

## Bharathidasan University Tiruchirappalli Tamil Nadu - India

# Programme: M.Sc Biotechnology Course Title: Plant Biotechnology Course code: 22BTCC12

Unit -1
Basics of Plant Tissue Culture
Virus elimination

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**Meristem culture** is a widely used technique for the elimination of viruses from plant materials, particularly when plants are infected with systemic viral diseases. The method relies on the principle that the meristematic tissue (the growing tip of the plant) is often free from viral infections, even though other parts of the plant may harbor the virus.

#### What is Meristem Culture?

- Meristem: The meristem is a region in the plant where active cell division occurs. It is found at the tips of roots, stems, and buds.
- Meristem Culture: Involves the excision (removal) and culture of meristematic tissue (typically from shoot tips) under sterile conditions. The cultured meristem gives rise to virus-free plantlets, which can be used for propagation.

#### **Virus-Free Status of Meristematic Tissues**

Meristematic tissues have the following characteristics that make them ideal for virus elimination:

- Viral Infections: Viruses typically infect the plant through systemic spread, affecting non-meristematic regions such as leaves, stems, and roots. However, the meristematic tissue generally remains free of viruses. This phenomenon is known as "meristematic resistance".
- Size of the Meristematic Cells: Viruses are usually unable to infect cells in the meristem because these cells are small and have a high mitotic activity, which makes it difficult for the virus to establish an infection.

**Excision of Apical Meristem**: The apical meristem, a specialized region of growth in the shoot tip, is most often used for virus elimination. The meristematic tissue is small and usually free from viral infection, thus excising and growing a plant from this tissue can yield a virus-free plan.

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#### **Steps in Virus Elimination by Meristem Culture**

- 1. Selection of the Plant Material: The first step is to select plant material that is suspected to be infected by a virus. The plant material typically comes from a field or greenhouse crop that shows symptoms of viral disease.
- 2. Isolation of the Meristem: The meristem is isolated from the plant by removing the topmost growing tip of the plant, which includes the shoot apical meristem. The size of the meristem used can vary, but it typically ranges from 0.2 to 0.5 mm in length.
- **3. Sterilization**: The isolated meristem is sterilized using disinfectants (e.g., sodium hypochlorite) to avoid microbial contamination. This step is critical as any contamination could affect the success of the culture.
- 4. Culturing the Meristem: The sterilized meristem is then placed in a sterile culture medium. This medium typically contains nutrients, hormones (like cytokinins and auxins), and growth regulators to encourage the growth and development of the plantlets. Growth regulators are important as they stimulate shoot proliferation and rooting from the meristem tissue.

- **5. Induction of Shoot Formation**: Under controlled conditions (light, temperature, and humidity), the meristem starts to grow and form new shoots. Over time, these shoots develop into healthy plants that are genetically identical to the original plant but free of viruses.
- **6.Acclimatization**: The virus-free plants produced in the culture media are then gradually acclimatized to external environmental conditions. This includes transferring the plants from the sterile culture medium to soil or a suitable growth medium.
- **7.Virus Testing**: Once the plantlets are grown, they are tested to ensure they are virus-free. Testing methods like **ELISA** (**Enzyme-Linked Immunosorbent Assay**), **PCR** (**Polymerase Chain Reaction**), and **electro-immunoassay** can be used for confirmation.
- **8.Propagation**: After successful virus elimination and testing, the plantlets can be propagated further. Since the original plant material was infected, meristem culture ensures that the newly grown plants will be free from viruses and can be used for future breeding programs or commercial production.

#### **Advantages of Meristem Culture for Virus Elimination**

- **.High Efficiency**: The technique is highly effective at eliminating viruses that are systemic in nature, and it results in the production of healthy, virus-free plant material.
- **Large-Scale Application**: It can be used to propagate large numbers of virus-free plants, especially in crops like potatoes, bananas, and ornamental plants.
- •Clonal Propagation: Since meristem culture produces clones, it ensures uniformity in plant characteristics such as yield, disease resistance, and quality.
- **Long-Term Benefits**: The virus-free plants produced can be used for further breeding and production of disease-free seedlings, helping to maintain crop health over several generations.

#### **Limitations and Challenges**

- •Technical Expertise: The technique requires skilled labor and technical expertise in tissue culture, particularly in sterile techniques and handling of plant tissues.
- •Costly and Time-Consuming: Meristem culture is labor-intensive and can be more expensive compared to other virus elimination techniques. It also takes time to culture and propagate the virus-free plantlets.
- •Potential for Contamination: The process involves handling plant tissue in a sterile environment, and any contamination (microbial or viral) can lead to failure.

**Limited to Certain Species**: While meristem culture is widely used in certain species, it may not be applicable or successful for all plant species, particularly those with less responsive meristematic tissue

#### **Applications of Virus Elimination by Meristem Culture**

- •Agricultural Crops: Meristem culture is widely used for virus elimination in important crops like potatoes (e.g., Potato Virus Y), bananas (e.g., Banana bunchy top virus), and sweet potatoes.
- •Ornamentals: Many ornamental plants, such as chrysanthemums, carnations, and orchids, are propagated through meristem culture to ensure virus-free plants for the floral industry.

**Fruit Trees**: In fruit trees like citrus and grapes, meristem culture is used to eliminate viral diseases like Citrus tristeza virus and Grapevine fanleaf virus, ensuring better yields and quality.