Lesson 2:
Plant Tissue Culture

The horticulture industry regularly uses a biotechnology technique called plant tissue culture as an effective method of propagating horticultural crops such as ferns and orchids. Plant tissue culture is also being used to grow whole plants from genetically modified plant cells. This lesson will introduce and explain the process of plant tissue culture. The steps in tissue culturing and the stages of tissue culture growth will also be examined.

Plant Tissue Culture

Plant tissue culture is an asexual method of reproduction. The plants produced will be exact clones of a single parent plant. Plant tissue culture involves selecting a piece of a parent plant and placing it in a sterile artificial media where it grows into a new plant.

Advantages and Disadvantages of Plant Tissue Culture

One of the main reasons that plant tissue culture was developed was that some plants are very difficult to propagate commercially. Tissue culturing allows plant growers to mass propagate clones of a highly desirable plant. They can produce thousands of plants from a few pieces of one plant. Another advantage of plant tissue culture is that pathogen-free plants can be produced since certain portions of a plant do not contain viruses that are found in the rest of the plant. A third advantage is the conservation of time, because plants can be propagated from tissue culture at any time during the year. A fourth advantage is the conservation of growing space. Tissue culture plants are divided multiple times and begin as very small plants that grow slightly more slowly than normal. The number of plants traditionally grown on one acre can therefore be grown on shelves in 50 to 60 square feet of space.

With the advantages come some disadvantages. Plant tissue culture is an expensive method of plant propagation requiring an extensive amount of sophisticated equipment and facilities. In addition, plant tissue cultures are susceptible to contamination by microorganisms. Tissue cultures are destroyed if contamination occurs. A third disadvantage is that commercial tissue culture requires skilled workers, which adds to the total cost of producing the plants.

Plant Tissue Culture Equipment

The equipment and supply needs for plant tissue culture can be broken down into three separate areas--preparation, transfer, and growth. Several types of equipment and supplies are needed when preparing to do a tissue culture. A refrigerator is needed to store the chemicals for the growing media. The pH of the growing media must be checked with a pH meter. A scale or balance is required to measure the quantities of the media ingredients. The ingredients are warmed on a heating plate. Finally, an autoclave is required to sterilize the media and the equipment used in transferring the plants. When transfer is carried out, a fume or air flow hood is used to reduce the movement of air (which may contain microorganisms) in the work area. The technician holds the plant tissue with a sterile forceps and uses a scalpel to divide the plant for culturing. Test tubes or petri dishes hold the growing media and the plant tissue after transfer. For some types of tissue culture, a dissecting microscope is used to help in the selection of the tissue for culturing. When the process is completed, the tissue cultures are put in a growth chamber, a room that controls exposure to heat and light.

Steps in Plant Tissue Culture

The first step in plant tissue culture is media preparation. The initial growing media varies slightly in composition depending on the plant species. However, it generally contains plant nutrients, mineral salts with vitamins, plant hormones that regulate growth, pure water, sugar, and agar (if a semi-solid media is needed).

The second step in the process is the selection and collection of the explant. The explant is the portion of the parent plant that will be used to grow new plants. Depending on the plant being propagated, a shoot tip, bud, section of a leaf with veins, node, or bud scale may be chosen. Different plants grow better from different types of explants. The explant is nearly always selected from rapidly growing tissue since this tissue is best able to produce the new plant. It is also important that healthy and disease-free parent tissue be used.

After the explant has been chosen, the next step is cleaning the explant. It must be disinfected. If the explant is woody tissue, alcohol is used as a disinfectant; for most other plant tissues, a 10 percent bleach solution is used. The explant is soaked in the bleach solution for only ten minutes. If it is soaked longer than ten minutes, damage to the plant tissue can occur. Often a drop or two of detergent is added to the bleach solution as a wetting agent to help the disinfectant apply to surfaces more effectively. After it is soaked in the disinfectant, the explant is rinsed at least three times in pure water to remove any remaining bleach solution.

The fourth step involves transferring the explant to the growing media. The collected plant tissues are trimmed, and some types of tissues can be divided for growing more plants. A dissecting microscope is sometimes needed if the explant is very small. The explant is then transferred to the growing media. This procedure must take place in a sterile environment.

Stages of Tissue Culture Growth

The first stage of tissue culture growth is called the initiation and establishment stage. This stage lasts about four to six weeks. A callus composed of rapidly dividing cells forms and grows in response to the wounding or cutting of the plant tissue. A shoot or immature stem begins to grow during this first stage.

The second stage of tissue culture growth is the proliferation or multiplication stage. It lasts one to three months. During this stage, the shoot multiplies into many shoots. These new shoots can be divided to increase the number of plants produced. A slightly different growing media is used in this stage.

The third stage is the pretransplant stage. It lasts about three weeks. Roots begin to grow. A slightly different growing media is used. The plants begin to photosynthesize and require more light. Near the end of this stage, the young plants are stressed slightly in a process called hardening off, which involves exposing the young plants to conditions outside the sterile container in which they grow.

The fourth stage of plant tissue culture growth is called the transplanting stage. The growing plants are put into pots and moved to a shady, humid greenhouse. After a several weeks, the plants are again hardened off and then moved to a regular greenhouse where they will receive full sun and less humidity.

The Use of Tissue Culture in Genetic Engineering

When a single plant cell or group of cells is genetically modified, tissue culture is often used to rapidly grow a set of plants. These plants are genetically identical to the plant cell or cells used to create them. A second use of plant tissue culture is for the screening of a large number of plants for certain characteristics. If a more drought-tolerant plant is desired, then hundreds of tissue cultures can be taken from a wide range of plant varieties and mutations to find this characteristic. The tissue cultures from the plants are grown and exposed to drought conditions. Those plants that show promise are grown to full size. This screening allows researchers to focus on plant varieties that might contain desirable genetic traits.

Summary

Tissue culture has become an important tool for plant breeders and researchers. It allows breeders to quickly produce large numbers of mature clones of a parent plant. Researchers are able to genetically modify a plant cell or group of cells and then grow these modified cells into a mature plant. However, plant tissue culture is an expensive process and can fail if the cultures become contaminated. Tissue culturing involves four major steps: media preparation, choosing the explant, cleaning the explant, and transferring the explant to the growth media. Growth also occurs in four stages: initiation and establishment, proliferation, pretransplant, and transplant.

Credits

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