DIRECT AND INDIRECT ORGANOGENESIS

Organogenesis:

Organogenesis is the process of morphogenesis involving the formation of plant organs i.e. shoots, roots, flowers, buds from explant or cultured plant tissues. It is of two types — direct organogenesis and indirect organogenesis.

Direct Organogenesis:

Tissues from leaves, stems, roots and inflorescences can be directly cultured to produce plant organs. In direct organogenesis, the tissue undergoes morphogenesis without going through a callus or suspension cell culture stage. The term direct adventitious organ formation is also used for direct organogenesis.

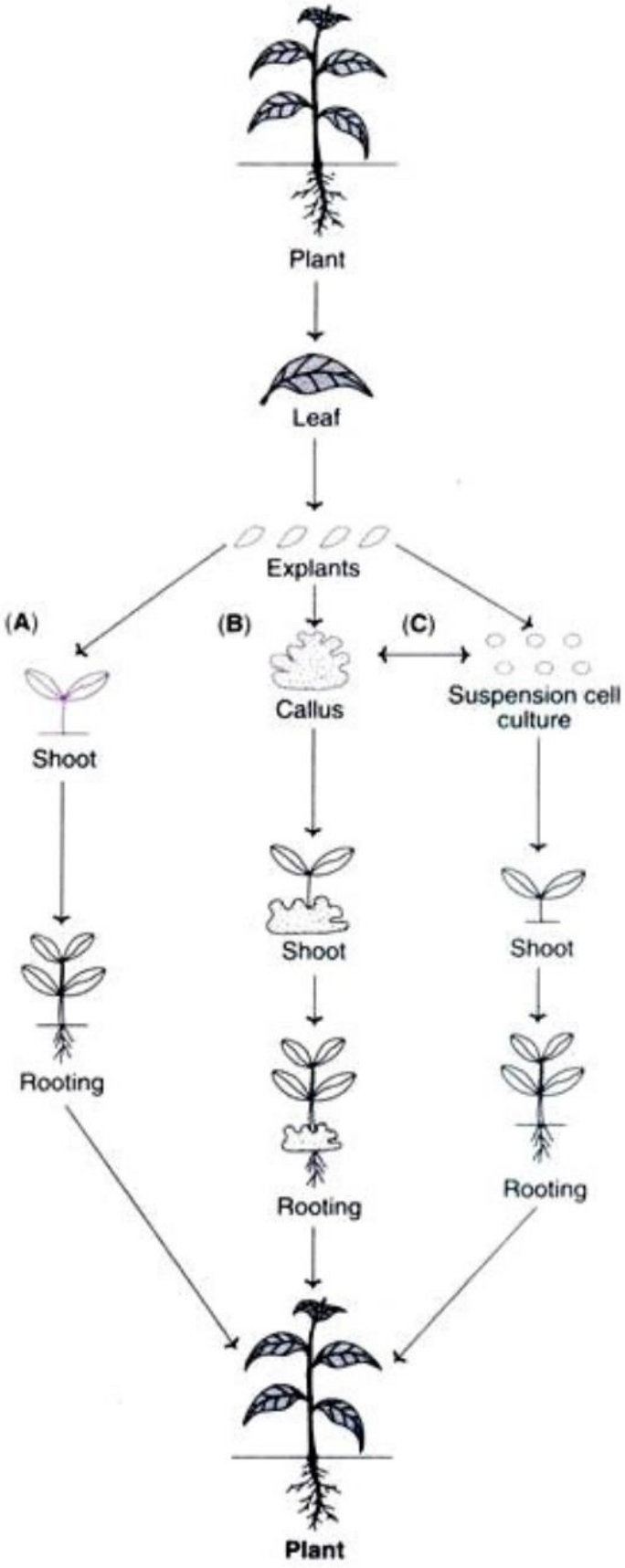
Induction of adventitious shoot formation directly on roots, leaves and various other organs of intact plants is a widely used method for plant propagation. This approach is particularly useful for herbaceous species. For appropriate organogenesis in culture system, exogenous addition of growth regulators—auxin and cytokinin is required. The concentration of the growth promoting substance depends on the age and nature of the explant, besides the growth conditions.

Indirect Organogenesis:

When the organogenesis occurs through callus or suspension cell culture formation, it is regarded as indirect organogenesis. Callus growth can be established from many explants (leaves, roots, cotyledons, stems, flower petals etc.) for subsequent organogenesis.

The explants for good organogenesis should be mitotically active immature tissues. In general, the bigger the explant the better the chances for obtaining viable callus/cell suspension cultures. It is advantageous to select meristematic tissues (shoot tip, leaf, and petiole) for efficient indirect organogenesis. This is because their growth rate and survival rate are much better.

For indirect organogenesis, the cultures may be grown in liquid medium or solid medium. Many culture media (MS, B5 White's etc.) can be used in organogenesis. The concentration of growth regulators in the medium is critical for organogenesis.



Micropropagation of plants by direct and indirect organogenesis

By varying the concentrations of auxins and cytokinins, in vitro organogenesis can be manipulated:

i. Low auxin and low cytokinin concentration will induce callus formation.

ii. Low auxin and high cytokinin concentration will promote shoot organogenesis from callus.

iii. High auxin and low cytokinin concentration will induce root formation.

4. Somatic Embryogenesis:

The process of regeneration of embryos from somatic cells, tissues or organs is regarded as somatic (or asexual) embryogenesis. Somatic embryogenesis may result in non-zygotic embryos or somatic embryos (directly formed from somatic organs), parthogenetic embryos (formed from unfertilized egg) and androgenic embryos (formed from male gametophyte).

In a general usage, when the term somatic embryo is used it implies that it is formed from somatic tissues under in vitro conditions. Somatic embryos are structurally similar to zygotic (sexually formed) embryos, and they can be excised from the parent tissues and induced to germinate in tissue culture media.

Development of somatic embryos can be done in plant cultures using somatic cells, particularly epidermis, parenchymatous cells of petioles or secondary root phloem. Somatic embryos arise from single cells located within the clusters of meristematic cells in the callus or cell suspension. First a pro-embryo is formed which then develops into an embryo, and finally a plant.

Direct Somatic Embryogenesis:

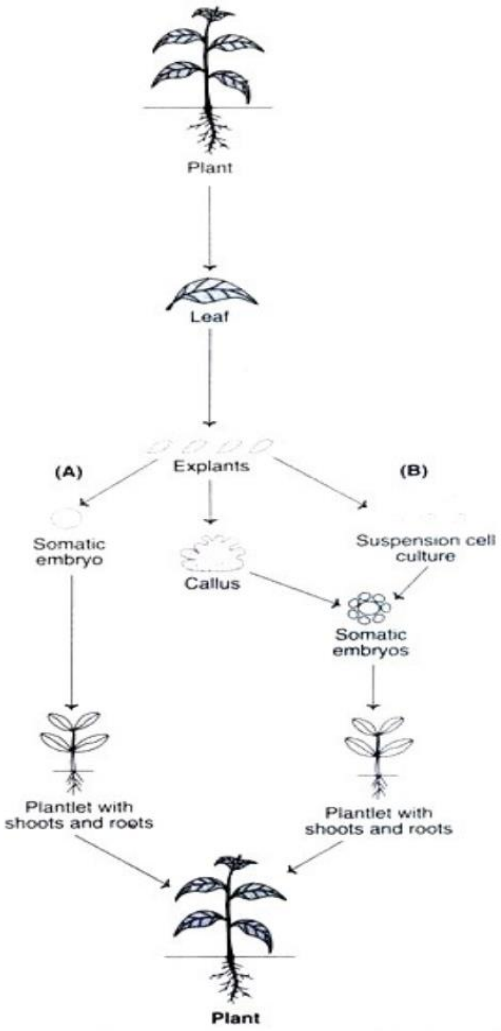
When the somatic embryos develop directly on the excised plant (explant) without undergoing callus formation, it is referred to as direct somatic embryogenesis (Fig 47.6A). This is possible due to the presence of pre-embryonic determined cells (PEDQ found in certain tissues of plants. The characteristic features of direct somatic embryogenesis is avoiding the possibility of introducing somaclonal variations in the propagated plants.

Indirect Somatic Embryogenesis:

In indirect embryogenesis, the cells from explant (excised plant tissues) are made to proliferate and form callus, from which cell suspension cultures can be raised. Certain cells referred to as induced embryo genic determined cells (IEDC) from the cell suspension can form somatic embryos. Embryogenesis is made possible by the presence of growth regulators (in appropriate concentration) and under suitable environmental conditions.

Somatic embryogenesis (direct or indirect) can be carried on a wide range of media (e.g. MS, White's). The addition of the amino acid L-glutamine promotes embryogenesis. The presence of auxin such as 2, 4-dichlorophenoxy acetic acid is essential for embryo initiation. On a low auxin or no auxin medium, the embryo genic clumps develop into mature embryos.

Two routes of somatic embryogenesis are known — direct and indirect



Indirect somatic embryogenesis is commercially very attractive since a large number of embryos can be generated in a small volume of culture medium. The somatic embryos so formed are synchronous and with good regeneration capability.

Artificial Seeds from Somatic Embryos:

Artificial seeds can be made by encapsulation of somatic embryos. The embryos, coated with sodium alginate and nutrient solution, are dipped in calcium chloride solution. The calcium ions induce rapid cross-linking of sodium alginate to produce small gel beads, each containing an encapsulated embryo. These artificial seeds (encapsulated embryos) can be maintained in a viable state till they are planted.

Factors Affecting Micro propagation:

For a successful in vitro clonal propagation (micro propagation), optimization of several factors is needed.

Some of these factors are briefly described:

1. Genotype of the plant:

Selection of the right genotype of the plant species (by screening) is necessary for improved micro propagation. In general, plants with vigorous germination and branching capacity are more suitable for micro- propagation.

2. Physiological status of the explants:

Explants (plant materials) from more recently produced parts of plants are more effective than those from older regions. Good knowledge of donor plants' natural propagation process with special reference to growth stage and seasonal influence will be useful in selecting explants.

3. Culture media:

The standard plant tissue culture media are suitable for micro propagation during stage I and stage II. However, for stage III, certain modifications are required. Addition of growth regulators (auxins and cytokinins) and alterations in mineral composition are required. This is largely dependent on the type of culture (meristem, bud etc.).

4. Culture environment:

Light:

Photosynthetic pigment in cultured tissues does absorb light and thus influence micro-propagation. The quality of light is also known to influence in vitro growth of shoots, e.g blue light induced bud formation in tobacco shoots. Variations in diurnal illumination also influence micro propagation. In general, an illumination of 16 hours day and 8 hours night is satisfactory for shoot proliferation.

Temperature:

Majority of the culture for micro propagation requires an optimal temperature around 25°C. There are however, some exceptions e.g. Begonia X Cheimantha hybrid tissue grows at a low temperature (around 18°C).

Composition of gas phase:

The constitution of the gas phase in the culture vessels also influences micro propagation. Unorganized growth of cells is generally promoted by ethylene, O_2, CO_2 ethanol and acetaldehyde.

Factors Affecting in Vitro Rooting:

A general description of the factors affecting micro propagation, particularly in relation to shoot multiplication is given above. For efficient in vitro rooting during micro- propagation, low concentration of salts (reduction to half to one quarter from the original) is advantageous. Induction of roots is also promoted by the presence of suitable auxin (NAA or IBA).

Applications of Micro propagation:

Micro propagation has become a suitable alternative to conventional methods of vegetative propagation of plants. There are several advantages of micro propagation.

High Rate of Plant Propagation:

Through micro propagation, a large number of plants can be grown from a piece of plant tissue within a short period. Another advantage is that micro propagation can be carried out throughout the year, irrespective of the seasonal variations. Further, for many plants that are highly resistant to conventional propagation, micro propagation is the suitable alternative. The small sized propagules obtained in micro propagation can be easily stored for many years (germplasm storage), and transported across international boundaries.

Production of Disease-free Plants:

It is possible to produce disease-free plants through micro propagation. Meristem tip cultures are generally employed to develop pathogen-free plants .In fact, micro propagation is successfully used for the production of virus-free plants of sweet potato (Ipomea batatus), cassava (Manihot esculenta) and yam (Discorea rotundata).

Production of Seeds in Some Crops:

Micro propagation, through axillary bud proliferation method, is suitable for seed production in some plants. This is required in certain plants where the limitation for' seed production is high degree of genetic conservation e.g. cauliflower, onion.

Cost-effective Process:

Micro propagation requires minimum growing space. Thus, millions of plant species can be maintained inside culture vials in a small room in a nursery. The production cost is relatively low particularly in developing countries (like India) where the manpower and labour charges are low.

Automated Micro propagation:

It has now become possible to automate micro propagation at various stages. In fact, bio-reactors have been set up for large scale multiplication of shoots and bulbs. Some workers employ robots (in place of labourers) for micro- propagation, and this further reduces production cost of plants.

Disadvantages of Micro propagation:

Contamination of Cultures:

During the course of micro propagation, several slow-growing microorganisms (e.g. Eswinia sp, Bacillus sp) contaminate and grow in cultures. The microbial infection can be controlled by addition of antibiotics or fungicides. However, this will adversely influence propagation of plants.

Brewing of Medium:

Micro propagation of certain plants (e.g. woody perennials) is often associated with accumulation of growth inhibitory substances in the medium. Chemically, these substances are phenolic compounds, which can turn the medium into dark colour. Phenolic compounds are toxic and can inhibit the growth of tissues. Brewing of the medium can be prevented by the addition of ascorbic acid or citric acid or polyvinyl pyrrolidone to the medium.

Genetic Variability:

When micro propagation is carried out through shoot tip cultures, genetic variability is very low. However, use of adventitious shoots is often associated with pronounced genetic variability.

Vitrification:

During the course of repeated in vitro shoot multiplication, the cultures exhibit water soaked or almost translucent leaves. Such shoots cannot grow and even may die. This phenomenon is referred to as vitrification. Vitrification may be prevented by increasing the agar concentration (from 0.6 to 1%) in the medium. However, increased agar concentration reduces the growth rate of tissues.

Cost Factor:

For some micro propagation techniques, expensive equipment, sophisticated facilities and trained manpower are needed. This limits its use.

In vitro Micrografting

Micrografting is an *in vitro* grafting technique which involves the placement of a meristem or shoot tip explant onto a decapitated rootstock that has been grown aseptically from seed or micropropagated cultures. Special techniques have been used for increasing the percentage of successful micrografts with the use of growth regulators, etiolation treatments, antioxidants, higher sucrose levels, silicon tubes, etc. The technique has great potential for improvement and large scale multiplication of fruit plants. It has been used on commercial scale for production of virus-free plants in fruit crops and viroid free plants. Micrografting has also been used in prediction of incompatibility between the grafting partners, histological studies, disease indexing, production of disease-free plants particularly resistant to soil borne pathogens and multiplication of difficult to root plants.

Stages of micrografting

Micro-propagation protocol for scion as well as rootstock needs to be standardized separately before performing the micrografting operation under in vitroconditions. Thus, micrografting can be divided into three main stages:

Establishment and multiplication of scion

Shoot or meristem tips intended for grafting can be taken from actively growing shoots in greenhouse, chambers, field or in vitro. Generally, apical shoot tips or nodal cuttings are used as explants for the establishment of in vitro cultures. Following establishment, microshoots are transferred to shoot proliferation medium where shoot number increases by the development of

new axillary shoots. Microshoots of desired thickness, age and length are used as scions for in vitrografting operations.

Establishment and multiplication of rootstock

Rootstocks used for micrografting are in vitro or in vivo germinated seedlings and rooted or unrooted micropropagated shoots. When seedling rootstocks are used and all stages of grafting are conducted in vitro, seeds are surface sterilized and germinated aseptically in vessels containing nutrient salts. The seedlings may be supported on agar medium. Seedlings can also be on a porous substrate, such as sterile vermiculite, which allows the growth of a branched root system. Preparation of rootstock and scion for micrografting Micrografting is affected by cutting off the top of the seedling rootstocks usually just above the cotyledons or top of the micropropagated shoot and placing small shoot apices of scion onto the exposed surface of decapitated rootstock in such a way that the cambium layer or vascular ring of the cut surfaces coincides with each other. This is called surface placement method. Wedge or cleft grafting is performed, incase thickness of rootstock and scion material is large enough to allow making of wedge on the scion material. Firm contact between rootstock and scion is extremely important at the graft junction for proper union of partners and callus formation. Several techniques have been developed for holding grafts together until fusion takes place such as translucent silicon tubing, elastic strip, filter paper bridge, and glass tubing, nylon bands, aluminum foil tubes, dual layer apparatus of aluminum foil and absorbent paper. When grafts are successful, rootstock and scion grow together to produce a plant. It is usually necessary to examine freshly grafted seedlings on a regular basis and remove any adventitious shoot arising on or below the graft union.

Applications of micrografting

- Virus and viroid elimination
- Production of plants resistant to pests and diseases
- Assessment of graft incompatibility
- Improvement of plant regeneration
- Mass multiplication
- Indexing viral diseases
- Safe germplasm exchange

Production of Disease-Free Plants:

Many plant species are infected with pathogens — viruses, bacteria, fungi, mycoplasma and nematodes that cause systemic diseases. Although these diseases do not always result in the death of plants, they reduce the quality and yield of plants. The plants infected with bacteria and fungi frequently respond to chemical treatment by bactericides and fungicides.

However, it is very difficult to cure the virus-infected plants. Further, viral disease is easily transferred in seed- propagated as well as vegetatively propagated plant species. Plant breeders are always interested to develop disease-free plants, particularly viral disease-free plants. This has become a reality through tissue cultures.

Apical Meristems with Low Concentration of Viruses:

In general, the apical meristems of the pathogen infected and disease harbouring plants are either free or carry a low concentration of viruses, for the following reasons:

i. Absence of vascular tissue in the meristems through which viruses readily move in the plant body.

ii. Rapidly dividing meristematic cells with high metabolic activity do not allow viruses to multiply.

iii. Virus replication is inhibited by a high concentration of endogenous auxin in shoot apices. Tissue culture techniques employing meristem-tips are successfully used for the production of disease-free plants, caused by several pathogens — viruses, bacteria, fungi, mycoplasmas.

Methods to Eliminate Viruses in Plants:

In general, plants are infected with many viruses; the nature of some of them may be unknown. The usage virus-free plant implies that the given plant is free from all the viruses, although this may not be always true. The commonly used methods for virus elimination in plants are listed below, and briefly described next.

i. Heat treatment of plant

ii. Meristem-tip culture

iii. Chemical treatment of media

iv. Other in vitro methods

Heat Treatment (Thermotherapy) of Plants:

In the early days, before the advent of meristem cultures, in vivo eradication of viruses from plants was achieved by heat treatment of whole plants. The underlying principle is that many viruses in plant tissues are either partially or completely inactivated at higher temperatures with minimal injury to the host plant. Thermotherapy (at temperatures 35-40°C) was carried out by using hot water or hot air for elimination viruses from growing shoots and buds.

There are two limitations of viral elimination by heat treatment:

1. Most of the viruses are not sensitive to heat treatment.
2. Many plant species do not survive after thermotherapy.

With the above disadvantages, heat treatment has not become popular for virus elimination.

Meristem-Tip Culture:

A general description of the methodology adopted for meristem and shoot tip cultures has been described. For viral elimination, the size of the meristem used in cultures is very critical. This is due to the fact that most of the viruses exist by establishing a gradient in plant tissues.

In general, the regeneration of virus-free plants through cultures is inversely proportional to the size of the meristem used. The meristem-tip explant used for viral elimination cultures is too small. A stereoscopic microscope is usually employed for this purpose.

Meristem-tip cultures are influenced by the following factors:

i. Physiological condition of the explant — actively growing buds are more effective.
ii. Thermotherapy prior to meristem-tip culture — for certain plants (possessing viruses in the meristematic regions), heat treatment is first given and then the meristem-tips are isolated and cultured.
iii. Culture medium —MS medium with low concentrations of auxins and cytokinins is ideal.

Chemical Treatment of Media:

Some workers have attempted to eradicate viruses from infected plants by chemical treatment of the tissue culture media. The commonly used chemicals are growth substances (e.g. cytokinins) and antimetabolites (e.g thiouracil, acetyl salicylic acid).

There are however, conflicting reports on the elimination viruses by chemical treatment of the media. For instance, addition of cytokinin suppressed the multiplication of certain viruses while for some other viruses, it actually stimulated.

Other in Vitro Methods:

Besides meristem-tip culture, other in vitro methods are also used for raising virus-free plants. In this regard callus cultures have been successful to some extent. The callus derived from the infected tissue does not carry the pathogens throughout the cells. In fact, the uneven distribution of tobacco mosaic virus in tobacco leaves was exploited to develop virus-free plants of tobacco. Somatic cell hybridization, gene transformation and somaclonal variations also useful to raise disease-free plants.

Elimination of Pathogens Other than Viruses:

Besides the elimination of viruses, meristem-tip cultures and callus cultures are also useful for eradication bacteria, fungi and mycoplasmas. Some examples are given
i. The fungus Fusarium roseum has been successfully eliminated through meristem cultures from carnation plants.
ii. Certain bacteria (Pseudomonas carophylli, Pectobacterium parthenii) are eradicated from carnation plants by using meristem cultures.

Merits and Demerits of Disease-Free Plant Production:

Among the culture techniques, meristem-tip culture is the most reliable method for virus and other pathogen elimination. This, however, requires good knowledge of plant pathology and tissue culture.

Virus-free plants exhibit increased growth and vigour of plants, higher yield (e.g. potato), increased flower size (e.g. Chrysanthemum), and improved rooting of stem cuttings (e.g. Pelargonium)

Virus-free plants are more susceptible to the same virus when exposed again. This is the major limitation. Reinfection of disease-free plants can be minimized with good knowledge of greenhouse maintenance.

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