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Need of tissue culture for conservation of medicinal plants

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Abstract

Plant are the basic important ingredient in man`s life style. Beside all useful plants in flora, medicinal herb/plants are majorly used all over the world due to curative property. Many pathies including Ayurveda use plants for treatment purpose. The database of medicinal plants is being added by new plants. But some plants are getting scared which are having great importance in medicine. Some plants have such a long germination period, while some having long reproduction time e.g. Santalum album after 20 yrs of germination, Abrus seed germinate after a long time. There is a need for conservation of such medicinal plants. Only cultivation of these plants is not sufficient to provide the demand. The modern techniques, new invention should be taken for grant. Tissue culture is one of such technique which is now used to develop best plant species, it is used in plant like pomegranate, Banana yield like cotton, brinjal etc .This method can also be used to develop plant species, which are distinct and waning day by day. A major step should be formulated in cultivation of medicinal plants by tissue culture, also their effectiveness in clinical practice.

Key word- Conservation, Tissue culture, Cultivation

Introduction

Plants are main source of life for living being direct or indirect mean. in the plant flora, some plants use for great extent because of having medicinal/ healing property. Such category of medicinal plants are diminishing day by day, whose conservation, protection is a need of era. New techniques are to be adopted for same. Tissue culture is a scientific boom for conservation of medicinal plant. The science of plant tissue culture takes its root from the discovery of cell followed by production of cell theory [1].

What is Tissue Culture?

Tissue culture is the vitro aseptic culture of cell, tissue, organ the whole plant under the controlled nutritional and environmental condition [2] .The controlled condition provides culture an environment conductive for their growth and multiplication .This condition includes proper supply of nutrients, pH medium, adequate temperature and proper gaseous liquid environment

Plant tissue culture technology is being widely used for large scale plant multiplication.Apart from their use as a tool of research, plant tissue culture techniques have in recent year, becomes of major importance in area of plant propagation, disease of secondary metabolites, small pieces of tissue culture (named explants) can be produce

thousands of medicinal plant in continuous process . A single explants can be multiplied in several thousand plant relatively short time period and space of under the controlled condition , irrespective in seasons and weather on year round basis .Endangered, threaded and rare species have successfully been grown and conserved by micro propagation because high coefficient of multiplication and small demand on number of initial plant and space[3] . In modern usage, tissue cultures refer to the growth of cells form tissue from multi cellular organism in vitro. The term tissue culture is after used interchangeably with the cell culture

The literal meaning of tissue culture refer to the culturing tissue pieces, i.e. explants culture.

The tissue culture is an important to for the study of the biology of cells multi cellular organisms. It provides an in vitro model of the tissue in a defined environment which can be easily manipulated and analysed.

Hence plant cell and tissue culture great promise for controlled production of myriad of useful secondary metabolism .Plant cell culture combine the merits of whole plant systems with those of microbial and animal cell culture for the production of valuable secondary metabolites. In the search of alternative to production of medicinal compound from plant, biotechnology approaches, especially culture are found to have potential as supplement to tradition agriculture in the industrial production of bioactive plant metabolism.

Hence the plant cell biotechnology has evolved as new era in the field of biotechnology, focusing on the production secondary plant product .Nowadays, one of the most important methods of producing plants and other medicinal substance, such as antibodies and vaccines is use of transgenic plant. Transgenic plant represent an economical alternative to fermentation based production system .Plant made vaccine on antibodies are specially striking ,as plants are from human disease , thus reducing screening cost for various and bacterial toxins. Hence tissue culture is important in medicinal plant.

Tissue Culture Technique [4]

Preparation of plant tissue for tissue culture is performed under aseptic conditions under HEPA filtered air provided by a laminar flow cabinet. Thereafter, the tissue is grown in sterile containers, such as petri dishes or flasks in a growth room with controlled temperature and light intensity. Living plant materials from the environment are naturally contaminated on their surfaces (and sometimes interiors) with microorganisms, so their surfaces are sterilized in chemical solutions (usually alcohol and sodium or calcium hypochlorite before suitable samples (known as explants) are taken. The sterile explants are then usually placed on the surface of a sterile solid culture medium, but are sometimes placed directly into a sterile liquid medium, particularly when cell suspension cultures are desired. Solid and liquid media are generally composed of inorganic salts plus a few organic nutrients, vitamins and plant hormones. Solid media are prepared from liquid media with the addition of a gelling agent, usually purified agar.

The composition of the medium, particularly the plant hormones and the nitrogen source (nitrate versus ammonium salts or amino acids) have profound effects on the morphology of the tissues that grow from the initial explant. For example, an excess of auxin will often result in a proliferation of roots, while an excess of cytokinin may yield shoots. A balance of both auxin and cytokinin will often produce an unorganised growth of cells, or callus, but the morphology of the outgrowth will depend on the plant species as well as the medium composition. As cultures grow, pieces are typically sliced off and subcultured onto new media to allow for growth or to alter the morphology of the culture. The skill and experience of the tissue culturist are important in judging which pieces to culture and which to discard.

As shoots emerge from a culture, they may be sliced off and rooted with auxin to produce plantlets which, when mature, can be transferred to potting soil for further growth in the greenhouse as normal plants.

Importance of Medicinal Plants [5]

India has 2.4% world's area with 8% of global biodiversity and it is one of the 12th mega-diversity hotspot countries of the world's with rich diversity of biotic resources. Out of 34 hotspot, India has two major hotspots namely Eastern Himalayas and Western Ghats.

India is also rich in medicinal plant diversity with all the three level of biodiversity such as species diversity, genetic diversity and habitat diversity. Across the country, the forest is estimated to harbour 90% of India's total medicinal plants diversity. Only about 10% of the known medicinal plants of India are restricted to non-forest habitat. It is estimated that in India 75,000 species of medicinal plants are present as compared to other countries.

Uses of Important medicinal plants of India [5]

Name	Family	Common Name	Uses
Aegel marmelos	Rutaceae	Bael tree	Diarrhoea, dysentery, malaria, fever, jaundice.
Acorus calamus	Araceae	Sweet flag, Bach	Anti-spasmodic, anti-helminthic properties also used for treatment of epilepsy, mental aliment, diarrhoea, dysentery.
Celestrus paniculatus	Celestraceae	Malkangani	Memory booster, depression, paralysis.
Commiphora mukul	Burseraceae	Guggulu	Astringent, expectorant, strong purifying and rejuvenating property and uterine stimulant.
Bacopa monnieri	Scrophulariaceae	Brahmi	Mental function longevity, disease fatigue and depression, energise the CNS.
Glycerrhiza glabra	Fabaceae	Liquorice	Ulcer, anti-spasmodic, asthma, cough.

List of Medicinal Plants in-vitro culture [5,6]

Sr. No.	Species Name	Explants	Ref.
1	Aegel marmelos	Nodal segment, shoot tip	Yadav & Singh, 2011
2	Acorus calamus	Rhizome tip & Rhizome segment	Yadav et.al., 2011
3	Celestrus paniculatus	Nodal segment	Sood & Choulin, 2009
4	Gymneme Sylvestre	Seed	Konalavalli & Rao, 2000.
5	Glycyrrhiza glabra	Nodal segment	Vadodaria et.al., 2007
6	Tinospora cordifolia	Nodal segment	Gururaj et.al., 2007

Choice of explants [5] The tissue obtained from a plant to be cultured is called an explant.

Explants can be taken from many different parts of a plant, including portions of shoots, leaves, stems, flowers, roots, single undifferentiated cells and from many types of mature cells provided are they still contain living cytoplasm and nuclei and are able de-differentiate and resume cell division. This has given rise to the concept of totipotentency of plant cells. However this is not true for all cells or for all plants. In many species explants of various organs vary in their rates of growth and regeneration, while some do not grow at all. The choice of explant material also determines if the plantlets developed via tissue culture are haploid or diploid. Also the risk of microbial contamination is increased with inappropriate explants.

The first method involving the meristems and induction of multiple shoots is the preferred method for the micropropagation industry since the risks of somaclonal variation (genetic variation induced in tissue culture) are minimal when compared to the other two methods. Somatic embryogenesis is a method that has the potential to be several times higher in multiplication rates and is amenable to handling in liquid culture systems like bioreactors.

Some explants, like the root tip, are hard to isolate and are contaminated with soil microflora that become problematic during the tissue culture process. Certain soil microflora can form tight associations with the root systems, or even grow within the root. Soil particles bound to roots are difficult to remove without injury to the roots that then allows microbial attack. These associated microflora will generally overgrow the tissue culture medium before there is significant growth of plant tissue.

Some cultured tissues are slow in their growth. For them there would be two options: (i) Optimizing the culture medium; (ii) Culturing highly responsive tissues or varieties. Necrosis can spoil cultured tissues. Generally, plant varieties differ in susceptibility to tissue culture necrosis. Thus, by culturing highly responsive varieties (or tissues) it can be managed.

Aerial (above soil) explants are also rich in undesirable microflora. However, they are more easily removed from the explant by gentle rinsing, and the remainder usually can be killed by surface sterilization. Most of the surface microflora do not form tight

associations with the plant tissue. Such associations can usually be found by visual inspection as a mosaic, de-colorization or localized necrosis on the surface of the explant.

An alternative for obtaining uncontaminated explants is to take explants from seedlings which are aseptically grown from surface-sterilized seeds. The hard surface of the seed is less permeable to penetration of harsh surface sterilizing agents, such as hypochlorite, so the acceptable conditions of sterilization used for seeds can be much more stringent than for vegetative tissues.

Tissue cultured plants are clones. If the original mother plant used to produce the first explants is susceptible to a pathogen or environmental condition, the entire crop would be susceptible to the same problem. Conversely, any positive traits would remain within the line also.

Applications

- Plant tissue culture is used widely in the plant sciences, forestry, and in horticulture. Applications include:
- The commercial production of plants used as potting, landscape, and florist subjects, which uses meristem and shoot culture to produce large numbers of identical individuals.
- To conserve rare or endangered plant species.
- A plant breeder may use tissue culture to screen cells rather than plants for advantageous characters, e.g. herbicide resistance/tolerance.
- Large-scale growth of plant cells in liquid culture in bioreactors for production of valuable compounds, like plant-derived secondary metabolites and recombinant proteins used as biopharmaceuticals.
- To cross distantly related species by protoplast fusion and regeneration of the novel hybrid.
- To rapidly study the molecular basis for physiological, biochemical, and reproductive mechanisms in plants, for example in vitro selection for stress tolerant plants.
- To cross-pollinate distantly related species and then tissue culture the resulting embryo which would otherwise normally die (Embryo Rescue).
- For chromosome doubling and induction of polyploidy, for example doubled haploids, tetraploids, and other forms of polyploids. This is usually achieved by application of antimitotic agents such as colchicine or oryzalin.
- As a tissue for transformation, followed by either short-term testing of genetic constructs or regeneration of transgenic plants.
- Certain techniques such as meristem tip culture can be used to produce clean plant material from virused stock, such as potatoes and many species of soft fruit.
- Production of identical sterile hybrid species can be obtained.

Conclusion:

- There are no. Of review published
- The rapid production and high quality, disease free and uniform planting stock is only possible through microprapogation. New opportunity has been cieated for the procedure.

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