

Propagating Plants by Tissue Culture

IMAGINE taking a plant cell and producing millions of plants from that single cell within a few years or even less. Not long ago the notion was pretty far-fetched. Today, this type of plant reproduction is happening across the country. It is being done by a method of asexual propagation known as tissue culture.



Objective:



Describe the tissue culture method of asexual propagation.

Key Terms:



agar
aseptic
autoclave
callous
explants
laminar airflow hood
plantlets
tissue culture

Tissue Culture

Tissue culture is a very technical method of asexual propagation that involves the growing of plant cells or tissues on artificial media under sterile conditions. It is also referred to as micropropagation.

Research is producing advances in tissue culture techniques. As a result, more and more types of plants are being propagated by tissue culture. Foliage plants, woody ornamentals, perennials, and cut flowers are commonly propagated by tissue culture methods.

ADVANTAGES TO TISSUE CULTURE

There are many advantages to tissue culture over other methods of propagation.

Large numbers of plants can be produced from a single plant in a relatively small space and in a short time. This reduces growing space, labor, and plant maintenance requirements.

Viruses and other systemic diseases are eliminated by propagating the quickly dividing cells of the shoot tip.

Tissue culture gives the grower a means to produce plants with identical traits.

Horticultural cultivars can be improved by selecting plants that vary slightly from the parent plant. Examples of characteristics subject to improvement are leaf shape, disease resistance, growth habit, and flower color.

Tissue culture is a required technique for producing genetically engineered plants. Genetically modified cells must be induced to divide and undergo cell differentiation in vitro, or in glass, before they are grown to maturity.

Tissue culture results in excellent basal branching of some plants. Some foliage plants produced by tissue culture have excellent multi-stem characteristics.



FIGURE 1. Tissue culture is a highly technical method of asexual reproduction. (Courtesy, Agricultural Research Service, USDA)

TISSUE CULTURE PROCEDURES

The tissue culture propagation process can be defined in four main stages.

Stage 1

In the first stage, small pieces of plant material, called **explants**, are carefully removed from the parent plant. Explants are obtained from actively growing parts of a desired plant. They may be taken from shoot tips, leaves, stems, roots, or embryos.

Bacteria or fungal spores are found on the tissue surface of explants. Therefore, the explants must be cleaned before being placed in the culture. A short bath in a 10 percent bleach solution, followed by a rinse in sterile water, is often effective in killing microorganisms. From this

point until stage 4, tissue culture must be done under **aseptic**, or sterile, conditions. The presence of any bacteria or fungal spores will contaminate the culture. Bacterial or fungal contamination results in microbial growth gone wild!

Tissue culture labs are designed to provide a clean environment. Technicians scrub much like surgeons do before surgery. Media, tools, and bottles or jars are sterilized in an **autoclave**. Autoclaving involves the heating of the materials to 245°F for 15 minutes to kill all bacteria and fungi. Also, to lessen the chance of contamination, work with the cultures is done under laminar airflow hoods. A **laminar airflow hood** filters bacteria and fungal spores from the air and produces a slight air current that blows toward the worker.

Explants are placed on sterile agar medium in glass bottles or test tubes. **Agar** is a gel that contains water, sugars, nutrients, and plant hormones to support and promote plant growth.

Stage 2

In stage 2, the cells of the explants multiply in one of two ways. They may form callous tissue or produce many plantlets.

Callous tissue is a group of cells with no particular function. Given the right hormones in the medium, the callous cells differentiate and develop into plantlets consisting of leaves and stems.

Stage 2 may involve the rapid multiplication of plantlets. Cytokinins placed in the medium encourage an increase in the number of buds on the explants, usually six to eight per shoot. Each bud is capable of becoming a



FIGURE 2. Explants are placed on an agar gel in glass containers. (Courtesy, Agricultural Research Service, USDA)



FIGURE 3. Tissue culture is carried out in a work area that is free of contamination.

plant and producing more buds. Branching occurs as these buds develop into shoots, or **plantlets**. Plantlets are divided and transferred to test tubes or jars. New plantlets are continually cycled to new containers. In this way, a single explant can produce millions of plantlets in a year.

Stage 3

When the plantlets have developed, they are ready for the third stage, the formation of roots. Shoots are transplanted to a medium containing a higher level of auxins. The auxins induce the growth of roots. The plantlets are also given higher light intensity in preparation for stage 4.

Stage 4

In stage 4, the plantlets are removed from the glass containers and divided. Agar is washed from the plants. They are planted in a sterile growing medium and placed in a greenhouse. Since the humidity in the greenhouse is much lower than in the glass containers, care must be taken during this transition to acclimatize the plants to a drier environment. Common practice is to place the young plants under a misting system until they grow accustomed to the environment and develop a stronger root system.



ON THE JOB...

CAREER CONNECTION: Plant Tissue Culture Technician

Research and technology have greatly changed the way many plants are reproduced. Tissue culture, or micropropagation, is a highly technical method of plant propagation that is widely used today. As the application of plant tissue culture propagation has increased, the need for skilled plant tissue culture technicians has grown.

Plant tissue culture work is done in laboratory-type settings where conditions are sterile. Plant tissue culture technicians must pay careful attention to cleanliness in the work environment. Technicians propagate foliage plants, perennial plants, cut flowers, woody plants, and genetically engineered plants.

Plant tissue culture technicians benefit from taking classes in a horticulture or agriculture program at the high school level. An associate's degree in horticulture, agriculture, or botany improves opportunities. Work experience in the agriculture or horticulture field is also helpful.



(Courtesy, Agricultural Research Service, USDA)

Summary:



Tissue culture is a very technical method of asexual propagation that involves the growing of plant cells or tissues on artificial media under sterile conditions. There are many advantages to tissue culture over other methods of propagation.

The tissue culture propagation process can be defined in four main stages. In the first stage, small pieces of plant material, called explants, are carefully removed from the parent plant. In stage 2, the cells of the explants either form a callous, which is a group of cells with no particular function, or plantlets rapidly multiply. In the third stage, the formation of roots is promoted on developed plantlets. In stage 4, the plantlets are removed from the glass containers, planted in a sterile growing medium, and placed in a greenhouse.

Checking Your Knowledge:



1. What is tissue culture?
2. What are some advantages to tissue culture over other methods of asexual reproduction?
3. Why are aseptic conditions so important in tissue culture?
4. What materials and equipment are used in tissue culture?
5. What are the four main stages of tissue culture?

Expanding Your Knowledge:



In the school laboratory, perform a tissue culture lab. Kits are available through scientific supply companies. Boston fern, African violets, and Venus flytrap are some plants available for this purpose. Practice aseptic techniques.

Web Links:



Plant Micropropagation Using African Violet Leaves

http://www.biotech.iastate.edu/lab_protocols/AV_Micropropagation.html

Plant Tissue Culture

<http://www.bio.davidson.edu/people/kabernd/seminar/2002/method/amy/aj.htm>

http://en.wikipedia.org/wiki/Plant_tissue_culture

Agricultural Career Profiles

<http://www.mycart.com/career-profiles>