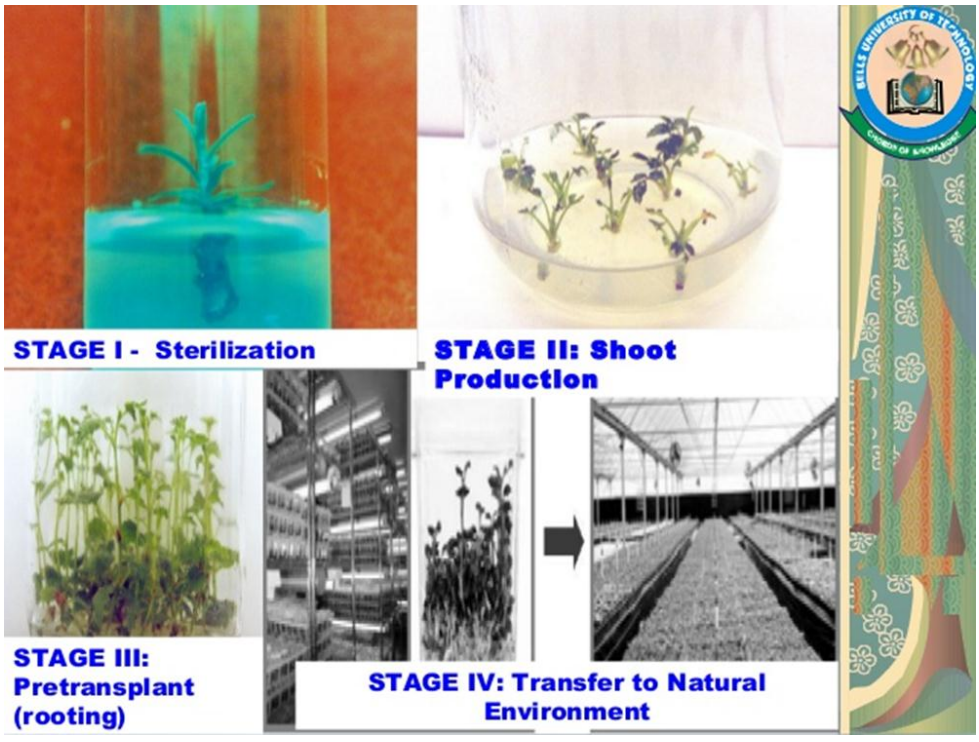
Lab.8: Plant Cell and Tissue Culture Techniques

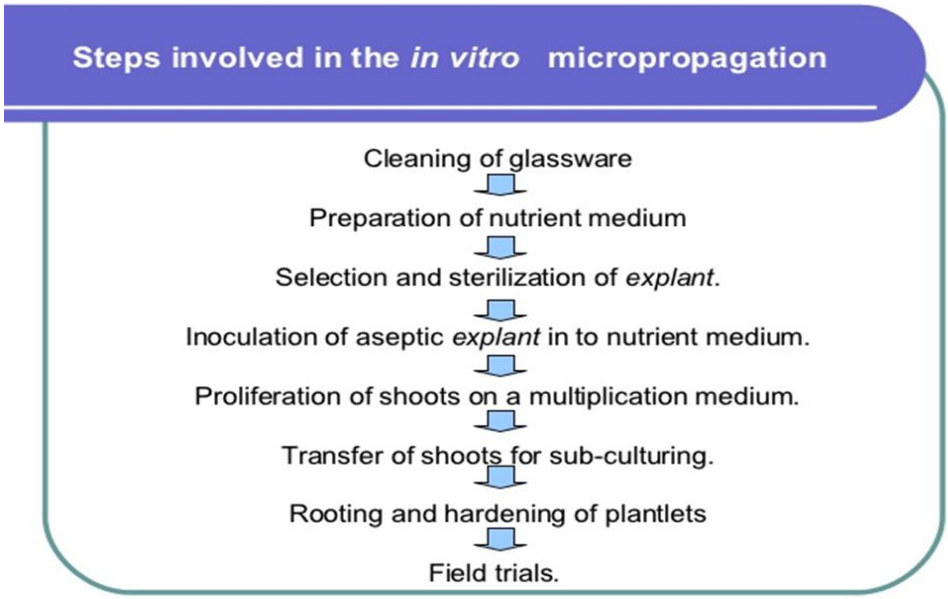
Plant cell culture is based on the unique property of the cell-totipotency. This property has been exploited to regenerate plant cells under the laboratory conditions using artificial nutrient mediums.

The whole plants can be regenerated virtually from any plant part (referred to as explant) or cells.

Plant tissue culture techniques involve the following steps:

1. Preparation and selection of suitable nutrient media.
2. Selection of explants such as shoot tip.
3. Surface sterilization of the explants by disinfectants e.g. sodium or calcium hypochlorite solution (0.3- 0.6%) followed by washing the explants with sterile distilled water.
4. Inoculation or Transfer of the explants onto the suitable nutrient medium (sterilized by autoclaving) in culture vessels under sterile conditions (using laminar flow hood).
5. Incubation or growing the cultures in the growth chamber or plant tissue culture room at optimum physical conditions of light (16 hours of photoperiod), diurnal illumination, temperature (25+/- 2^0C and relative humidity (50%-60%).
6. Regeneration of plants from cultured plant tissues.
7. Hardening: it is the gradual exposure of plantlets for acclimatization to environmental conditions.
8. Transfer of plants to the field conditions following the acclimatization/ hardening of the regenerated plants.





Nutrient media

The composition of plant tissue culture medium depends upon the type of plant tissues or cells that are used for culture.

A typical nutrient medium consists of the following components:

1. Inorganic nutrients (micronutrients, such as Fe and macronutrients, such as N, K, Ca, P, S and Mg).
2. A carbon source and energy source, sucrose is the most preferred.
3. Organic supplements to achieve good growth of cells; vitamins, amino acids and organic acids. Sometimes antibiotics are also added to the medium to prevent the growth of the microorganisms.
4. Plant Growth regulators(PGRs);The growth hormones included:

- Auxins (for root formation), the most commonly added auxins is 2,4-dichlorophenoxy acetic acid.
- Cytokinins (for shoot formation), the most commonly added cytokinins are Kinetin and benzyl-aminopurine.
- Gibberellins, gibberellin A3 (GA3) is the most commonly used for tissue culture. GA3 enhances callus growth and induces dwarf plantlets to elongate.

The ratio of auxins and cytokinins play an important role in the morphogenesis of culture systems.

- Intermediate ratio =callus formation.
- Low auxin to cytokinin ratio = shoot formation.
- High auxin to cytokinin ratio = root formation.

5. Solidifying agents like agar is added to the liquid medium for its solidification, because in the liquid medium, the tissue will be

submerged and die due to lack of oxygen. Cells are grown in suspension medium without agar but such cultures are aerated regularly either by bubbling sterile air or by gentle agitation.

6. Other compounds like casein hydrolysate, coconut milk, malt extract, yeast extract, tomato juice, etc. may be added for specific purposes.
7. pH - An optimum pH (usually 5.7) is also very important. At pH higher than 7.0 and lower than 4.5, the plant cells stop growing in cultures.

Major types of media:

- **White's medium** - is one of the earliest plant tissue culture media
- **MS medium** - formulated by Murashige and Skoog (MS) in 1962 is most widely used for many types of culture systems
- **B5 medium** - developed by Gamborg for cell suspension and callus cultures and at present it's modified form used for protoplast culture
- **N6 medium** - formulated by Chu and used for cereal anther culture
- **Nitsch's medium** - developed by Nitsch and Nitsch and used for anther culture

Preparation of media

The methodology for media preparation involves preparation of stock solutions (in the range of 10x to 100x concentrations) of highly purified chemicals and demineralized water. The stock solutions are stored in glass or plastic containers and frozen till further requirement. Now days, plant tissue culture media are commercially prepared, and are available in the market as dry powder.

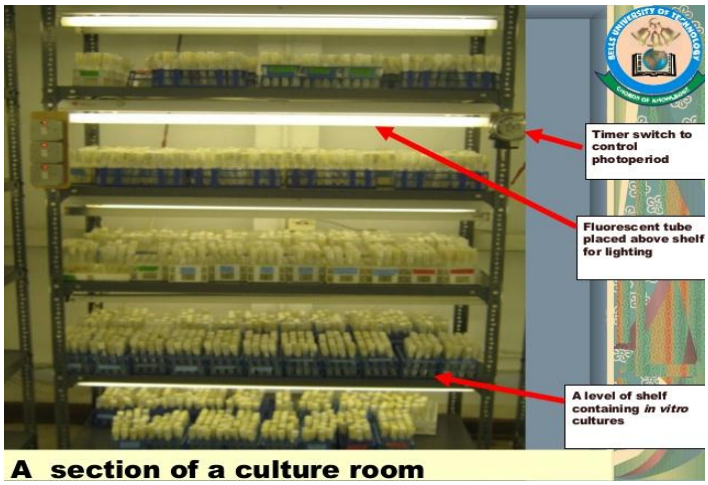
Maintenance of Aseptic Environment

- Sterilization of Glassware- The glassware can be sterilized in a hot air oven at 160-180^{0}C for 2-4 hours.
- Sterilization of instruments- The metallic instruments are incinerated by dipping them in 75% ethanol followed by flaming and cooling.
- Sterilization of nutrient media- The culture media are transferred into glass container, plugged with cotton or sealed with plastic closures and sterilized by autoclaving at 15 psi (pounds per square inch) for 30 min. The autoclaving denatures the vitamins, plant extracts, amino acids and hormones therefore the solution of these compounds are sterilized by using Millipore filter paper with pore size of 0.2 micrometer(Mm) diameter.
- Sterilization of plant materials- The surface of the plant material is made sterile by using disinfectants e.g. sodium hypochlorite, hydrogen peroxide, mercuric chloride, or ethanol. The transfer of sterile plant material on to the nutrient medium is done under the cabinet of laminar airflow.

- Sterilization of Culture room and transfer area- the floor and walls of the culture room should be washed with detergent followed by 2% sodium hypochlorite or 95% ethanol. The sterilization can also be done by exposure to UV light. The cabinet of laminar air flow is sterilized by exposing to UV light for 30 min. and 95% ethanol 15 min. before starting the work.

General laboratory & media

Transfer area (GRC IITA IBADAN)



A section of a culture room