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Plant Tissue Culture: A Powerful Tool for Mass Multiplication of Plants

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Plant biotechnology is the technology which is used for getting modern product with high yield and at a faster rate. It emerged at the beginning of the 20th century and involves growing plant cells and tissues *in vitro*, regenerating and cloning new plants, and modifying their genetic characteristics. A technology known as plant tissue culture is widely used to rapidly produce large numbers of plants with improved genetic traits under controlled environmental conditions. This technique involves the *in vitro* cultivation of plant cells and organs, which then divide and regenerate into callus or specific plant organs.

The technique of plant tissue culture relies on several key factors: (i) the totipotency - the inherent capacity of the individual cells of an organism to develop into a complete organism, (ii) the explants - which is a small tissue excised from any part of the plant, (iii) the aseptic environment -to avoid contamination from microorganisms and (iv) the nutrient media - that strongly govern the growth and morphogenesis of plant tissues (Anonymous, 2020).

Plant tissue culture can also be defined as a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. The plant material to be cultured may be cells, tissues or plant organs such as excised root tip, shoot tip, shoot bud, leaf petiole, inflorescence, anther, embryo, ovule or ovary. Thus, using the appropriate growing conditions for each explant type, plants can be induced to rapidly produce new shoots, and with the addition of suitable hormones, new roots. These plantlets or microplant can also be divided, usually at the shoot stage, to produce large numbers of new plantlets or microplants (Sub-culturing). The new plants can then be placed in soil and grown in the normal manner.

The various techniques of *in vitro* culture includes:

a) Seed Culture

Growing seed aseptically *in vitro* on artificial media is called seed culture. It increases the efficiency of germination of seeds that are difficult to germinate or do not germinate well *in vivo*. It is used to raise the sterile or aseptic seedlings and to identify the plants which are resistant or tolerant to various stresses. Research on *in vitro* seed culture in vanilla (Kumar *et al.*, 2014). E.g., Orchids, Vanilla, Tomato, Chilli, etc

b) Meristem culture

It involves the culturing of apical meristems, especially of shoot meristem *in vitro* on artificial media. It is also known as Meristematic Mericlonning. Three to five mm shoot apices having several leaf primordial are selected as explants. However, when the objective is virus free plant production, the size of explant should be < 1mm. It makes use of single nodes or axillary buds. Meristem Culture of Potato (*Solanum tuberosum* L.cv. Desiree) for production of virus-free plantlets (Zaman *et al.*, 2018).

E.g., Capsicum, Tomato, Brinjal, Potato, etc

Applications

- Plant propagation.
- Production of virus free planting material.

c) Bud culture

It involves isolating a single bud or meristem from a parent plant and culturing it in a nutrient-rich medium under sterile conditions. The bud or meristem contains undifferentiated cells capable of generating a whole new plant. *In vitro* Micropropagation of potato cultivars (*Solanum tuberosum* L.) (Xhulaj *et al*., 2019).

Bud culture is of 2 types:

- **Single Node Culture (SNC)**: Here, a nodal segment is isolated from the third and fourth nodes from the stem apex. The bud is then allowed to develop on a nutrient media, with the purpose of forming a shoot. Most commonly used method for propagating plants in vitro.
- **Axillary Bud Culture:** Here, an axillary shoot bud is isolated from a plant. The bud is then allowed to develop under the influence of a relatively high cytokinin concentration. High cytokinin concentration stops the apical dominance and allows axillary buds to develop.

E.g., Potato, Tomato, Chilli, Capsicum, etc

Applications

- Simple and quick method of plant propagation
- In most cases, organogenesis occurs directly i.e., without callus formation.
- Favors high multiplication frequency coupled with genotypic uniformity of the plants produced.

d) Callus culture

Callus is an undifferentiated, tumor-like mass of cells. *In vitro* culturing of callus tissue aseptically on artificial media is known as Callus culture. *In vitro* micropropagation of *Capsicum chinense*Jacq. (Gayathri *et al*., 2015).

Regeneration via callus culture involves 2 important processes;

- **De-differentiation** – the non-dividing quiescent cells of explant are reverted to meristematic state by placing on nutrient media. It results in the formation of undifferentiated mass of cells (Callus).
- **Re-differentiation** – the de-differentiated of cells or callus undergo differentiation i.e., shoot & root formation and develops capacity to regenerate into the complete plant.

E.g. Potato, Tomato, Chilli, Capsicum, Brinjal, etc.

e) Cell culture

It is also called Cell Suspension Culture. It consists of single isolated cells or cell aggregates dispersed and growing in moving liquid media. It is normally initiated by transferring pieces of explant/ undifferentiated and friable calluses to a liquid medium which is continuously agitated by a rotary shaker to provide aeration and dispersion of cells.

E.g., *Capsicum frutescens* (CapsaicinPungency), *Dioscorea* spp. (Diosgenin), *Vanilla spp.* (vanillin- flavouring chemical), 3-N-Butyl-pthalide in Celery (Effective against hypertension), etc.

Applications

- Large scale clonal propagation through embryogenic cell suspension.
- Somatic embryos from cell suspensions can prove useful for long-term storage in germplasm banks. Somatic embryos from cell suspensions produce the same flavour compounds or secondary metabolites as present in the mature plant.

f) Organ culture

In organ culture, two *in vitro* methods have been used, Ovule culture – it refers to the culture of excised ovaries and ovules. Anther culture – It refers to the culture of excised anthers and pollens. Studies on Anther Culture in tomato (*Solanum lycopersicum* L.) (Shere *et al.*, 2009)

Applications

- Simple, quick and efficient technique of haploid production.
- Reduction of time in developing variety of cross-pollinated crop.
- Fixation of heterosis through dihaploid production.
- Induction of genetic variability.

g) Microspore culture

Microspore or the immature pollen can be used as the explant to get the haploid plants directly. For pollen or microspore culture, the flower buds are collected, surface sterilized and the anther lobes are dissected out from the flower buds. Then the anther lobes are squeezed with the help of a scalpel within a tube or small beaker to collect the microspore or pollen in nutrient media. Then the anther tissue debris is removed by filtering the suspension through a nylon sieve with a diameter slightly larger than the pollen size (40μ-100μ) allowing the microspore only to pass through it.

Then, the microspore-suspension is washed and concentrated to a plating density. The microspores obtained are then mixed with an appropriate culture medium at a density of 103 - 104 microspore ml-1 , and plated in small petriplate. To ensure good aeration, the layer of liquid in the dish should be as thin as possible, and sealed with parafilm to avoid dehydration. The responsive pollen will divide and form embryos or calli which directly or indirectly will form the haploid plantlet. By following the method of subculturing the whole plant suitable for soil transfer can be obtained.

Applications

- The explants i.e., microspores or pollens are all haploid cells.
- The sequence of androgenesis can be observed starting from a single cell.
- The microspores are ideal for uptake, transformation and mutagenic studies, and the microspores are evenly exposed to chemicals and physical mutagens.
- Higher yields of plants/anther could be obtained.

h) Double haploidy

Haploid plants obtained either from anther or ovule culture may grow normally under *in vitro* conditions up to the flowering stage but viable gametes are not formed. Also, there is no seed set due to the absence of one set of homologous chromosomes.

The only mechanism for perpetuating the haploids is by duplicating the chromosome no. in order to obtain homozygous diploids. Diploidization is achieved by immersing very young haploids in a filter sterilized solution of colchicine (0.4%) for 2-4 days, followed by their transfer to the culture medium for further growth. In this procedure, chromosome or gene instabilities are minimal compared to other methods of chemical treatment.

i) Embryo culture

It consists of isolation of immature or mature embryos under aseptic conditions and culturing it on nutrient media.

E.g., Legumes (Green gram, Black gram, French bean, Soybean, etc.), Tomato, Brinjal, Potato, Turnip, etc.

Applications

- Embryo rescue in case of F1 hybrids obtained through wide/ distant hybridization.
- Propagation of seeds having short viability i.e., low to negligible amount of endosperm.
- Shortening of breeding cycle.

Advantages of plant tissue culture technique

1) Mass multiplication of elite clones.
2) Beneficial when conventional propagation is difficult.
3) Plants can be produced or multiplied in large numbers in a shorter period of time from small vegetative parts.
4) Micropropagation is not season dependent because the controlled conditions in the Tissue Culture Laboratory permits the year round production of tissue culture plants in season-controlled growth rooms, where environmental conditions are set for optimal regeneration and growth.
5) Plants produced through micropropagation may have increased branching and flowering, greater vigour and higher yield, mainly due to the possibility of elimination of diseases. Plant cultures in approved media are easier to export than the soil- grown plants.

Disadvantages of plant tissue culture technique

1) During the course of micro propagation, several slow-growing microorganisms (e.g. *Eswinia sp*., *Bacillus sp*.) contaminate and grow in cultures that will adversely influence propagation of plants.
2) Micro propagation of certain plants is often associated with accumulation of growth inhibitory substances in the medium. Chemically, these substances are phenolic compounds, which are toxic, turns the medium into dark colour and can inhibit the growth of tissues (Browing of media).
3) During the course of repeated *in vitro* shoot multiplication, the cultures exhibit water soaked or almost translucent leaves. Such shoots do not grow and even may die.

Conclusion

Tissue culture is rapidly becoming a commercial method for propagating new and rare species, difficult-topropagate plants, healthy, virus free and true to-type plants. Plant production can be carried out throughout the year, irrespective of season and weather, which solves farmer climatic plantation problems. Permits germplasm exchange and distribution throughout the world. It has been estimated that in India more than three hundred and fifty million tissue cultured plants are being produced annually through tissue culture method. Plant cell and tissue cultures provides a way for controlled production of myriad of useful flavor compounds and secondary metabolites. E.g. Capsaicin (Capsicum frutescens), etc.

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