Lecture-6

The physiological basis of adventitious root formation in cutting and layering

Learning objective

- To know about the physiological, anatomical and biochemical basis of root formation

Introduction

Adventitious root formation is an organized developmental process involving discrete biochemical, physiological and histological events in the induction, initiation, development and elongation of root primordia. In general, regeneration of a new plant from a cutting basically depends on two fundamental properties of the plant cell.

a) **Totipotency:** which states that individual cell contains all the genetic information required for producing a new plant of same kind.

b) **Dedifferentiation:** which means the capacity of mature cell to return to a meristmatic condition and develop into a new growing point.

In some species, cuttings root very easily as in grapes, while in some species, rooting takes place only after some treatments, where as others do not root at all. Therefore, it becomes mandatory to study the anatomical and physiological basis involved in the process of rooting of cuttings.

Anatomical basis of rooting of cuttings

- Adventitious roots in stem cuttings of many woody plant species have been reported to originate from various tissues. ARF in woody plants generally originate in young secondary phloem, but also arise from vascular rays, cambium or pith (Hartmann and Kester, 1983).
- The origin of adventitious roots has been reported to be callus tissue in difficult-to-root species. Poor rooting in stem cuttings of certain woody species has been correlated with extensive sclerification.
- Thick lignified walls of sclerenchyma tissues were physiological or mechanical barriers to adventitious root formation in poor-to-root species like *Fagus, Prunus and Quercus.* Generally, four anatomical changes are observed in adventitious root formation.

These anatomical changes are:

- Dedifferentiation of specific mature cells

- Formation of root initials from the dedifferentiated specific mature cells
- Development of root initials into organized root primordia and formation of vascular connection between root primordia and conducting tissues of the cuttings emerging through the cortex and epidermis
- Emergence of roots outside the cuttings

In herbaceous plants, adventitious roots generally appear just outside and between the vascular bundles. In woody plants, one or more layers of xylem and phloem are present and adventitious roots are formed in the stem cutting from the living parenchyma cells. After emergence, the roots develop root cap and other tissues of the root. Adventitious root and shoot usually arise within the stem (endogenously) near vascular tissue, outside the cambium.

Dedifferentiation of specific mature cells: It is also referred as callus formation and rooting process. When the cuttings are placed in a suitable medium, a mass of undifferentiated parenchymatous cells, called as **callus**, is usually developed at the base of the cutting and only then the root initiation process takes place. There was a belief that callus formation is necessary for rooting of cutting but now it has been established that callusing and root initiation are two independent phenomenons and can occur simultaneously. Though, excess of callus formation may hinder root initiation in some species.

Formation of root initials: Root initials are sometimes developed in the intact stem of certain woody plants, even before cuttings are made from them. These root initials remain dormant in the stem. These dormant root initials are called as **preformed or latent root initials**. When the cuttings made from such stems are placed in favorable environmental conditions, these root initials become active and roots are developed rapidly and easily from them. Occurrence of root initials is quite common in willow, hydrangea, poplar, jasmine, currant and citron. In some clonal apple and cherry rootstocks and old trees of apple and quince, the preformed root initials show a swelling (outgrowth) on the stem and are more often called as burr knots. Cuttings taken from plants having burr knots usually root better and easily as compared to those having no burr knots.

Stem structure and rooting: It has been found that certain type of stem structures or tissue relationship plays a vital role in adventitious root formation in the cuttings. The development of continuous sclerenchyma ring between the phloem and cortex of the stem is generally considered as an anatomical barrier to the rooting of cutting as in olive and in some leaf cuttings. Similarly, presence of sclerenchyma fibers in the cortex of stem may cause difficulty in the rooting of cuttings in some species as in English Ivy and *Hedera helix.*

Physiological basis of rooting of cuttings

Prerequisites for adventitious root formation to occur include:

- Availability and receptivity of parenchyma cells for regenerating new meristmatic regions
- Various modifications of the rhizocaline complex of phenolics (inhibitors, promoters, rooting co-factors) auxin, enzyme system
- Substrate needs such as carbohydrate accumulation and partitioning and changes in nitrogen and amino acids.

Several physiological processes occur during rooting of cuttings such as:

Growth regulators: Various growth regulators such as, auxins, cytokinins, gibberellins, abscisic acid and ethylene influence rooting of cutting. In addition, various other natural occurring promoters and inhibitors may also take part in the root initiation process.

Auxins: During 1934, indole-3-acetic acid (IAA) was identified as the first naturally occurring auxin having activity, which takes part in inducing rooting in the cuttings. Later on Indole Butyric Acid (IBA) and Naphthalene Acetic Acid (NAA) were found to be more effective in inducing rooting in cuttings.

- For initiation of rooting in the cuttings, continuous supply of auxin is basically required for the first 3-4 days.
- Usually, the cuttings do not respond to exogenous application of auxin once the rooting process has initiated.
- IBA treatments may control endogenous auxin levels of cuttings either through direct regulation of the IAA oxidase system or indirectly through the transport of auxin protectors.

Cytokinins: Cytokinins are responsible for growth and differentiation of the cells. Various natural and synthetic chemicals like kinetin, zeatin and 6-benzyl adenine have cytokinin activity. In general, cuttings having high endogenous levels of cytokinins have more difficulty to root than those having low cytokinin activity.

- Similarly, if cytokinins are applied artificially, root initiation process is inhibited. However, cytokinins at very low concentration promote root initiation process, while high concentrations inhibit it.
- Cytokinins strongly promote bud initiation in root cuttings of some species as in *Bryophyllum* and C*onvolvulus.*

Giberellins: Giberellins are a group of naturally occurring compounds first isolated in Japan during 1939. They are primarily known for their elongation effects in the intact stems.

- The high concentrations of gibberellins (10^{-3}M) usually inhibit root initiation process in cuttings but lower concentration ($10^{-11}$to 10^{-7}M) promotes it.

- The inhibitory effect of higher doses of GA_3 is mainly due to prevention of early cell division, involvement in transformation of mature stem tissues to the meristmatic conditions. Therefore, many gibberellins antagonist chemicals like Alar, Arrest or Abscisic acid which usually promote rooting in cuttings.

Abscisic acid: The role of abscisic acid in rooting of cuttings is not very clear and reports are highly contradictory. However, its effects on rooting depend upon concentration and nutritional status of the stock plant from which the cuttings have been prepared.

Ethylene: Ethylene, gaseous material produced by the plants, has a promoting effect on rooting of cuttings at low concentration. Ethylene application at 10ppm induces root formation in stem and root cuttings but at higher concentration (100 ppm or above) decrease or inhibit it. Root promotion activity of ethylene is supposed to be due to its synergistic effects on auxin synthesis, which alternately leads to root initiation process.

Role of vitamins: Endogenous optimum level or exogenous application of vitamin promotes root initiation process in the cuttings. Thiamine (vit B_1), pyridoxine (vit B^6), niacin and biotin B-complex vitamins and vitamins K or H all are known to stimulate the rooting process in different plants. Vitamin, if applied with some auxins like IAA, NAA or IBA have synergistic effect on root initiation in cuttings of different plant-species.

Presence of buds and leaves: It has been demonstrated by many workers that presence of leaves on the cuttings exerts a strong stimulating influence on the root initiation process in cuttings. The leaves produce carbohydrates, which are translocated downward to the base of stem, where it promotes root formation. Further, the leaves and buds are powerful auxin producers and their transport to basal parts may initiate rooting faster as in poplar, grape and currant. If the buds are removed from the cuttings, the root formation may be hindered adversely.

Rooting co-factors: Many rooting co-factors have been isolated from cuttings of different plants. These co-factors are naturally occurring substances that appear to act synergistically with auxin, primarily IAA, for root formation in cuttings. Bouillenne and Went were the first to name the root- forming factors of leaf, cotyledon and buds as rhizocaline in 1933. Later, a new hypothesis was proposed, highlighting that rhizocaline is a complex of three components viz:

- A specific rooting factor, which is translocated from buds, leaves, characterized chemically as *ortho-dihydroxy phenol*
- A non-specific factors (auxin) in low concentration, which is also translocated from leaves and the buds, and

- A specific enzyme, probably *polyphenol oxidase* present in pericycle, phloem or cambium.

According to this hypothesis, ortho-hydroxyphenol reacts with auxin, giving rise to rhizocaline wherever the enzyme is present. The formation of rhizocaline leads to the reactions involved in the root initiation process.

Another hypothesis proposed by Hess (1968) indicated that a number of co-factors interact with IAA, resulting in IAA co-factors complex, which subsequently initiates rooting(Fig.6.2).

- Generally, the easily rooted plants have a large number of co-factors and in higher concentration than in difficult-to-root plants.
- One of these co-factors (no 4) consists of highly active substances characterized as oxygenated terpenoids.
- Another co- factor (no.3) was identified as chlorogenic acid. Later, it was postulated that phenolic compound more especially catechol, reacts synergistically with IAA in root formation.
- This oxidation of ortho-dihydroxy phenol is one of the first steps leading to root initiation process.

Similarly, ABA was also found to be associated as one of the co-factors in root initiation. The action of phenolic compounds in root initiation is mainly in protecting the natural occurring auxin IAA from destruction by indole acid oxidase. Rooting co-factors have been found in *Hedera helix*, Crab C, MM-106 and M-26 apple rootstocks and Old Home pear.

Nutritional factors: The nutrient status of plant from which cuttings are taken, also plays vital role in root initiation process of the cuttings.

- Low nitrogen and high carbohydrate resources usually favour root initiation process compared to high nitrogen and low carbohydrate reserves.
- High nitrogen content usually favours luxuriant growth and hinder root initiation process but extreme N-deficiency also hinders the rooting process.
- Thus, high C: N ratio should be maintained in the stock plants before taking cuttings from them.

Endogenous rooting inhibitors: In addition to root promoting substances, certain endogenous root-inhibiting substances are also present in some plant species, inhibiting the process of root initiation.

- Therefore, cuttings of certain plants do not root easily mainly because of presence of endogenous inhibitors.

- For example, the cuttings of V*itis berlandier*i do not root easily as compared to the cuttings *of Vitis vinifera* because of presence of higher concentration of abscisic acid.
- Similarly hardwood cuttings of Bartlett pear do not root easily as compared to Old Home cultivar.
- Placing these cuttings in water before planting help in leaching of inhibitors and enhance the root initiation and subsequent development of roots.

