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Analytical Study of Plant Tissue Culture Techniques

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ABSTRACT

Plant tissue culture techniques are essential to many types of academic inquiry, as well as to many applied aspects of plant science. In the past, plant tissue culture techniques have been used in academic investigations of tot potency and the roles of hormones in cytodifferentiation and organogenesis. Plant research often involves growing new plants in a controlled environment. These may be plants that we have genetically altered in some way or may be plants of which we need many copies all exactly alike. These things can be accomplished through tissue culture of small tissue pieces from the plant of interest.

Key words: Plant, Tissue, Explant, Environment.

1. Introduction

Plant tissue culture techniques are essential to many types of academic inquiry, as well as to many applied aspects of plant science. In the past, plant tissue culture techniques have been used in academic investigations of tot potency and the roles of hormones in cytodifferentiation and organogenesis. Plant research often involves growing new plants in a controlled environment. These may be plants that we have genetically altered in some way or may be plants of which we need many copies all exactly alike. These things can be accomplished through tissue culture of small tissue pieces from the plant of interest. Plant tissue culture technology is being widely used for large scale plant multiplication [2]. Apart from their use as a tool of research, plant tissue culture techniques have in recent years, become of major industrial importance in the area of plant propagation, disease elimination, plant improvement and production of secondary metabolites. Small pieces of tissue (named explants) can be used to produce hundreds and thousands of plants in acontinuous process. Single explants can be multiplied into several thousand plants in relatively short time period and space under controlled conditions, irrespective of the season and weather on a year round basis [3]. Endangered, threatened and rare species have successfully been grown and conserved by micro propagation because of high coefficient of multiplication and small demands on number of initial plants and space.

2. Required Conditions for Plant Tissue Culture

Tissue culture is a technique of growing plant cells by culturing explants aseptically on a suitable nutrient medium [4].

Media: Plant tissue culture is a technique of *in vitro* cultivation of plant cells and organs, which divide and regenerate into callus or particular plant organs. This technique relies on the following conditions:

- Explant
- Aseptic environment
- Nutrient medium

A small tissue excised from any part of the plant is called explant which is the starting point. It can be initiated from any part of plant- root, stem, petiole, leaf o flower, choice of explant varies with species. Meristems are more responsive and give better success as they are actively dividing. The physiological state of the plant also has an influence on its response to initiate tissue culture. Therefore, the parent plant must be healthy and free from obvious signs of disease or decay.

Aseptic Conditions (Sterilization) Nutrient medium contains ample sugar which increases growth of microorganisms such as bacteria and fungi [4]. These microbes compete with growing tissue and finally kill it. It is essential to maintain aseptic conditions of tissue culture. Thus sterilization means complete destruction or killing of microorganisms so that complete aseptic conditions are created for in vitro culturing.

Nutrient Medium

The composition of plant tissue culture medium can vary depending upon the type of plant tissues or cell that are used for culture. A typical (generalized) nutrient consists of inorganic salts (both micro and macro elements), a carbon source (usually sucrose), vitamins (e.g., nicotonic acid, thiamine, pyridoxine and myoinositol), amino acids (e.g., arginine) and growth regulators (e.g., auxins like 2,4-D or 2,4-dichlorophenoxyacetic acid and cytokinins such as BAP = benzlaminopurine and gibberellins). Other compounds like casein hydrolysate, coconut milk, malt extract, yeast extract, tomato juice, etc. may be added for specific purposes [5].

Aeration of the Tissue

Proper aeration of the cultured tissue is also an important aspect of culture technique. It is achieved by occasionally stirring the medium by sterring or by automatic shaker.

Plant Material—the Explant

Any part of a plant taken out and grown in test tube under sterile conditions in special nutrient media is called explants.

3. Methods of Plant Tissue Culture

Plant tissue culture includes two major methods:

(A) Type of in vitro growth-callus and suspension cultures.

(B) Type of explants— single cell culture, shoot and root cultures, somatic embryo culture, meristem culture, anther culture and haploid production, protoplast culture and somatic hybridisation, embryo culture, ovule culture, ovary culture, etc.

4. Types of Plant Tissue Culture

Cultures are generally initiated from sterile pieces of a whole plant.-These pieces are termed ‘explants’, and may consist of pieces of organs, such as leaves or roots, or may be specific cell types, such as pollen or endosperm.-Many features of the explants are known to affect the efficiency of culture initiation.-Generally, younger, more rapidly growing tissue (or tissue at an early stage of development) is most effective.-Several different culture types most commonly used in plant transformation studies[6].

Callus and Suspension Cultures:

Callus

Tissues and cells cultured on an agar-gelled medium form an unorganized mass of cells called callus shown in figure 1. In callus culture, cell division in explants forms a callus. Callus is irregular unorganized and undifferentiated mass of actively dividing cells. Darkness and solid medium gelled by agar stimulates callus formation [7]. The medium ordinarily contains the auxin, 2, 4-D, (2, 4- dichlorophenoxy acetic acid) and often a cytokinin like BAP (Benzyl aminopurine). Both are growth regulators. This stimulates cell division in explants. Callus is obtained within 2-3 weeks.

Suspension Cultures

Tissues and cells cultured in a liquid medium produce a suspension of single cells and cells clumps of few too many cells; these are called suspension cultures. Liquid cultures must be constantly agitated, generally by a gyratory shaker at 100-250 rpm (revolution per minute), to facilitate aeration and dissociation of cell clumps into smaller pieces.

Suspension cultures grow much faster than callus cultures, need to be sub-cultured about every week, allow a more accurate determination of the nutritional requirements of cells and are the only system amenable to scaling up for a large scale production of cells and even somatic embryos (SEs).

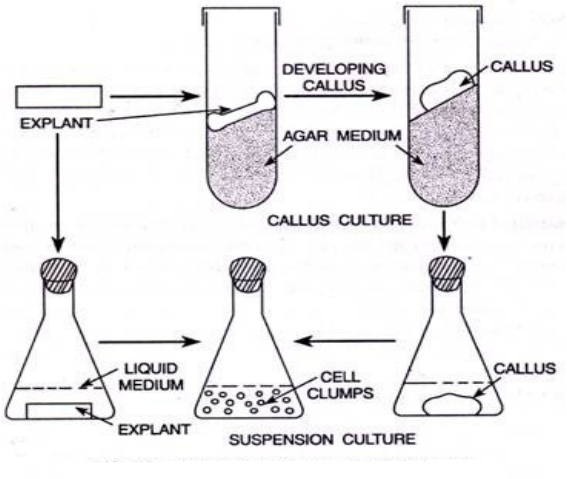

Figure 1: Callus and Suspension Cultures

Sub culturing

Sub culturing is the aseptic transfer of micro-organisms from a culture to fresh medium. The freshly inoculated medium is then incubated at the temperature appropriate for growing the organism's here are four sub culturing procedures with which you should become familiar in figure 2. They are:

a. Solid to solid: The transfer of bacteria or fungi from an agar slope or plate culture to an agar plate.
b. Solid to liquid: The transfer of bacteria or fungi from an agar slope or plate culture to a broth.
c. Liquid to solid: The transfer of bacteria or fungi from a broth culture to an agar slope or plate.
d. Liquid to liquid: The transfer of bacteria or fungi from a broth culture to a broth.

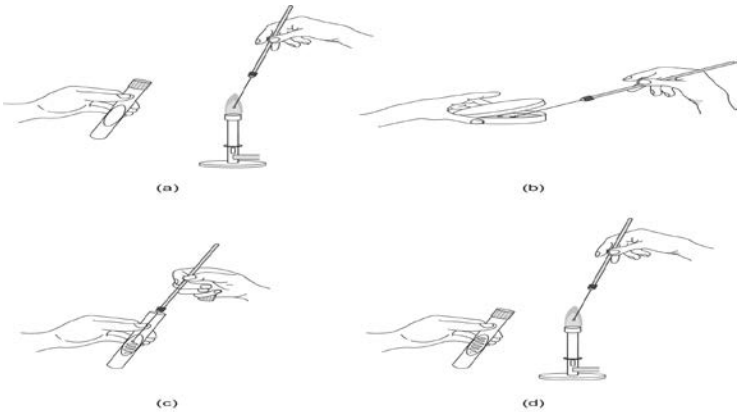

Figure 2: Sub culturing procedures.

Endosperm Culture

Endosperm is a distinctive tissue in its origin, development and ploidy. Angiosperms the endosperm is the main nutritive tissue for the embryo. The endosperm is the product of double fertilization during which out of the two male gametes, one fertilizes the egg to form zygote and other fuses with secondary nuclei to form triploid endosperm. Hence, triploid nature of endosperm is the characteristic feature of angiosperms [8]. Both mature and immature endosperm can be used for culture initiation. A key factor for the induction of cell divisions in mature endosperm cultures is the initial association of embryo but immature endosperms proliferate independent of embryo. The endosperm tissue often shows in figure 3 for corn and figure 4 shown of endosperm in coconut a high degree of chromosomal variations and polyploidy. Triploid plants are used for the production of seedless fruits (e.g., corn, coconut. etc.). The technique of endosperm culture involves the following:

(i) The immature seeds are dissected under aseptic condition. Endosperms along with embryos, are excised. Sometimes, mature seeds can also be used.

(ii) The excised endosperms are cultured on a suitable medium and embryos are removed after initial growth.

(iii) The initial callus phase is developed.

(iv) The shoots and roots may develop and complete triploid plants are formed for further use.

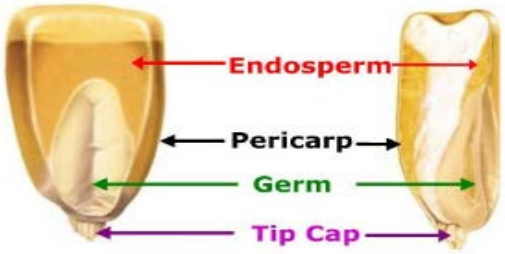

Figure 3: Endosperm Culture in corn

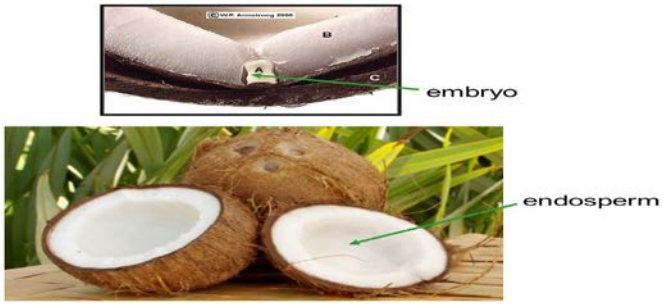

Figure 4: Embryos & Endosperm in coconut

Meristem Culture

A group of identical cells which are in a continuous state of cell division. Some of the cells from the meristematic tissue stops dividing and exhibit certain changes to become permanent tissues of the plant. This change from meristematic to permanent state is called as differentiation [9]. The rest of the cells in the meristematic tissues persists their meristematic activity. The meristematic tissues are self-perpetuating.

Features of meristematic cells

The meristematic cells may be round, oval, polygonal or rectangular in shape. These cells are arranged closely without intercellular spaces. They have dense cytoplasm with large nucleus. They have smaller vacuoles scattered throughout the cytoplasm. Their cell walls were thin, elastic and made up of cellulose.

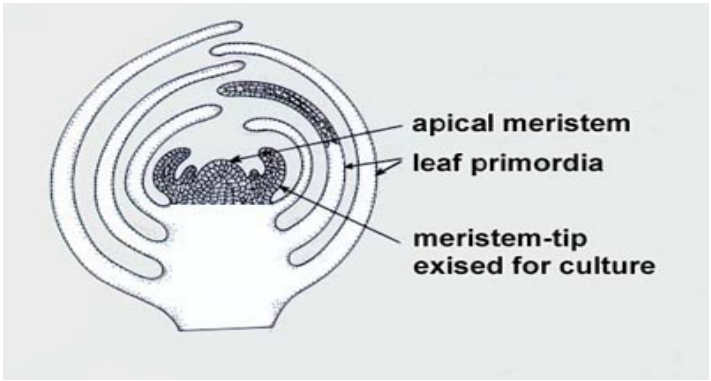

Figure 5: Meristem Culture in perimordia leaf

Anther Culture

The technique was discovered in 1964 by Guha and Maheshwari. This technique can be used in over 200 species, including tomato, rice, tobacco, barley, and geranium. Some of the advantages which make this a valuable method for obtaining haploid plants are:

- The technique is fairly simple
- It is easy to induce cell division in the immature pollen cells in some species
- A large proportion of the anthers used in culture respond (induction frequency is high)
- Haploids can be produced in large numbers very quickly.

In experiments using *Datura innoxia*, induction frequencies of almost 100% and a yield of more than one thousand plantlets or calluses have occurred under optimal conditions from one anther [10]. Success can be determined within 24 hours as cells begin to divide.

Some disadvantages of using anther culture to obtain haploids are:

- when working with some species, the majority of plants produced have been non-haploid
- in cereals, very few green plants are obtained; many of the plants are albinos or green-albino chimeras
- it is tedious to remove the anthers without causing damage
- sometimes a particular orientation is necessary to acheive a desired responce

Anter culture is the process of using anthers to culture haploid plantlets.

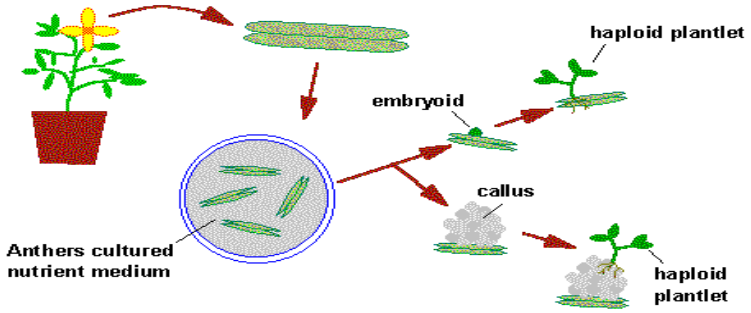

Figure 6: Anter culture process of using anthers to culture haploid plantlets

Embryo Culture

Embryo culture involves isolating and growing an immature or mature zygotic embryo under sterile conditions on an aseptic nutrient medium with the goal of obtaining a viable plant [11]. The basic premise for this technique is that the integrity of the hybrid genome is retained in a developmentally arrested or an abortive embryo and that its potential to resume normal growth may be realized if supplied with the proper growth substances. The technique depends on isolating the embryo without injury, formulating a suitable nutrient medium, and inducing continued embryogenic growth and seedling formation.

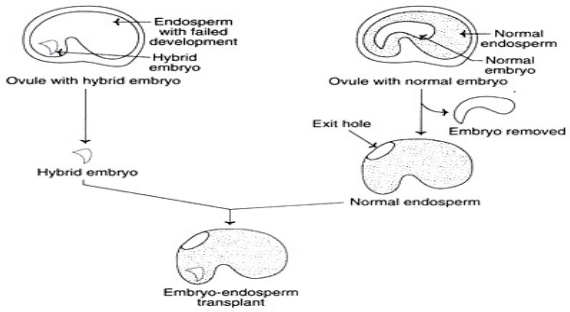

Figure 7: Embryo Culture

Ovule Culture

Ovule culture technique is utilized for raising hybrids which normally fail to develop due to the abortion of the embryos at an early stage [12]. Ovules can easily be excised from the ovary and cultured on the basal medium. The loss of a hybrid embryo due to premature abscission of fruits may be prevented by ovule culture [13]. In some cases, addition of fruit/vegetable juice increase the initial growth.

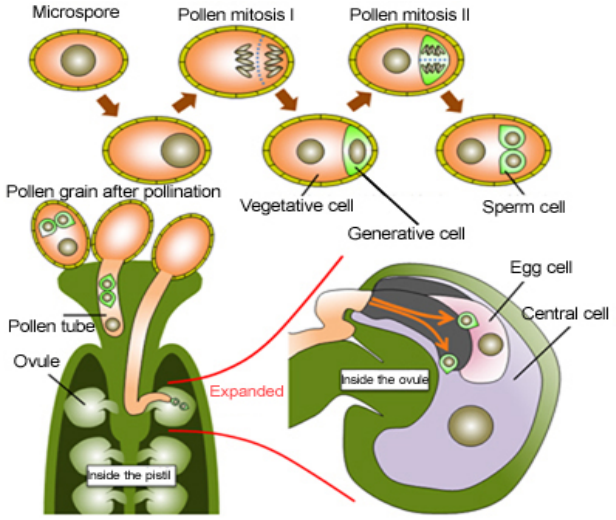

Figure 8: Ovule Culture

Ovary Culture

Ovary culture is a technique of culture of ovaries isolated either from pollinated or un-pollinated flowers. Ovary is a ovule bearing region of a pistil. Excised ovaries can be cultured in vitro [14]. For many species e.g. tomato, gherkin (Cucumis anguria) excised ovaries grow in culture and form the fruits that ripen and produce viable seeds. This development takes place on a simple nutrient medium containing only mineral salts and sucrose, provided the flowers have been fertilized two or more days before excision shown in figure 9.

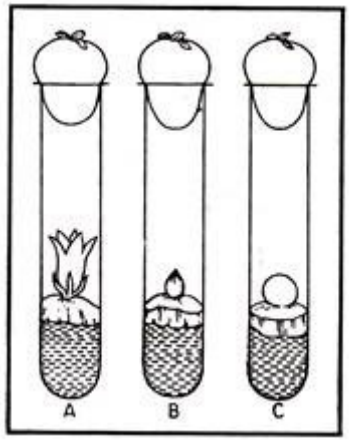

Figure 9: Ovary culture of Lycoperslcon Esculentum

5. Conclusion

Plant tissue culture is now a well established technology which has made significant contributions in different types of cultures techniques. Plant tissue culture represents the most promising areas of application at present time and giving an out look into the future.

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