Bharathidasan University
Tiruchirappalli
Tamil Nadu - India

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

Unit -1
Basics of Plant Tissue Culture
Adventitious shoot production

Dr.M.Manickavasagam
Associate Professor
Department of Biotechnology

Adventitious shoot production

- **Adventitious shoot production** refers to the formation of shoots from non-meristematic tissues (i.e., tissues not originally designated to form shoots).
- This phenomenon is a common technique in plant tissue culture, where shoots are induced to form from various plant tissues, including leaves, stems, and roots, under controlled laboratory conditions.
- This process plays a crucial role in plant regeneration, clonal propagation, and genetic studies.

Adventitious shoot production

- **Adventitious shoots** are shoots that develop from parts of the plant that are not typically involved in shoot formation. These include tissues like leaves, stems, roots, or callus (undifferentiated plant cells)
- The formation of adventitious shoots is primarily influenced by the plant's **regenerative capabilities** and the **exogenous application of plant growth regulators (PGRs)** such as cytokinins (e.g., BAP, kinetin) and auxins (e.g., IAA, NAA).

Adventitious shoot production

Regulation of Adventitious Shoot Proliferation

Adventitious shoot formation is a complex process regulated by genetic, hormonal, and environmental factors.

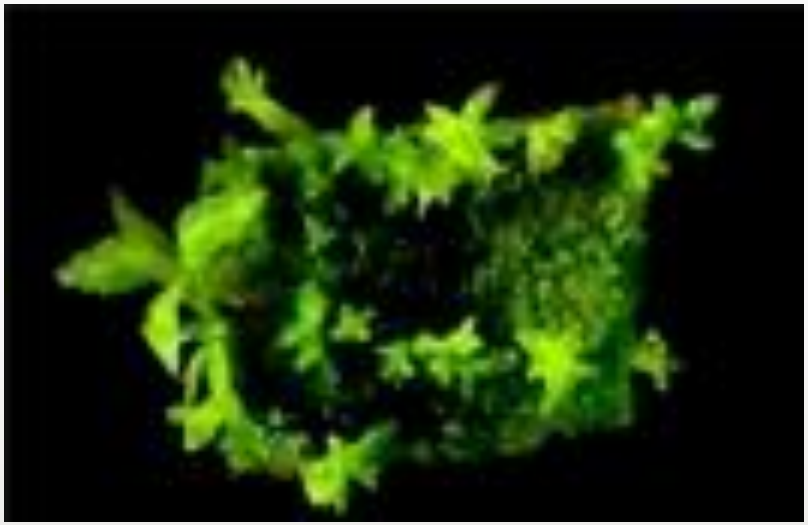
a. Hormonal Regulation

The balance between cytokinins and auxins plays a critical role in adventitious shoot formation:

Cytokinins: These hormones promote cell division and shoot initiation. When applied in the right concentration, cytokinins stimulate the formation of adventitious buds from callus tissue or leaf explants.

Auxins: Auxins are important for root initiation but can inhibit shoot formation if present in high concentrations. However, in certain cases, they can induce shoots when used in very low concentrations or in combination with cytokinins.

The proper ratio of **cytokinin to auxin** is crucial for the success of shoot regeneration. High cytokinin levels favor shoot formation, while an imbalance can lead to the formation of roots or callus instead.



Adventitious shoot production

b. Explants and Their Role

The type of explant (the tissue from which the adventitious shoots are induced) is a critical factor:

Leaf Explants: Leaves are commonly used for adventitious shoot regeneration. The epidermis and mesophyll tissue contain meristematic cells capable of producing shoots when exposed to the right hormonal environment.

Stem Explants: Adventitious shoots can also form from the nodes or internodes of stems. Meristematic cells in these regions, when cultured on nutrient media, can differentiate into adventitious shoots.

Root Explants: In some species, adventitious shoots can be induced from root tissues, particularly in certain species of dicots and monocots.

C. Callus Formation

•Callus is an undifferentiated mass of plant cells that can be induced at the site of injury on an explant. In many cases, adventitious shoots form from callus tissue when the right hormonal signals are applied.

•Callus formation is often a precursor to shoot regeneration, as the callus cells can redifferentiate into meristematic tissue that gives rise to adventitious shoots.

Stages of Adventitious shoot production

Stages of Adventitious Shoot Proliferation

The process of adventitious shoot proliferation can be broken down into several stages:

a. Induction

In the first stage, explants (such as leaf or stem sections) are placed on a nutrient medium containing appropriate plant growth regulators.

Cytokinins, particularly **benzylaminopurine (BAP)**, **kinetin**, or **zeatin**, are typically used to stimulate shoot initiation. If auxins are used, they are often at very low concentrations to avoid root induction.

b. Callus Formation

For many species, an intermediate step of **callus formation** may occur. This mass of undifferentiated cells can be cultured and treated with hormones to induce shoot formation.

Stages of Adventitious shoot production

c. Shoot Induction

As the callus or explant is exposed to the right combination of cytokinins and sometimes low auxin concentrations, **adventitious shoots** begin to form.

The shoots may appear as small buds on the explant or callus.

In this phase, the plant cells begin differentiating into shoot meristems, which grow into functional shoots.

d. Shoot Elongation

Once adventitious shoots have formed, they need to be **elongated** and developed into viable plantlets. This often involves transferring the newly formed shoots to a medium with low cytokinin and high nutrient content.

e. Rooting

After shoot elongation, plantlets are transferred to a rooting medium that may contain auxins to encourage the formation of roots.

The establishment of roots is essential before the plantlets are transferred to soil or acclimatized to external conditions.

Factors affecting Adventitious shoot production

Several factors influence the efficiency and success of adventitious shoot proliferation:

a. Plant Species

Different plant species exhibit varying capacities for adventitious shoot formation. Some species, particularly those with high regenerative abilities, are more responsive to tissue culture techniques. Species like **Arabidopsis**, **tobacco**, **petunia**, and certain fruit trees are well-known for their ability to regenerate adventitious shoots.

b. Explant Type

The choice of explant significantly affects shoot induction. Some explants, like young leaves or shoot tips, are more likely to produce adventitious shoots compared to older or more differentiated tissues.

c. Medium Composition

The composition of the nutrient medium is crucial. A standard medium used in adventitious shoot proliferation is **Murashige and Skoog (MS) medium**, which contains essential macro- and micro-nutrients, vitamins, and sugars. The pH of the medium, the strength of nutrients, and the addition of specific vitamins can influence shoot formation.

Factors affecting Adventitious shoot production

d. Environmental Factors

Light: Light intensity and photoperiod can affect shoot regeneration. Many plants require a specific light condition (such as fluorescent light or darkness) to encourage shoot initiation.

Temperature: The temperature at which the cultures are maintained is critical. A consistent temperature range of around 22-25°C is often ideal for shoot proliferation.

e. Culture Vessel

The type of culture vessel used (e.g., Petri dishes, culture bottles, or jars) can influence the growth of adventitious shoots by affecting humidity, oxygen exchange, and light exposure.

Applications of Adventitious shoot production

Adventitious shoot proliferation has a wide range of practical applications in plant science and agriculture:

Clonal Propagation: The process is widely used for cloning plants with desirable traits, ensuring uniformity and the maintenance of genetic characteristics.

Germplasm Conservation: Rare or endangered plant species can be propagated via adventitious shoot regeneration to ensure their preservation.

Genetic Engineering: Adventitious shoot formation is a critical step in producing genetically modified plants, as it allows for the regeneration of whole plants from transformed tissues.

Commercial Horticulture: Many ornamental plants, fruit trees, and medicinal plants are propagated using adventitious shoot cultures to quickly generate large numbers of plants.

Biotechnology and Pharmaceutical Production: Plants can be cultured in vitro to produce valuable secondary metabolites, pharmaceuticals, and other bioactive compounds.

Disadvantages of Adventitious shoot production

Despite its potential, adventitious shoot regeneration can present challenges:

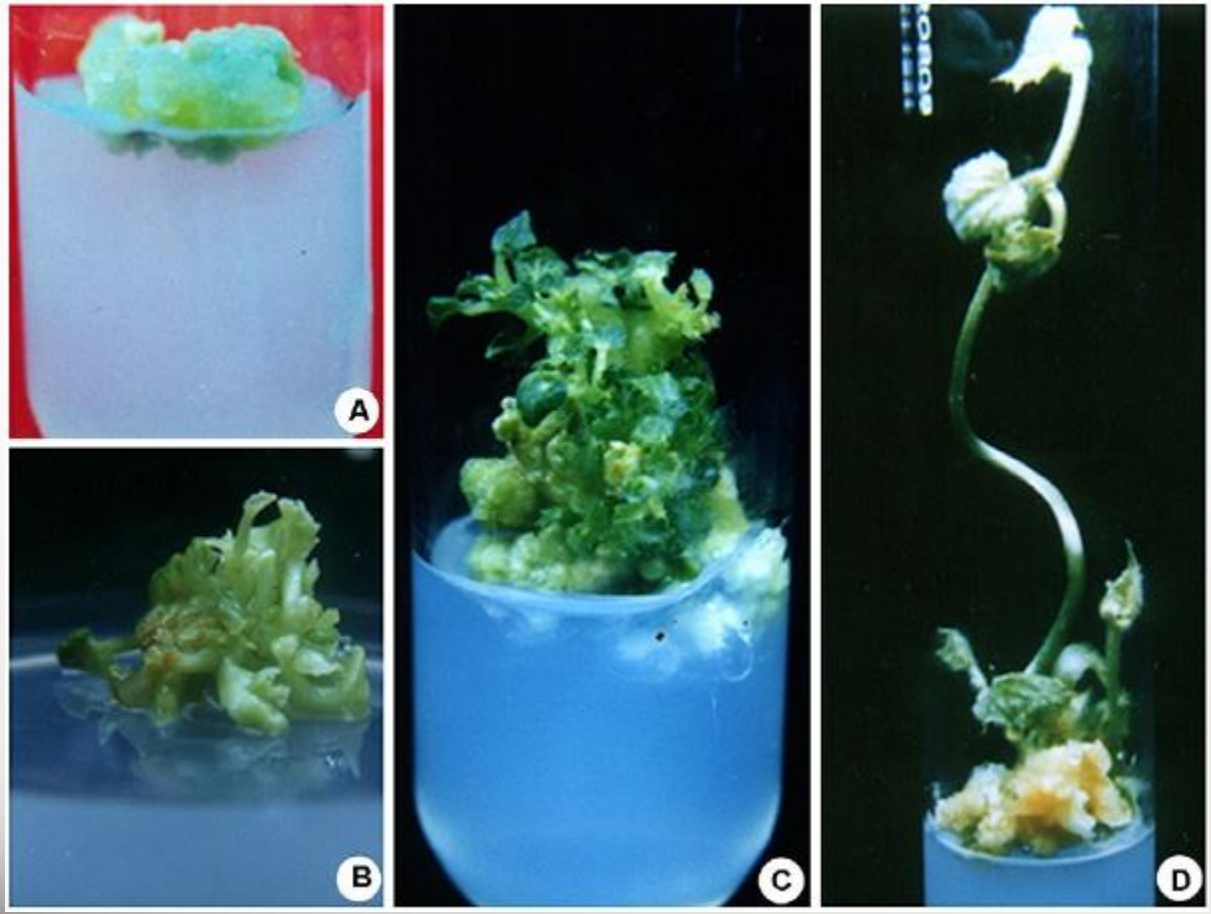
Species-Specific Response: Not all plant species respond well to adventitious shoot induction, and optimizing protocols for each species can be time-consuming and labor-intensive.

Somaclonal Variation: Clonal plants generated through tissue culture may exhibit genetic variations (somaclonal variation), which can affect plant quality or performance.

Cost and Labor: In vitro culture requires specialized equipment, materials, and skilled labor, making it an expensive process, especially for large-scale applications.

Adventitious shoot production from cotyledonary Nodes of Soybean

Adventitious shoot production from cotyledon Derived callus of Cucumis sativus



References

Smith (2013) Plant Tissue culture : Techniques and Experiments. Academic Press. London, U.K.

Bojwani and Datu (2013): Plant tissue culture : An Introductory Text. Springer, New Delhi, India

Gamborg and Philips (1995)Plant cell Tissue and Organ culture: Fundamental methods Springer Verlag, New York .

Thank you