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PLANT TISSUE CULTURE

Saja. J. S. Baday

College of Administration and Economics, University of Baghdad. Iraq

ABSTRACT

Plants tissue culture is one of the most important and modern techniques that is used in plant propagation and improvement of plants in quantity and quality. Plant tissue culture playing an increasing role in field plant production in industrial countries. Plant tissue culture depends on several factors that help the success of the plant propagation program and the types of tissue that can be grown and propagation. Plant tissue culture is applied to plant research for many purposes such as propagation of plants and production of disease-free plants.

Keywords: Plants tissue culture, plant cells, tissue farm

INTRODUCTION

Plant tissue culture is a technique that means the growth of plant cells or tissues in glass or plastic containers containing industrial nutrient media contain the required nutrients under complete conditions of sterilization. Pots contain the media and a plant material called (tissue farm) preserved in incubators with controlled temperatures and lighted according to the appropriate needs of the plant (1). The technology of plant tissue culture provides the best way to propagate plants in high quality and free of diseases and in large quantities in a short period compared to traditional methods of breeding that takes (10-15 years) (2). The limited availability of plant seeds in a timely manner, which takes time to obtain them, as well as that some seeds begin to deteriorate due to exposure to bio-stresses so plant tissue culture technology provided alternative ways to improve crops. Plant tissue culture refers to the process in which parts of plant tissue are introduced into a media that contains nutrients and nutrients necessary for plants to continue to function or grow. The tissue parts are often used as leaves, stems and seeds and any part of the plant parts can be used for tissue culture purposes. Various materials such as plant growth regulators and agar are used to facilitate this process. Tissue culture is applied to plant research for many purposes such as propagation of plants and production of disease-free plants. Any part of the plant is taken during tissue culture and grown in a controlled media where environmental conditions and sterilization. Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant (totipotency). Single cells, plant cells without cell walls (protoplasts), pieces of leaves, stems or roots can often be used to generate a new plant

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on culture media given the required nutrients and plant hormones (3). In this review, we will discuss the areas of plant tissue culture, the factors that help in the success of the plant culture program, the types of tissue that can be grown and propagation.

Evolution of plant tissue culture

The history of plant tissue cultivation dates back to 1902 when Haberlandt published his attempt to plant a single plant cell. However, in 1934, White was able to achieve some success in the same field when he succeeded in producing a whole plant of tomatoes from planting part of a plant root Tomato in an industrial diet, and in late 1939 a number of researchers, individually and separately, produced whole plants from the cultivation of specialized plant tissues. Both Gautheret and researcher White produced tobacco in the same way. In 1957, researchers Miller and Skoog explained that the ratio of auxin and cytokine in the food medium plays an important role in determining the nature of the growth and specialization of the plant part planted in the food medium or one. In 1958, Steward and others produced the carrot plant by developing a mass of non-specialized cells are cited by researcher Gautheret. In 1962 researchers Murashige and Skoog were able to make a quantum leap in the development of this modern science by reaching a special combination of the nutritional media for growing the tissues of the tobacco plant, which was later examined from the most famous food media used today, whether for single or double housing. In 1965 researchers, Hildebrandt and Vasil were able to use the food media (MS) for Murashige and Skoog to produce a whole tobacco plant by cultivating a single plant cell. In 1969, Nitsch found a way to grow hundreds of tobacco plants that carry half of the haploid chromosome Cultivation of pollen. In the early 1970s in several experiments, researchers were able to isolate and extract protoplast from the middle layer of leaves and then cultivate it into a food medium to produce a whole plant. The researchers also controlled the growth and specialization of protoplasts due to the varying conditions of the experiment. In 1972, Carlson and others succeeded in producing the first hybrid tobacco plant asexual when they were able to extract and synthesize protoplast from two types of tobacco and develop them on an artificial food medium to produce a whole plant. This step promised to make bells alert to the importance of this science. To carry out studies on propagation of plant tissue culture (4).

Applications of plant tissue culture

1 . **Plant breading and germplasm preservation**

The most current researches in the field of plant tissue culture deals with genetics and plant breeding. Through breeding new varieties can be devised with special specifications in terms of production and disease resistance. There are still many problems facing plant breeders such as time factor and plant incompatibility different crosses to transfer a specific characteristic between plants, include:

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- **Cell culture**

Mutations are important sources needed by plant breeders as a source of genetic variations that can result in plants superior to their origins. Mutations can occur in sexual cells that are easily transmitted to subsequent generations. If the mutations occur in Somatic cells, to the next generation that have been cultivated, and since the rate of occurrence of genetic mutations very little in nature so it became necessary to use different techniques to develop mutations in both genomes in vivo or in vitro. Mutagenesis in vitro is one of the technologies that can be used in this field. Through this technique, millions of cells can be exposed to stress, whether physical such as emulsions or chemical, such as Ethel methane sulphonate (EMS) Selection of mutant cells and replanting of plants. This technique can be used for breeding purposes to obtain plants with specific specifications such as NaCl salt tolerance or pesticide and toxin resistance by adding such media to the food media used in the development of cells showing resistance to this stresses (5).

- **Protoplast fusion**

The technique of isolating, cultivating and integrating protoplast has provided a solution to the problem of incompatibility between species and species and the development of embryos that can not be obtained in nature, as well as the possibility of transferring new genetic material from one cell to another. This technique is carried out by the cell wall either chemically or by cell membrane enzymes such as cellulose, then isolate the protoplast and promote the integration of protoplast desired varieties with the presence of some chemicals such as sodium nitrate or calcium ions or polyethylene glycol (PEG) and can be used to short-range electric stimulation to promote the process of integration and called this technique electrofusion (6).

- **Micro sperm and anther culture (Haploids)**

Plant breeders seek to obtain homozygous strains because of their importance in breeding programs. This technique is slow and time-consuming in traditional breeding. Researchers have isolated and grown the immature grain of pollen and planted it on certain media to obtain homogenous plants containing half the original number of the chromosomes in a short period of time and then multiply the chromosome number of these sterile plants by colchicine to obtain homogenous and fertile plants. In such cases the alteration of pollen grain growth from the composition of the pollen tube to cell division and re-differentiation into embryos have the ability to grow into full plants and there are many factors that affect the pollen most important meiosis stage of pollen grain when grown in addition to the physiological condition of the mother plant the composition and genetic nature of the food center and the conditions of keeping zygote there are still many obstacles facing this technology since many of the plants did not succeed in this technique so far and the solution alternative to the cultivation of grain of the

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vaccine, the researchers resorted to isolate anther in its entirety and its cultivation in the stage of evolution . The technique is much easier to cultivate than the pollen, however, there are some caveats to use as plants may be created from the anther wall, which consists of body cells containing the original number of chromosomes. Thus, the resulting plants are double-chromosome diploid as well as plants containing half the number of haploid chromosomes originating from on this basis; all the plants produced from the growth of the stalks should be examined to ascertain the number of chromosomes (7).

- **Culture of embryos, ovules and ovaries**

Embryo culture is the most successful technique of the tissue culture techniques used by plant breeders. Embryo abortion is one of the common cases that occur after cross breeding between species and sometimes after cross breeding between species. In such cases, hybrid seeds unless the embryo is isolated in early stages and grown on a diet to grow and develop into a plant. In cases where the abortion takes place at very early stages after direct fertilization, in which the embryo cannot be isolated because of its small size, the researchers isolate the whole ovule and fertilize it media appropriate until the development of the embryo. Ovules technology is also used to eliminate incompatibilities, especially in genetically divergent species. Pollen may mature at an early time before mites are ready to be vaccinated. The vaccine tube does not grow on the flowerbed due to the presence of some inhibitors in the flowering season. In vitro pollination and fertilization this problem can be overcome by isolating the oocytes and then fertilizing them with pollen from the desired plant in order to obtain the seeds of live embryos that grow later to seedlings or ovarian implants can be used in studies that relate to the appearance of fruits separation , stages of development and food needs (8).

2. Production of specific pathogen-free plants

The vegetative methods of plant propagation, such as the use of mind, bulbs and tubers, are also helpful in the spread of pathogens, and the resulting plants are infected if the mother plant is infected. Pathogens play a major role in determining the transfer of genetic resources among countries. Plants are not allowed in the world unless they are equipped with a health certificate that is free of pathogens and by the development of different tissue culture techniques, it is possible to eliminate the various pathogens, viruses, fungus or bacteria, include:

- **Shoot tip culture**

Although the technique is common, it is not guaranteed. The results are obtained in terms of obtaining healthy plants from infection. Usually, the length of the developing summit is between (0.1-20) mm. One plant is therefore relatively slow and its success depends on the size of the

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cultivated part. The larger the size, the greater the chance of success and the less chances of obtaining healthy plants (9).

- **Shoot tip grafting in vitro**

This technique can be used in cases where it is difficult to root the developing summit easily, especially in trees. This technique has been used to obtain citrus free of many viruses. This method is the removal of the developing summit consisting of the leg bone meristem with (1-2) of leaf primordio and the installation of a small-scale initiative growing on the center of a sterile food and then develops the taste and original as a whole plant is then transferred to the soil and requires this technique and skill and accuracy is finite (10).

- **Shoot apical meristem culture**

It is one of the ways to secure the results in obtaining healthy plants from infection with viruses, but the achievement requires extreme skill and accuracy in this technique is eradicated meristem leg length of (0.05-0.1) mm without any leaf primordio and grown on a diet to stimulate growth and development into a leg can In order to ensure the success of the seedlings, the developing summit should be rapidly isolated and transported to the media before drying and the success rate is low (11).

3. Production of drugs and secondary substances

Many pharmaceuticals and pharmaceuticals such as phenols, steroids and pigments are produced by plant cells as metabolic products of metabolic activity. In most cases, these compounds cannot be produced laboratory, but must be obtained from their natural sources. Plant tissue culture technology can be used to produce these substances rather than plant by isolating certain parts of the plant and cultivating them on food media to stimulate and sustain their growth and production of these substances, using this technology, these compounds can be obtained with higher purity than those separated from the whole plant. Their production is quick and does not depend on a given season. It reduces the amount of land needed for planting, as well as producing some important compounds used in anesthesia such as morphine, include:

- **Root culture**

Roots cultivation technique has contributed to the study of the construction of many phenolic compounds, alkaloids, vitamins and amino acids. This technique involves root removal from plant plantations and planting on a sterile liquid media times. The roots are then cut and replanted periodically, where the roots produce the spinach and produce it into the center where the active substances are extracted from the medium or extracted directly from the roots (12).

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- **Cell suspension culture**

It is commonly used technique in obtaining medically important substances from plant cells. In this technique, the callus is stimulated from the part known to produce the required materials. The callus then transferred to a liquid food medium, which is continuously circulating with a shaker at a certain speed so that cells can be separated from each other, this method is characterized by a lack of output compared to other methods in addition to the high costs and rapid pollution in these plants (13).

- **Callus culture**

The researchers resort to the induction of callus from the plant known to produce the required material in cases where it is difficult to plant special members of the plant or that these materials produced in small quantities and then extract these materials from the callus tissue has been extracted many alkaloids this technique in addition to the release of enzymes inhibitors Antibiotics and antimicrobial agents (14).

4. Rapid clonal propagation

Plant propagation is one of the most important practical applications for growing plant tissues. It is possible to obtain very large numbers of homogenous plants in a short period of time compared to conventional methods, as well as multiplication of hybrid plants and the rescue of endangered species as well as being the only way to reproduce plants that can not be propagated by roads Other vegetables, the MS food medium identified by Murashige and Skoog in 1962 has had a significant impact on the development of plant tissue culture, include:

- **Adventitious bud formation**

The occasional buds are the buds that grow outside their normal places (peripheral buds or capillaries and axillary buds in the leaf follicles). It is possible to encourage the formation of occasional buds on callus tissue in specific food media and subsequently grow into legs that can be easily rooted. Between auxins and cytokines. When cultivating callus tissue in a food medium is equipped with a quantity of auxin higher than cytokinesis, this leads to the development of the root of the callus. If the food medium is equipped with a higher amount of cytokine than auxin, this leads to the development of the Legs of callus (15).

- **Enhancement of axillary branching**

This method is based on the Thimann and Wickson observations of 1958 on the elimination of cytokines by cytokines. Because of the absence of dominion, axillary buds begin to grow and develop into legs that can be isolated from specific food groups (16).

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- **Induction of somatic embryogenesis**

This method is one of the fastest and most commonly used methods as well as the huge numbers of plants that can be produced in this way. It is possible to develop callus tissue from any part of the plant in the center of a food containing the auxin and re-planting on a new diet free of dioxins and this is done induction of somatic embryogenesis (17).

Factors affecting the success of plant tissue culture

1. The nutrient media

The composition and concentration of mineral salts and organic materials suitable for the growth of cells and plant tissues vary according to species, varieties and the most famous food medium in Murashige and Skoog in 1962, food medium White in 1963 and food medium Gamborg in 1968.

2. Temperature

Temperature is a determinant of the development of the cultivated plant part. The temperature at which the vegetable part or plant is preserved depends mainly on the type of plant and the type of part used in agriculture. In general, the development of the plant part at 25 ° C is appropriate in most cases.

3. Light

Cultured tissues do not need light in the early stages of agriculture because the fabric or plant part does not process photosynthesis and is entirely dependent on the nutrient medium of the elements and materials needed for growth, while light is very important in the final stages of cultivation, include:

- **Photoperiod**

The effect of the photovoltaic period varies on the composition of the organs according to the plants. 16 hours of lighting a day is suitable for most plants, but some callus farms grow better in the dark.

- **Kind of light**

White light stimulates the formation of buds, while red light stimulates the formation of flowers, while the dark and distant red rays stimulate the formation of roots, and found that blue light has a catalytic effect on the emergence of branches from the pulp of tobacco plant.

- **Light intensity**

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The lighting intensity of 1000 lux is suitable for the stage of emergence of seedlings and multiplication of propagation stages, while increasing the intensity of the light to 10000 lux suitable for the growth of the parts of the plant in the rooting stage (18).

4. The explant include:

- **Size of the explant**

The chance of success of the small plant segment is weak whether it is a shoot tip or callus, and the size of the shoot tip is planted on a larger number of leaf primordia whenever the chance of success is greater.

- **Source of explant**

For plant cells, the ability of embryogenesis and efficiency was noted. This susceptibility varies according to the plant tissue within each plant and different plant species, the cells lose their ability to embryogenesis, but they regain their ability to organogenesis.

- **The physiological**

The young tissue has a higher ability to restore the virtual configuration compared to older tissue, whether these tissues are inherited from plants, grass or wood, and the season of taking the plant part and the stage of growth of the mother plant affects the success of the growth of the plant part.

5. Genetics

Genetics of species and species within a species plays a large role in their response to tissue culture. There are cultivars that are easy to propagate, while other varieties are difficult to reproduce. This may be due to variation in hormonal content, which is reflected in plant growth plant needs.

6. Disinfestation

The food medium is also suitable for the growth of pathogens, so the sterilization process is very necessary to prevent the presence of these organisms, which if they exist, they will compete as well as attacking him causing death, and the process of surface sterilization is not feasible to get rid of organisms that grow within the fabric Planted.

7. Donor plant

The physiological state of the mother plant has a significant role on the behavior of the cultivated part. For example, the plant part taken from mature trees often requires a food medium that differs in its components from mature trees (19).

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Types of Tissue Culture

1. Seed Culture

The development of tissue culture from a single cell gives a great chance to characterize the properties of the planted cells and to study photosynthesis within the cell and to know how the biological processes in the cell and in the plant improvement. Seed culture is the type of tissue culture; in this method, explants are obtained from an in-vitro derived plant and introduced in to an artificial environment, where they get to proliferate. In the event that a plant material is used directly for this process, then it has to be sterilized to prevent tissue damage and ensure optimum regeneration (20).

2. Embryo Culture

Embryo culture is the type of tissue culture that involves the isolation of an embryo from a plant for in vitro growth. The term embryo culture is used to refer to sexually produced zygotic embryo culture. Embryo culture may involve the use of a mature of immature embryo. Mature embryos for culture are essentially obtained from ripe seeds. Immature embryo involves the use of immature embryos from unripe/hybrid seeds that failed to germinate. In doing so, the embryo is able to produce a viable plant. The ovule, seed or fruit from which the embryo obtained were sterilized. Salt sucrose was used to provide the embryo with nutrients. The culture is reinforced with organic and inorganic compounds, inorganic salts growth regulators (21).

3. Callus Culture

Callus the term used referring to unspecialized, unorganized and a dividable mass of cells. A callus is produced when explants are cultured in an appropriate medium. Callus culture includes the growth of a callus (differentiated and non- differentiated cells), which is then followed by a procedure that induces organ differentiation. The culture is often sustained on a gel medium, which composed of agar and a mixture of given macro and micronutrients depending on the type of cells. Different types of basal salt mixtures such as Murashige and Skoog medium are used in addition to vitamins to enhance growth (22).

4. Organ Culture

Organ culture is a type of tissue culture involving the isolating of an organ for in vitro growth. Any organ plant can be used as an explant for the culture process (Shoot, root, leaf, and flower). Organ culture, the method is used for preserve their structure or functions, which allows the organ to still, resemble and retain the characteristics they would have in vivo. New growth (differentiated structures) continues given that the organ retains its physiological features (23).

5. Protoplast Culture

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Protoplast are cells without cell walls (naked cells). Protoplast culture is an important method that provides numerous cells (single cells) that can be used for various studies. The protoplast has regenerated a cell wall, and then it goes through the process of cell division to form a callus, which then cultured for continuous growth (24).

6. Anther culture

Anther culture is one of the oldest and most widely used methods for the production of single-chromosomal plants. This method was first applied to the Datura plant. It was applied to several plants such as rice, wheat, barley, maize, potatoes and tobacco. Only the growth and development of pollen can be achieved with minimal growth of the surrounding tissue or lack of growth. The pollen develops into the embryos as regular as in tobacco or develops into an irregular mass of callus as in the grains. Sterilize the non-open floral shoots containing the stalks at the appropriate surface and remove the petals and leaves. The stalks are grown directly on the appropriate food media (25).

Plant propagation stages using plant tissue culture technique

1. Establishment stage

This stage involves all steps related to the selection of cultivated plant area (knots, interned and leaf) identification of sterilization material and the time required for it, control of the conditions surrounding the transplant, selection of the appropriate medium for agriculture in terms of type and components.

2. Multiplication stage

It is an important and critical stage where the success or failure of the propagation process is determined and depends on the total number and quality of the resulting plants, and at this stage the propagation of the plant part that was cultivated in the first stage.

3. Rooting formation stage

The branches produced from the occasional buds or axillary buds and growing in a food medium (stored on cytokines) are usually not rooted. For complete plants, branches should be moved to other food media to encourage the formation of transverse roots for the purpose of rooting, the branches are separated by a length of (1 cm) and transferred to the center of rooting, and those branches that have roots called plantlets.

4. Acclimatization stage

This process is important for plants resulting from the cultivation of plant tissues that are not ready to with stand the external environmental conditions. The resulting plants are very sensitive

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due to their growth and development in farms with a special environment where all plant needs are met. On the other hand, the process of photosynthesis in tissue cultures is very small compared to the growing plant in the field (26). These stages are explained shape (1).

Shape (1) Stages of propagation of palm in plant tissue culture

Steps of Agriculture

Selection of a mother plant
↓
Explant
↓
Trimming
↓
Surface sterilization
↓
Washing
↓
Establishment an appropriate growth media
↓
Transfer to multiplication media
↓
Rapid shoot formation

↓

Transfer to rooting media

↓

Rooted shoots (plantlet)

↓

Shoots or plantlets transferred to gradual weaning processes

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