IN VITRO METHODS FOR PATHOGEN ELIMINATION AND INTERNATIONAL DISTRIBUTION OF SWEET POTATO GERMPLASM

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Introduction

The introduction of plant material to in vitro culture conditions requires a prior surface sterilization step to remove superficial contaminants such as bacteria and fungal spores. If the internal tissues of the plant material are not infected the explant can then be easily introduced to a sterile culture environment with little difficulty. However, plant pathogens that will not grow on the nutrient medium such as viruses and viroids cannot be simply removed by surface sterilization. The use of in vitro culture methods for eradication of viruses is not a particularly new technique, the first successful use of meristem culture for virus eradication was reported by Morel and Martin in 1952 (1).

In the case of a vegetatively propagated crop such as sweet potato there are two principal reasons for eliminating pathogens. Firstly, if material is to be distributed internationally then quarantine regulations normally require that the material is of a pathogen-tested nature. Secondly, it has been shown in many crops that pathogens can significatly reduce yield and marketable quality of the crop. A successful pathogen elimination program required a close cooperation between tissue culture specialists who are responsible for meristem culture and regeneration of plantlets, and virologists and bacteriologists who are responsible for testing the regenerated plantlets. A fundamental criteria for this type of study must be a detailed knowledge of the pathogens since a pathogen elimination program can only be as good as the sensitivity of the testing methods.

Elimination of Bacteria/Fungi/Mycoplasmas

These types of pathogens are either on the surface of the plant material or can be internal, tissue infections transported within the

vascular bundles. A number of pretreatments can be used that normally involved watering or soaking the plant material in antibiotic solutions for several hours. The following have been used.

- Gentamycin
- Rifampicin
- Nystatrin + Carbenicillin
- Gentamycin + Amphotericin B
- Vancomycin-HCl + Mycostatin
- Steptomycin - Carbenicillin

Care must be exercised in the use of antibiotics since reports in the literature have shown that some antibiotics show phytotoxicity at the appropriate concentration.

One technique now being applied routinely at CIP for potatoes is the use of filter paper wicks infiltrated with antibiotics, these wicks are inserted into the culture medium and the meristem or single node is inoculated onto the surface of the filter paper wick.

Pregrowth of the plantlets obviously plays a major role in the phytosanitary status and it is always preferable to use plants grown in screenhouses or growth rooms to field grown material. The plant materials should be used while at active growing stage. Routine spraying of plants with fungicides and bactericides for several weeks prior to in vitro introduction can be of great assistance.

Meristem Culture and Virus Eradication in Sweet Potato

As stated in the introduction, it was shown many years ago that isolating meristems could be a successful way of eradicating virus infections.

Success in virus elimination by meristem culture depends on virus type, host plant species, and size of the meristems used for culture. In general, virus-free plants produced is inversely proportional to the size of the meristem used. The mechanism involved in virus elimination by meristem culture is not clear.

Several hypothesis have been evolved. It is postulated that the meristem and even the young leaf primordia which have not been in contact with the main vascular system, therefore, the virus particles which may be present in the vascular system can not easily reach the meristem. It is also possible that the meristematic cells can produce virus-inhibitory substances. The presence of hormones in the culture media may also have contributions to the virus elimination.

It has been reported that sweet potato virus diseases such as mosaic virus, feathery mottle virus (2, 3), internal cork virus (2, 4), sweet potato virus (SPVD) (5) and some unidentified viruses (6, 7) were eliminated from sweet potato plants by meristem culture alone or in combination with thermotherapy.

Thermotherapy

It has since been shown that heat treatment (thermotherapy) can greatly increase the success rate of virus elimination. The logic behind thermotherapy is to raise the temperature to a level at which the plant meristem is still able to grow but replication of the virus is inhibited. In the case of potato, CIP uses a thermotherapy regime of $37/25^0$ 16 hr/8 hr in continuous light, whereas IITA uses a thermotherapy at 37^0 constant temperature at 16 hr light for cassava. CIP is also testing the use of thermotherapy on sweet potato clones at a range of temperatures up to 40 grades C.

In recent years interest has developed in the possibility of carrying out thermotherapy on materials that are already in vitro (8). The advantages of in vitro thermotherapy would appear to be the volume of plantlets that can be cleaned and a significant reduction in unit cost for the clear up process.

Chemotherapy

For many years there has been interest in the use of antiviral chemicals for virus elimination (9, 10). The most commonly used antiviral chemicals are virazole and adenosine arabinoside, these compounds do however exhibit pronounced phytotoxic effects at their effective antiviral

concentrations. The use of these chemicals has been highly effective for reduction of the concentration of virus in many cases although true eradication was not achieved. This reduction in virus titre can be an important pretreatment to produce stronger plants for entry to thermotherapy.

Scheme for Disease Elimination in Sweet Potato

Meristem culture technique is routinely used at IITA to eliminate virus diseases from sweet potato (11), it was found that about 85% of the regenerated plants were free from SPVD by applying this technique alone.

The sweet potato tubers harvested from the field are cleaned, planted in pots and grown in the greenhouse. When sprouted, both apical and later buds are excised for culturing. After disinfection process, meristem with one to two leaf primordia are excised and cultured in vitro. Six to eight weeks after incubation, plantlets are obtained and can be transplanted to jiffy peat pellets and then to sterile soil in pots in isolation room for virus testing.

At IITA, several virus testing methods are used. (1) After one to grafted to I. setosa , an indicator plant for a number of sweet potato virus diseases. This type of grafting are repeated three times for each plant. (2) A complementary grafting test is also carried out on the cuttings of the plant since the virus disease that we have in Nigeria consisted of two viruses and that if two viruses are presented at the same time, severe disease symptoms are observed whereas if appears singly, it is latent. (3) Serology tests such as, enzyme linked immunosorbent assay (ELISA) and serologically specific electron microscopy (SSEM) are carried out on the leaf extract of the sweet potato plants.

Problems of Genetic Stability During pathogen Elimination Procedure

Genetic stability of vegetatively propagated plants is of primary importance or the characters of the clone will be lost. In the case of potato many examples exist of changes in plant phenotype as a result of meristem culture, these are normally chimeras that result in change of tuber skin color. It is important that testing procedures to screen such changes be carried out.

At IITA, sweet potato materials entering pathogen elimination are well characterized in terms of plant type, storage root colour (skin and flesh), growth habit and other morphological characters. The meristems regenerated plants are also evaluated for these characters while being planted in isolation room for virus indexing. Besides, a number of randomly selected varieties which are maintained in pathogen tested collections are periodically being transplanted for such evaluation. The experience in CIP using electrophoresis of soluble proteins and isoenzyme analysis with potato will also be used at IITA for the evaluation of the in vitro sweet potato collections.

In the case of potato it is well known that the formation of callus and subsequent plant regeneration will cause minor or major genetic abberations (12, 13). Sweet potato is a crop that is known to exhibit somatic mutation so care must be taken during meristem regeneration and plantlet propagation to keep callus formation to minimum, ideally complete avoidance of callus formation should be maintained in a clonal propagation program.

Routine Testing

Once plantlets have been regenerated and fully tested against known pathogens the material can then enter an in vitro pathogen tested collection. If handling procedures are appropriate it should not be possible for these plantlets to become reinfected. However, if the materials in the pathogen tested collection are to be routinely distributed internationally it is a good fail-safe procedure to routinely screen the collection once every year for known viruses. It should be remembered that in vitro does not necessarily indicate that the materials are pathogen tested, it is important therefore that pathogen tested material is clearly identified with the routine testing carried out and the types of methods used for the testing.

International Distribution of Sweet Potato Germplasm

The international movement of sweet potato germplasm is subject to quarantine regulations, there regulations vary from country to country but in most cases there exists a list of quarantine hazards for any given crop.

In vitro cultures are the most effective way of removing quarantine problems for insect pests, bacteria, fungi and mycoplasmas. The in vitro plantlets can also be tested by indicator plants and serologically against viruses and the plants are not likely to be attacked by aphids.

Applying meristem culture technique and virus testing, IITA is able to eliminate diseases from its improved sweet potato varieties. The in vitro cultures have been distributed to more than fifty countries throughout the world. The distribution is mainly by hand carry. However in same cases, the cultures are despatched via air freight by our collaborator, The Research Institute for Plant Protection (IPO), Netherlands, to the requesting national programs. Superior varieties that are adapted to different ecologies are selected by IITA collaborators in many countries and are distributed to the farmers (14).

The major drawback of exporting in vitro plantlets is that the cultures need to reach their destination in a period of less than 14 days. A successful alternative may be the production of in vitro swollen roots that would store well in the dark. However, more research is needed on in vitro storage root induction before this would become a possiblity.

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