Pathogen-free Plants b

Meristem-tip Culture

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Vegetative propagation of plants conserves not only desirable germ plasm but viruses and other pathogens as well. Meristem-tip culture has proved to be a reliable approach to eliminating these pathogens.

What is Meristem-tip Culture?

The meristem is a dome of actively dividing cells at the apex of shoots and roots. The meristematic dome varies in size and shape among species and among cultivars of the same species, but it can be estimated to be 1 mm in diameter and 0.25 mm long. Approximately 30 yr ago, viruses were shown to be decreased or absent in this meristem region. Apical and axillary meristems and shoot tips for many plant species, therefore, have been excised to avoid virus-infected portions. The resultant explant is transferred to a nutrient medium to stimulate shoot elongation and root development. This process is known as meristem-tip culture and also has been called meristem culture, tip culture, culture of shoot apices, and shoot-tip culture. I have chosen the terminology proposed by Hollings (1).

Plantlets can be regenerated from individual cells and from nonmeristematic tissue of many plant species, but the developmental and differentiation processes generally are complex. Additionally, for some species, plantlets derived from meristem-tip culture are more stable genetically than are plantlets derived from other tissue culture procedures. When the objective is to eliminate a virus from an existing cultivar, returning to the propagator a plant that does not vary phenotypically from the original plant is of the highest importance.

In practice, buds 0.1–1 mm long are excised for meristem-tip culture. The smaller explants often fail to grow and the larger ones usually remain virus-infected. An intermediate size of 0.3–0.7 mm generally gives the highest proportion of virus-free regenerated plantlets. Buds this size are composed of the meristematic dome plus one to two leaf primordia. Bud excision and subsequent steps must be done aseptically, normally in the contaminant-free atmosphere of a transfer room or hood. Buds are usually excised with a sterile razor blade and with the aid of a stereomicroscope (10–40 × magnification).

The explants are placed in contact with an agar or liquid nutrient medium and supported by a filter-paper bridge or, on the latter medium, allowed to float. The nutrient medium is composed of basal salts (major and minor elements) and organic supplements. Although the organic supplements may vary considerably, all include a carbon source, such as sucrose; vitamins; and plant growth regulators, such as auxins, gibberellins, and cytokinins.

Other factors can affect the success of plantlet regeneration, including age of the donor plant, time of year the explant is obtained, temperature, and length, intensity, and quality of light. Fungal and bacterial contamination of meristems can retard or kill regenerating explants; when detected by visible colony growth in the test tube, these contaminated cultures should be discarded.

After plantlets have generated roots (Fig. 1), they are transferred to soil. They are quite tender at this stage, and care must be taken not to stress them after transplantation. Covering transplants with a beaker during the first week reduces transpirational water loss and increases survival.

Plantlets are indexed initially for viruses at the time they are transferred from the culture medium to soil. A single leaflet is removed from the plantlet and tested by mechanical sap inoculation onto indicator plants, by serologic tests, by electron microscopic examination, or by other appropriate methods. The tests selected are determined by the investigator's knowledge of the viruses infecting the cultivar.

Plantlets are indexed a number of times before they are declared or considered to be virus-free. Although viruses remaining in most plants after meristem-tip treatment usually are detected during the initial index, virus concentration may be reduced below the level of detection for nearly a year. When eradication or elimination is difficult, heat therapy or antimetabolites may be used preceding meristem-tip culture. By inhibiting virus invasion of the meristem region, these treatments provide a larger margin of error in the size of the meristem-tip that may be excised.

A Potato Model

Once a plantlet is determined to be virus-free, the problem becomes one of propagation without recontamination. The seed potato program in Wisconsin is one approach to dealing with this problem. The University of Wisconsin-Madison operates an elite foundation seed potato farm that provides an annual supply of disease-tested seed stocks to private growers who participate in the certified seed potato program. The foundation farm is isolated some 15 km from other potato fields, and seed production is strictly controlled. All cultivars currently grown for production on the farm have been through the meristem-tip culture process. The cultivars are increased as clonal lines for 3–4 yr before sale. Ample time, therefore, is assured to test and retest cultivars for viruses and other pathogens and to evaluate clones

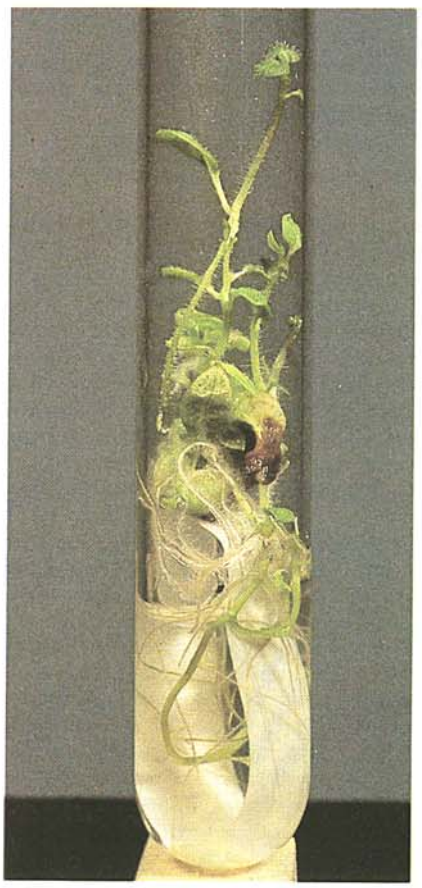

Fig. 1. Potato plantlet regenerated from excised meristem-tip is ready to be transferred to soil.

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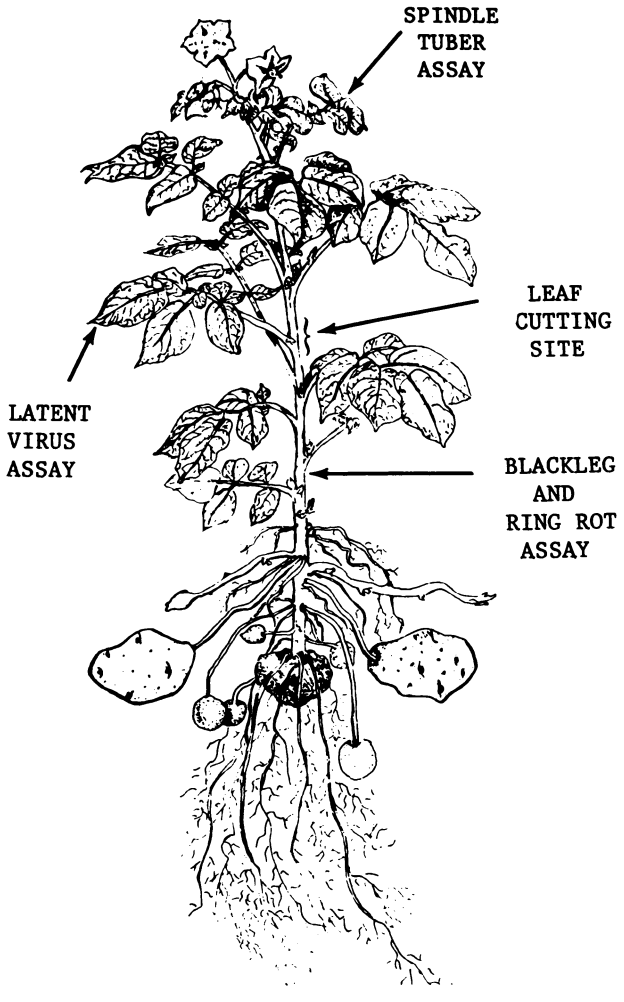

Fig. 2. Mother plant in the first generation of a seed potato increase scheme. The plant is indexed for the diseases indicated and is then processed into leaf-bud cuttings for rapid multiplication.

for "true-to-type" horticultural characters.

Tubers from selected plants are hand-dug each fall, treated to break dormancy, and planted in 114-L containers in a greenhouse. The mother plants are allowed to grow until five to six nodes have developed. At that time, the tops of the plants are removed to induce lateral shoot growth from the remaining axillary buds. The plants are then allowed to grow for 2–3 mo. After growth is adequate, the mother plants are divided into leaf-bud cuttings, each cutting consisting of 2–3 cm stem sections with an axillary bud and a leaf (2). Each mother plant yields 25–100 leaf-bud cuttings, which are placed in a moist sand bench with the stem and axillary bud covered but the leaf exposed. Intermittent misting is supplied to the sand bench to keep the cuttings moist. After 6–8 wk, the axillary buds develop into small tubers that can be harvested and replanted in the field for the summer season. The advantages of the leaf-bud cutting procedure are that 1) microorganisms normally infesting potato tubers are eliminated, 2) mother plants can be tested for latent infections by specific pathogens, and 3) pathogen-free stocks may be increased rapidly.

All mother plants that comprise this first generation of nuclear-class seed stock are tested for potato spindle tuber viroid by polyacrylamide gel electrophoresis, for latent potato viruses S and X, and for bacterial blackleg (*Erwinia carotovora* var. *atroseptica*) and ring rot (*Corynebacterium sepedonicum*) by serologic tests (Fig. 2). Mother plants are also inspected carefully for symptoms of these and any other diseases, and infected plants are eliminated from the program. In the Wisconsin scheme, meristem-tip culture is not repeated once a cultivar has been freed from known potato viruses. The procedure is repeated only if total recontamination of a cultivar is encountered.

The program does not guarantee that seed stocks will not become reinfected as they are increased or protect against insect-transmitted or soilborne pathogens. The selection procedures, however, do enhance the probability that the clonal lines will be free from all known plant pathogens. If seed stocks are initially pathogen-free, the pathogen must be reintroduced for recontamination to occur. Because the nuclear seed stocks are reselected and tested every year, the input of pathogen-tested stock into the seed program is constant. Similarly, certified seed is being sold at the end of the clonal increase system. This "flushing out" of older seed stocks with continual reintroduction of new seed stocks is the best available mechanism to minimize the incidence of latent potato viruses S and X, which have been found universally in the older North American cultivars, and of the blackleg bacterium, which may be carried within tuber lenticels without producing disease symptoms.

A Worldwide Procedure

Virus-free plants have been obtained by meristem-tip culture for the species that have been studied (1,3). The procedure has been successfully applied in the ornamental industry to *Chrysanthemum*, *Dianthus*, *Dahlia*, and various orchids. Potatoes and strawberries are prominent examples of industries where productivity of cultivars has been increased through meristem-tip culture and through clonal increase programs designed to minimize recontamination. Similar benefits can be expected in the *Citrus* and forestry industries, where rather recent progress has been made. Each species, however, presents its own technical difficulties. *Citrus*, for example, must be grafted onto young seedling rootstocks because attempts to initiate root development have failed. The procedure is successful in *Narcissus*, but the rate of plant multiplication is prohibitive.

A recent modification of meristem-tip culture called multi-meristem culture, developed by researchers at the International Potato Center (CIP) at Lima, Peru, (5) has increased the multiplication rate of potato meristem-tips about fiftyfold. Excised meristem-tips from plants previously freed from viruses were induced to produce multiple shoots in shake culture. Plantlets regenerated from nodal cuttings of multi-meristem shoots were then shipped in culture tubes from CIP to cooperating countries (4).

Shipment of plantlets in aseptic culture should enhance the international transfer of germ plasm because culture tubes can be inspected easily for insects and plant pathogenic microorganisms. Other species, such as sugarcane and asparagus, have also been shipped in this manner. In the CIP program, plantlets are indexed for known potato viruses before shipment, which should facilitate international acceptance of plant materials. The receiving authority responsible for plant quarantine determines whether retesting or additional testing is necessary. Obviously, however, the groundwork for uniform guidelines governing rapid international transfer of vegetatively propagated germ plasm has been established.

Other Prospects

A number of exciting prospects for tissue culture systems exist, including

germ plasm preservation as well as transfer; maintenance of disease-free stocks; crop improvement; and manipulation of genetic resources through cell and tissue culture. These prospects are recognized widely; a survey of the contributors to a recent book on plant tissue culture (3) shows that 18 countries are represented.

Meristem-tip culture has been and continues to be a valuable tool for obtaining pathogen-free stocks of germ plasm maintained through vegetative propagation. The widespread utilization of meristem-tip culture in potato production and its use for international potato improvement testify to its importance in plant protection and disease management.

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