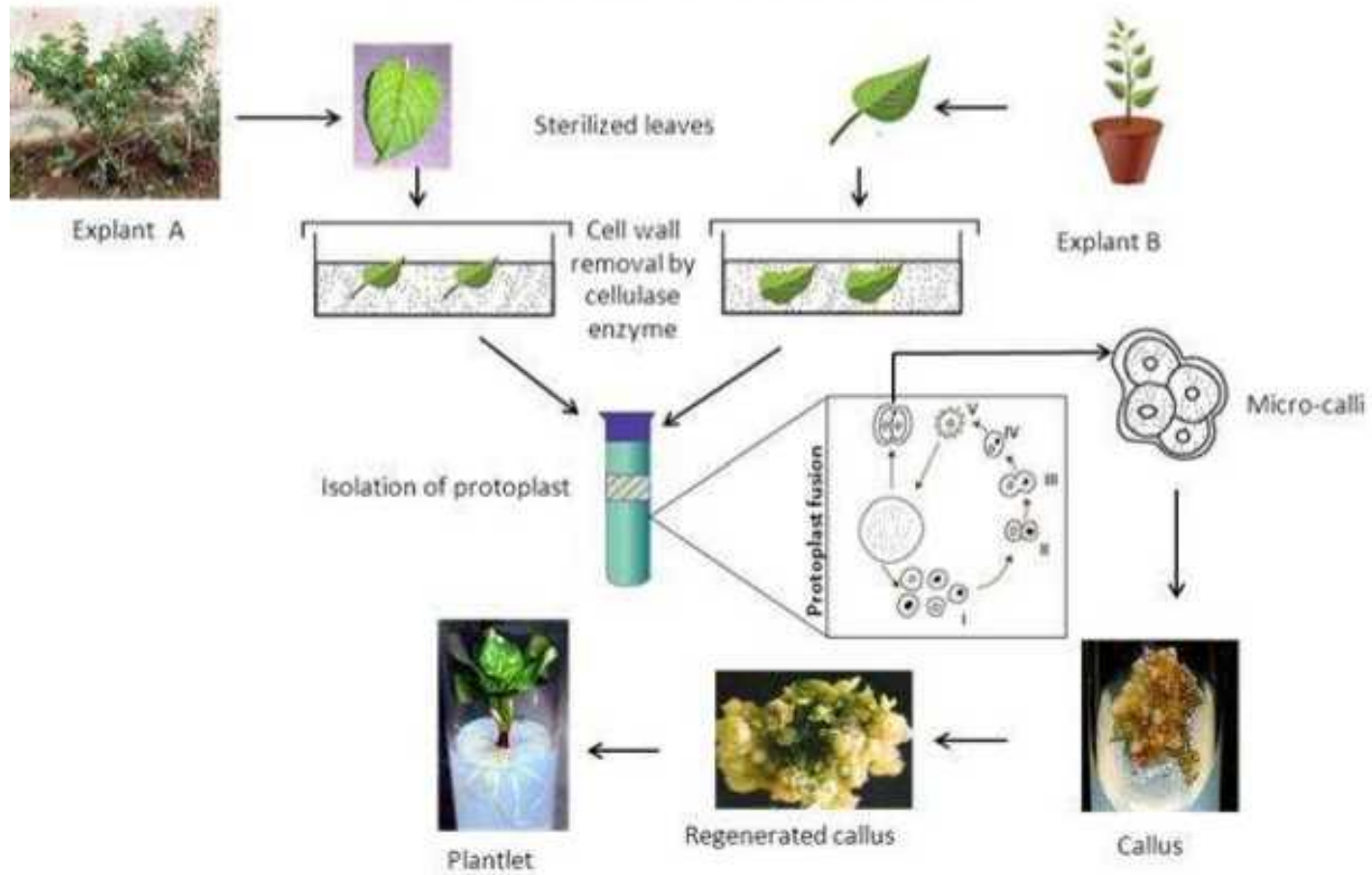
Mechanical Fusion

Gentle tapping of protoplasts suspension in a depression slide results in protoplasts fusion. The giant protoplasts of *Acetabularia* have been fused mechanically by pushing together two protoplasts. This fusion doesnot depend upon the presence of fusion inducing agents

Induced Fusion

Freshly isolated protoplasts can be induced to undergo fusion, with the help of a range of fusogens .e.g., NaNO_3 , artificial sea water, lysozyme, high pH/ Ca^{++} , PEG, polyvinyl alcohol, electrofusion.

Schematic Representation of Protoplast Fusion



PROTOPLAST CULTURE

Isolated protoplast are cultured in either liquid or semisolid agar media plates. They require somatic protection in culture medium until they generate a strong cell wall. Methodology of Protoplast culture is described below:

Isolated protoplast is cleaned by centrifugation and decantation method

Protoplast solution ($\sim 10^5$ protoplasm/ml) is poured on sterile medium

Mix it gently and allowed the medium to set

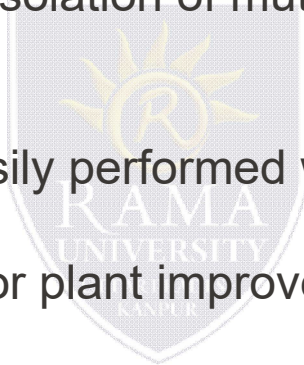
Protoplasts capable of dividing undergo cell divisions from callus within 2-3 weeks

Callus is subculture on fresh medium

Embryogenesis begin from callus when it is transferred to a proper nutrient medium and subsequently whole plant develops

Applications of protoplast culture

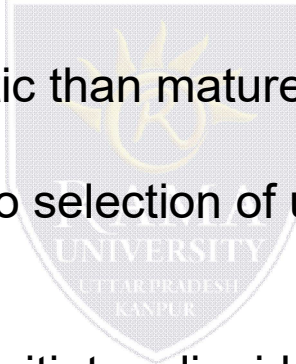
1. To develop Novel hybrid plant through protoplast fusion.
2. In single cell derived colony, isolation of mutants through mutagens is easier.
3. Single cell cloning can be easily performed with protoplasts.
4. Regeneration of entire plant or plant improvement through protoplast culture.
5. Genetic transformation through DNA uptake can be achieved.



CALLUS CULTURE

- A tissue that develops in response to injury caused by physical or chemical means, most cells of which are differentiated although they may be and often are highly unorganized within the tissue.
- Callus differs in compactness or looseness, i.e. cells may be tightly joined and the tissue mass is one solid piece or cells are loosely joined and individual cells readily separate (friable). This can be due to the genotype or the medium composition.
- A friable callus is often used to initiate a liquid cell suspension culture.
- Callus is formed at the peripheral surfaces as a result of wounding and hormones (auxin, high auxin/low cytokinin).

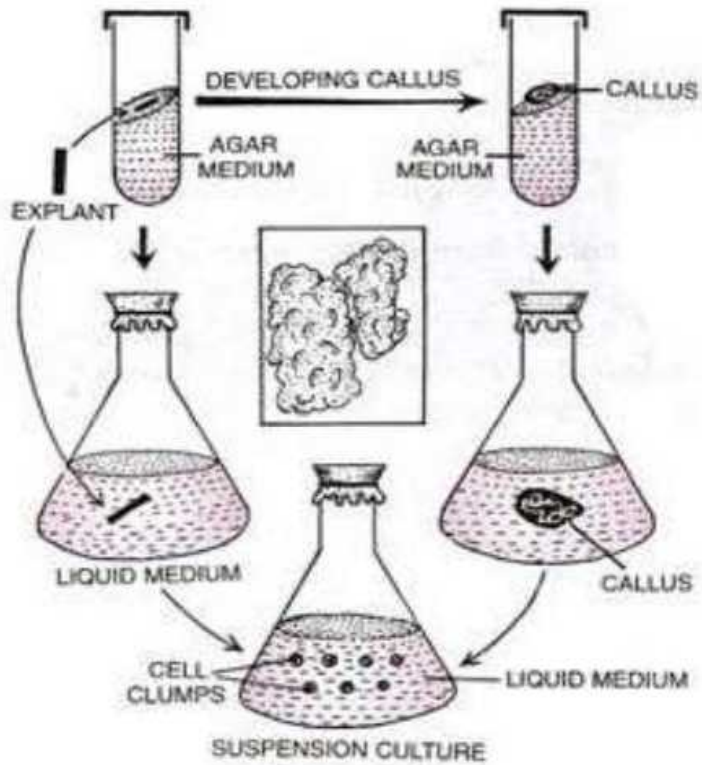
- Genotype, composition of nutrient medium, and physical growth factors are important for callus formation.
- Explants with high mitotic activity are good for callus initiation.
- Immature tissues are more plastic than mature ones.
- Callus is ideal material for in vitro selection of useful somaclonal variants (genetic or epigenetic).
- A friable callus is often used to initiate a liquid cell suspension culture for production of metabolites
- Friable callus is a source of protoplasts.



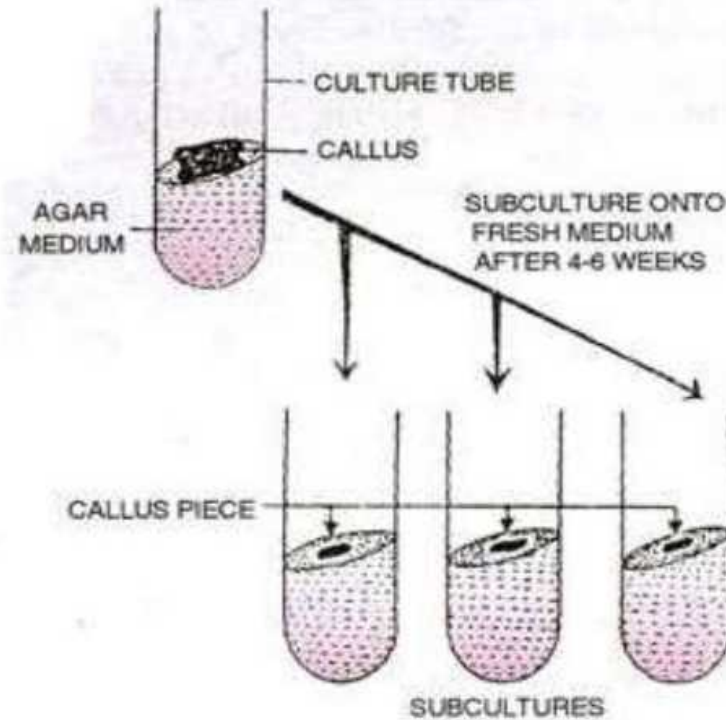
Steps involved in Callus Culture

- Some callus growths are heavily lignified and hard in texture whereas others break easily into small fragments. Most explants produce sufficient callus in an appropriate medium to allow for subculture within 3-7 weeks.
- When cultured for several weeks, callus will show signs of ageing. This can be noted as deceleration of growth, necrotic or browning and finally desiccation.
- The newly formed callus will be removed from the initial explant at this stage by cutting with the sterile scalpel.
- Once well established, most callus cultures will require regular subculture at approximately 4 weeks interval.

SUMMARY OF CALLUS CULTURE AND SUBCULTURE



Initiation of callus and suspension cultures.



Schematic representation of subculturing.

QUIZ

