Lecture-13

Micropropagation and handling of plants in nurseries

Learning objective

- Significance of micropropagation
- Techniques of micropropagation

Introduction

Micropropagation refers to the production of plants from very small plant parts, tissues or cells, grown aseptically in a test tube or containers under controlled nutritional, environmental and aseptic conditions. All the biological principles of micropropagation techniques are based on the phenomenon or totipotency of cell, which implies that a plant cell has the capacity to generate into a full-fledged plant having different organs.

Micropropagation versus other propagation methods: Micropropagation propagation differs from other vegetative propagation methods as:

- A very small plant part (explants) is used as a starting material
- The explants are maintained in small containers having well defined culture medium
- Highly aseptic conditions are required; and
- A large propagating material is produced in a very short-time

The various **applications** of micro propagation are:

1. Rapid rate of multiplication of a plant clonally
2. Production of disease-free and disease resistant plants
3. Induction of mutants and selection of mutants
4. Production of haploids through anther culture
5. Wide hybridization through excised embryo and ovule culture
6. Somatic hybrids and cybrids through protoplast fusion
7. Transformation through uptake of foreign genome
8. Nitrogen fixation
9. Cryopreservation of germplasm types

Advantages

- ✓ Production of large propagating material in shorter time

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- ✓ Production of disease-free plants
- ✓ Clonal propagation of parental stock for hybrid seed production
- ✓ Year-around nursery production
- ✓ Useful in propagation of dioecious plants
- ✓ Breeding cycle is reduced
- ✓ Useful in hard-to-propagate plant species
- ✓ Germplasm preservation

Disadvantages

- Expensive and sophisticated facilities, trained personnel and specialized techniques are required
- High cost of production results from expensive facilities and high labour inputs
- Contamination or insect infestation can cause high losses in a short time
- Higher level of somatic variation
- Poor establishment of the plantlets in the field

Principles and practices

A. **Totipotency and plant regeneration:** The term totipotency was coined by Germen plant physiologist Haberlant in 1902. This is achieved by excising the explants and placing it on culture medium, which involves the following consecutive events:

   1) Differentiation of source tissue or organ, as a result of which, all physiological mechanism activate in the cell, which leads to cell division.
   2) Active cell division in the entire cut surface of the explants, resulting in proliferation of the callus tissue.
   3) Organization of defined meristems, resulting in formation of shoot or root meristems or both.
   4) Regeneration and differentiation of new organs through organogenesis or somatic embryogenesis.

B. **Axillary bud proliferation**

   - It is considered a convenient route for micropropagation, because it does not include a callus stage and is considered safe for the preservation of clonal characteristics.
   - It has been observed by many workers that a large number of axillary shoot buds grow extensively when shoot-tips or even smaller apical meristems are cultured in an appropriate culture medium, containing higher concentration of cytokinin.

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- Both the excision of the explants and the cytokinin rich medium activate the bud, leading to proliferation of many side shoots, which can later be separated for further culture.

C. **Organogenesis (Development of organs)**

- Organogenesis from explants results in the formation of both shoots and roots.
- Generally shoots are formed first than roots.
- The regeneration of other organs occur later on, either in the same media or after sub-culturing in another medium, supplemented with different concentration of salt under different environmental conditions.

D. **Somatic embryogenesis**

- Somatic embryogenesis is different from organogenesis in that the regeneration and organization are bipolar i.e. the shoots and root meristems in the explants are formed simultaneously from pre-embryonic masses.
- Differentiation and organization of somatic embryogenesis usually, a two stage culture is involved.
- First induction of pre-embryonic masses, which is usually favoured by 2,4-D or other auxins and second, by the transfer of the pre-embryonic masses to a differentiation medium in which the somatic embryos fully differentiate into organs.

Stages of micropropagation

Micro propagation is an integrated process in which cells, tissues or organs of the selected plants are isolated, surface sterilized and incubated in growth promoting, sterile medium and environment to produce a large number of plants. The different stages are:

Stage 0: Selection of mother plant for explants isolation: The mother plant from which explants has to be excised should be

a.) A certified and true to type representative of the desired species and or cultivar.

b.) Healthy and free from insect pest and disease

c.) Should be quite vigorous.

Stage1: Explant establishment in culture medium: During this stage the explant is cultured in a suitable culture medium, preferably agar based media for tissue activation and multiplication.

Stage2: Proliferation and multiplication: In this stage, repeated sub-culturing is done to encourage more proliferation, which largely depends upon the combination of growth regulators. The duration of this stage is unlimited and largely depends on the choice of propagator.

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Stage 3: Plant establishment and rooting: In this stage the selected plants are forced for root formation, which can be achieved by media modification and modifying the concentration of growth regulators. The concentration of cytokinins and sugars are reduced and concentration of auxins and light intensity in the laboratory is increased to start with photosynthesis and other physiological activities.

Stage 4. Acclimatization or hardening: The plantlets developed in the culture tubes are acclimatized to specific environment having a high humidity, a low light level and a constant temperature (Plate13.2). Besides, the roots developed *in vitro* are hairless and hence delicate, requiring care during transfer from culture medium. To have better survival rate, the plantlets may be transferred to container kept in mist chambers where relative humidity is maintained at higher order. Once new growth is seen, the plants may be slowly transferred to outside by exposing to increased light intensity in stages.

Once plantlets are well rooted, they must be acclimatized to the green house environment. *In vitro* rooted plants are removed from the culture vessel and the agar is washed away completely to remove a potential source of contamination. Plantlets are transplanted into a standard pasteurized rooting or soil mixture in small pots or cells in a more or less conventional manner. Initially, micro-plants should be protected from desiccation in a shaded, high humidity tent or under mist or fog. Several days may be required for new functional roots to form.

Plantlets should be gradually exposed to a lower relative humidity and a higher light intensity. Any dormancy or resting condition that develops may need to be overcome as part of the establishment process. These conditions help the plantlets in getting acclimatized to the natural conditions, which would help them in getting established in the field easily.

Culture Techniques

Various culture techniques such as (i) meristem culture (ii) callus culture (iii) shoot bud regeneration (iv) somatic embryogenesis (v) ovule culture (vi) embryo culture (vii) anther culture and (viii) protoplast culture are employed in micropropagation.

1. Meristem culture: Meristem culture involves culture of both shoot-tip and axillary-bud. The use of small shoot-tips comprising of the apical dome with one or two leaf primordia (0.1-0.5 mm) is the basis for the technique known as meristem-tip-culture, pioneered by Morel in the 1950s. Meristem tip culture is now being routinely used, mainly in horticultural crops, for the elimination of virus from

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infected material. Virus apparently either does not easily invade or rapidly multiply in the young meristematic tissue. A simple nutrient medium consisting only of salts, sucrose and vitamins is used in order to minimize the formation of callus. Gibberellic acid is often needed to promote adequate growth and NAA may be required to stimulate root formation.

2. Callus culture: *A* piece of sterile plant tissue with living cells is transferred to a culture medium to induce callus proliferation. Sub-culturing is then done onto a medium with or without altered growth regulator concentrations, ultimately resulting in the induction of adventitious organs or embryos. In the last stage, regenerated plants are removed from *in vitro* culture and slowly exposed to outer environment so that the plants can be fully autotrophic.

3. Cell culture: The cells are maintained in suspension cultures so as to produce free cells and are then sub-cultured to regenerate complete plant from single cells. This technique is now useful to induce variability in plant cells and to select desirable cell variants and regenerate complete plants from these variants.

4. Embryo culture: It involves aseptic excision of the embryo and its transfer to a suitable medium for development under optimum culture conditions. After the embryo has grown into a plantlet *in vitro,* it is transferred to sterile soil or vermiculite and grown to maturity in a green house. This technique is useful in the production of intetspecific and intergeneric hybrids which could not be otherwise accomplished and also in overcoming embryo abortion.

5. Protoplast culture: From different sources, protoplasts (the plant without any rigid cellulose wall but with plasma membrane only allowed to fuse to form a somatic hybrid) are cultured in suitable media to regenerate the cell wall and are again cultured in suitable medium for differentiation and morphogenesis.

6. Anther culture: The culture of anthers is of considerable value to breeders as it is possible to produce haploid plants which reveal recessive alleles. These haploid plants can be used for the production of homozygous diploids, thus avoiding generations of inbreeding. Added benefits, such as small flowers and prolonged flowering time, might be ensured from the use of haploid plants as they are usually smaller than their diploid counterparts and being sterile there will be no pollination-induced senescence. Anther culture has been used in *Pelargonium* spp. to eliminate virus, in *Lilium* spp. to produce haploid plants and in *Gerbera* to obtain different flower colour.

7. Somatic embryogenesis: The greatest potential for clonal multiplication is through somatic embryogenesis, where technically a single isolated cell can produce first an embryo, then a complete plant. Somatic embryogenesis and plantlet regeneration has been reported in various species of horticultural plants by using mid-rib, leaf and stem callus on modified MS basal medium

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supplemented with 1.0 – 2.0 mg/l 2,4-D and 0.25-0.50 mg/l BA or kinetin.

Culture medium

Success in the technology and application of tissue culture methods depends on the selection of proper culture medium, which meets the nutritional requirements of cultured cells and tissues. The basic components of all nutrient media are inorganic salts, carbohydrates, vitamins, growth regulators, agar (for solid medium) and water. Other components including organic nitrogenous compounds, organic acids and complex substances can be important but are optional.

The inorganic nutrients required in macro-amounts are N,P, K, Ca, S and Mg. The optimum concentration of each nutrient for achieving maximum growth rates varies considerably. The essential nutrients required in micro-molar concentration include Fe, Mn, Zn, B, Cu and Mo. The concentration of inorganic nitrogen in the culture medium varies from 25 to 50 mM. Nitrate is commonly used in the range from 25-40 mM and the amount of ammonium varies between 2 and 20 mM. Potassium is supplied in the range of 20 mM or higher and the optimum concentrations of P, Mg, Ca and S for most tissues are 1-3 mM.

The standard carbon source is sucrose or glucose at a concentration of 2-3%. Other carbohydrates which have been tested include fructose, lactose, maltose, galactose and starch but these compounds are generally much inferior to sucrose or glucose as a carbon source. Most media contain myo-inositol. There is no absolute requirement of myo-inositol, but its inclusion (100 mg/l) generally improves tissue growth. MS medium is the most popularly used nutrient medium in tissue culture.

The nutrient media are supplied with certain vitamins. There is an absolute requirement (0.1-0.4 mg/1) for thiamine. Growth is also improved by the addition of nicotinic acid and pyridoxine; sometimes biotin, vitamin B_{12}, folic acid, choline chloride, riboflavin and ascorbic acid are also added to the culture medium, depending on the type of tissue to be cultured.

The pH of the culture medium is an important factor for maintenance of growth of cultured plant tissues. In general, plant tissues require an acidic pH, and an initial pH of 5.5 - 5.8 is optimum. The physical form of the culture medium is also an important factor determining the success or failure of tissue culture. Both liquid and solid form of culture medium are useful for initiation and proliferation of callus tissue and initial organogenesis, but for further growth of organs solid medium is essential. When culturing in a liquid medium, gentle agitation of the liquid is necessary for aeration of culture medium, rotating apparatus is used for this purpose.

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