Methods of Virus Eradication

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Introduction

The eradication of viruses by excision of meristems was first demonstrated almost 30 years ago (Morel and Martin, 1952). The principle is based on the uneven distribution of virus particles throughout the plant.

In the case of vegetatively-propagated crops such as potato and sweet potato, there is clear evidence that the eradication of virus infection to produce so called "pathogen-tested" plants enhances the yield of the crop (Mori, 1971; Morel and Martin, 1955; Stace-Smith and Mellor, 1970; Pennazio, 1978).

Pathogen-tested materials are the basis of modern "seed schemes" whereby clean material is propagated under conditions of zero or minimal reinfection. In this way, higher quality planting material can be produced for farmers, that will in turn give both higher yields and enhanced tuber quality.

Although the excision of meristems has been a key technique in virus eradication, a few important developments have followed this procedure. First, the quality of a pathogen eradication program is only as good as the detection methods used to show that the pathogen has been eradicated. In this area, great advancements have been made recently in both routine serological testing (Clark and Adams, 1977) and nucleic acid hybridization techniques (Owens and Diener, 1981).

Secondly, a number of additions have been made to virus eradication procedures that enhance the probability of cutting meristems that are virus-free. These additions are normally chemical treatments (chemotherapy), heat treatments (thermotherapy), or a mixture of the two.

The use of thermotherapy on both potato and sweet potato is now routine at CIP. Sometimes chemotherapy can be applied.

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Chemotherapy

In the case of sweet potato, we have found the plants to be highly susceptible to virazole and other antiviral chemicals. This is similar to the situation with cassava*. Thus to date, chemotherapy has not been used on sweet potato.

In potato, antiviral chemicals were sometimes used in the old protocols as a pretreatment of intact plants before placement in the growth chamber for thermotherapy. With the new protocol, the optimal treatment to date is a mixture of in vitro heat treatment with the inclusion of ribavirin in the medium. Table 1 shows the effect of a wide range of antiviral chemicals when used in the in vitro system.

Table 1. The effect of a range of antiviral chemicals when included in a tissue culture medium as a way of enhancing the virus elimination process.

Chemical	Concentration (nM)	Desiree/PVS % Titer reduction	Russet Burbank/ PLRV % Titer reduction
1. Autoclaved chemical			
Adenine arabinoside	0.01,0.1,0.2	0	0
	0.4,0.8	40	0
5-azacytidine	0.1,0.5	17	52
6-azauracil	1.0,2.0	-	-
3-deazauridine	0.05	25	33
	0.1,1.0	-	-
2,3 diaminopyridine	1.0	0	0
5-diazouracil	1.0	27	0
2,4 dimercaptopyrimidine	0.15	65	-
5-fluorouracil	2.0,3.0	-	-
Tubercidin	0.1	0	0
2.Filter sterilized			
Adenine arabinoside	0.01, 0.10	0	0
	1.0,1.5,2.0	0	-
5-azacytidine	0.1	-	-
6-azauracil	1.0	-	-
cordycepina	0.04,0.06,0.08	0	-
5-diazouracil	1.25,1.5	0	-
Dicyandamide	0.6	0	-
	1.0	-	-
	2.0	0	74

*W. Roca, personal communication.

Thermotherapy

Thermotherapy at elevated temperatures (37°C) does not eliminate the potato spindle tuber viroid (PSTVd). PSTVd consists of a single-strand, ring-shaped RNA that is twisted in the form of a supercoil. In this form it is resistant to nucleases. Elevated temperature, far from decreasing the concentration of the viroid, favors its multiplication (Sanger and Ramm, 1975). Therefore, a first test for PSTVd should be carried out at the end of the thermotherapy period.

A method that permits the eradication of PSTVd (Lizarraga et al., 1980) is based on the observation that in plants grown at low temperatures, the viroid concentration is low. In an experiment, plants were grown at 8°C for four months. Then apical domes were excised. Thirty percent of the plantlets regenerated were free of PSTVd, even in the second tuber generation. A clear (negative) relationship between meristematic explant size and eradication success was observed (Lizarraga et al., 1982).

This method, however, is not suitable as a routine technique, since it is time-consuming and costly. It may be useful in specific cases in which a valuable clone is not available without PSTVd infection.

Experiments carried out with different virus host systems have shown that treatment of plants with elevated temperatures (thermotherapy) leads to a reduction in virus concentration (titer) in the plant (Kassanis, 1957; Quak, 1977). Different reasons have been given to explain this phenomenon; most likely, the reduction in virus titer is caused by a combination of several factors. These can include competition for sites of synthesis of nucleic acids and proteins between the fast-dividing host cells and the virus particles, which may lead to a change in the balance between synthesis and degradation of virus particles. Also, the nucleic acid of the virus, the carrier of its genetic information, is usually protected from attacks by degrading enzymes by a coat of many protein subunits. At elevated temperatures, the linkage between these subunits becomes weaker, temporary holes may open and permit the attack of nucleases, leading to virus inactivation and decreased virus concentration.

Thermotherapy has been applied to dormant potato tubers and, as a result, a reduction of virus concentration, mainly of potato leafroll virus (PLRV), has been observed. However, elimination was not achieved except for PLRV.

Thermotherapy applied to the whole plant as well as to sprouted tubers followed by meristem culture has been successfully used as a standard procedure for elimination of many viruses in potato (Stace-Smith and Mellor, 1970; Pennazio and Redolfi, 1973).

In the standard procedure used at CIP, best results have been obtained when the plant is decapitated before introduction into thermotherapy and axillary buds grown while undergoing heat treatment. A daily temperature regime of 36°C for 16 hours and 30°C for eight hours and

continuous light of high intensity (10000 lux) improved elimination rates. Plants are kept under these conditions for four weeks. Meristems are isolated and cultured both from axillary buds and apical buds.

In vitro thermotherapy is an innovative method that is gradually replacing the standard procedure explained above. Nodal cuttings from in vitro plants are placed in plastic boxes with propagation medium using agar as gelling agents. When the plantlets reach 3 to 4 cm and have a good root system, they are ready for thermotherapy treatment in an incubator. After a 1-month treatment, apical meristems are isolated and cultured. Figure 1 shows a low-cost incubator that can be constructed for in vitro thermotherapy. The optimal treatment to date is the application of heat and the inclusion of antiviral chemicals in the culture medium.

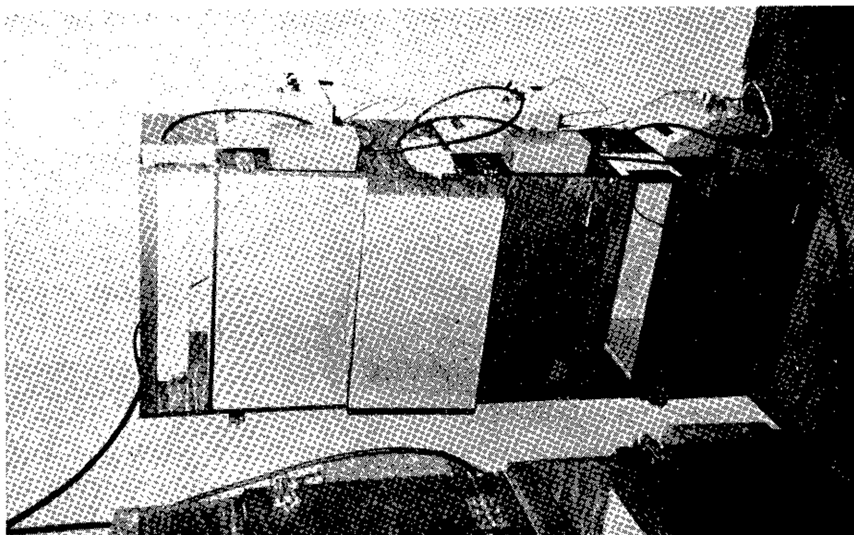

Figure 1. Low cost construction thermotherapy chamber. A wooden box construction, insulated with polystyrene, fitted with a heat tape and fire thermostat. A small fluorescent light is used for illumination.

Meristem Isolation and Culture

The active growing point of the plant shoot is the meristem. It is a small region composed of rapidly dividing (meristematic) cells.

The dome of a shoot apical meristem contains the truly meristematic cells and is surrounded by leaf primordia and primary leaves. Since the differentiated vascular tissues are located far from the meristem (towards the older stem tissue), the vascular elements of the leaf primordia

are still incipient, and have not yet made contact with the main strand of the vascular system in the stem. Therefore, virus particles, which may be present in the vascular system, can reach the meristematic region of the apex only through cell-to-cell movement; a slow process. This is one of the main reasons why in a virus-infected plant, virus concentration decreases acropetally toward the meristem of both the apical and the axillary buds.

The apical portion, called the meristem tip, when isolated under aseptic conditions and cultured on an adequate aseptic nutrient medium, leads to the development of plantlets. This developmental sequence, in principle, simulates normal plant growth.

The aseptic dissection of the meristem is a delicate process requiring many hours of practice.

Stems are cut from the plant that has just undergone thermotherapy into segments which each contain one node with its axillary bud. The leaves are carefully removed. The stem segments are disinfected for 30 seconds in 70% alcohol, followed by 2.5% calcium hypochlorite for 15 minutes. Then the stems are washed four times for five minutes each time with sterile distilled water to remove excess hypochlorite. If in vitro heat treatment is carried out, then the meristems are isolated directly without the need for surface sterilization.

Under a binocular dissecting microscope, the leaflets surrounding the growing point are removed until only the apical dome and a few leaf primordia remain. The dome and two leaf primordia are excised and transferred to the meristem culture medium. The excised apical dome is transferred weekly to a fresh medium. After six to eight weeks, the small plantlets are subcultured for further growth and micropropagation.

After regeneration from the cultured meristems, plants are tested (indexed) to detect any persisting virus infection.

Genetic Stability of Regenerated Plantlets

Once the plants have been through the pathogen elimination process it is important to check that no abnormalities have occurred during the culture/regeneration process. At CIP, the standard tests for this are the analysis of morphological characters and the running of protein electrophoresis patterns from mature tubers. These tests are carried out prior to the international distribution of the pathogen-tested genotype.

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