PRODUCTION OF VIRUS-FREE BANANA PLANTLETS IN TAIWAN

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EXTENSION BULLETIN 460

November 1998

FOREWORD

Banana is one of the most important fruit crops in the region. However, production is seriously threatened by virus diseases. This important Bulletin describes how such diseases have been controlled on thousands of small banana plantations in Taiwan.

A key technology is the production of disease-free seedlings by tissue culture (TC). The Bulletin discusses the production system for TC plantlets, and the benefits and problems in using planting materials of this kind. TC plantlets are not only disease-free, they also have other benefits. They have uniform growth, and give higher yields of better quality. However, an important disadvantage is that TC plantlets are more vulnerable to herbicide damage than ordinary plants. This problem can be overcome by using mulch to control weeds. The Bulletin also emphasizes the importance of early detection and removal of diseased plants.

In many countries, advanced technology of the kind described by the authors is practiced only on large plantations. This Bulletin shows it is also effective and feasible for smallholders, provided they are given the necessary technical support. The Bulletin is based on a paper first presented at an international seminar on "Disease Management of Banana and Citrus: The Use and Management of Disease-Free Planting Materials", held in Davao City, Philippines on October 14-16, 1998.

The co-sponsors were the International Network for the Improvement of Banana and Plantain (INIBAP), the Philippine Council for Agriculture, Forestry and Natural Resources Research and Development (PCARRD), and the Davao National Crop Research and Development Center (Bureau of Plant Industry, Department of Agriculture, Philippines).

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(*Chinese Abstract*)

摘要

本文討論台灣以組織培養所生產的香蕉苗之使用狀況。香蕉組織培養計畫始於 1983 年。最初是針對香蕉黃葉病（Fusarium wilt），目前則同時用於確保植物材料不帶病毒。目前，這個計畫已生產 2,600 萬株蕉苗。文中除談及其優點外，也討論其缺點，包含對殺草劑抗性弱，與偶發的發根問題。最後，描述一個防止無毒苗在田間再度感染病毒的生產體系。

(*Japanese Abstract*)

摘要

台湾における、組織培養を用いた、バナナのウイルス無病苗の生産について述べる。組織培養計画は 1983 年に着手された。当初はバナナの Fusarium wilt 無病苗を得るために組織培養がおこなわれたが、現在ではこの方法により、ウイルス病の無病化いる。これまで生産された無病苗は 2,600 万本にのぼる。組織培養苗の長所とともに、その欠点、例えぼ除草剤によ害を受けやすいこと、不定根が出やすいことなどについて述べる。また無病苗を圃場に栽植した後の、ウイルス病感染を防ぐための耕種的対策について述べる。

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This Bulletin discusses the use of banana plantlets produced by tissue culture in Taiwan. The tissue culture program for banana was begun in 1983. Originally started to produce plantlets free of fusarium wilt, the program is now also used to ensure that banana planting materials are free of virus. A total of 26 million plantlets have been produced under the program. The advantages of TC plantlets are discussed, and also their disadvantages, such as their susceptibility to herbicide damage and occasional rooting problems. Finally, the Bulletin describes a series of cultural methods to protect disease-free plantlets from virus reinfection in the field.

Keywords : Banana, banana bunchytop, banana streak, fusarium wilt, Taiwan, tissue culture, virus

INTRODUCION

Banana is one of the most important fruit crops in many tropical and subtropical countries. The commercial cultivation of banana in Taiwan involves a large number of small growers. Fruit is produced both for local consumption and for export to Japan. In the past, when bananas were grown as an annual crop, farmers traditionally used sword suckers as planting material. Each mother plant supplied one or two suckers during the planting season from March to May. Inevitably, many important diseases, including viruses and Fusarium wilt, were readily transmitted from one crop cycle to the next.

Fusarium wilt of Cavendish bananas, caused by race 4 of *Fusarium oxysporum* Schl. f. sp. *cubense* (E. F. Smith) Snyder & Hensen, appeared in Taiwan in the late 1960s, and is now the most serious problem affecting banana production in Taiwan (Su et al. 1986). The solution was to develop a profitable rotational system with paddy rice (Hwang 1985).

The key was cheap and efficient production of clean planting material through tissue culture (TC). Fusarium wilt was the driving force leading to the development of a meristem culture technique for mass propagation of disease-free banana plantlets for commercial planting in Taiwan, beginning in 1983 (Hwang et al. 1984). Since then, micropropagation of bananas for both the rapid production of planting material and the storage and transfer of germplasm has become common all over the world.

Many viruses are known to infect banana, and cause serious losses in many countries. Viruses are usually spread from plant to plant in nature by insect vectors, but often are also transmitted over long distances and from one crop cycle to another in vegetative planting material. It is known that viruses can be readily transmitted through the tissue culture process. To produce virus-free plantlets, the source plants for tissue culture must be completely free of virus. Virus indexing technology is an essential component of virus-free seedling production.

Prior to 1990, the identification and detection of virus in bananas was largely based on observed symptoms and electron microscopy, but these methods were not reliable. Advances in biotechnological studies on banana viruses at the Department of Plant Pathology, National Taiwan University, have made possible the development of reliable and sensitive methods of detecting viruses. Use of these new virus indexing techniques has greatly improved the efficiency of tissue culture, and minimizes the risk of virus being distributed through TC plantlets.

Banana Viruses, and Methods to Detect Them

To date, viruses that have been identified as pathogens in banana (*Musa* spp.) are abaca mosaic potyvirus (AbMV), banana bract mosaic potyvirus (BBMV), banana bunchy top virus (BBTV), cucumber mosaic cucumovirus (CMV), and banana streak badnavirus (BSV). Of these five viruses, AbMV is reported to infect abaca (*Musa textilis*), which yields the Manila hemp of commerce in the Philippines. The other four cause yield losses of considerable economic importance in various banana cultivars (Stover 1972).

To establish a program for producing virus-free banana TC plantlets, we have to know the kinds of virus present in a country, so we can select a suitable method of virus detection. Detailed information on the symptoms, host range, geographical distribution and transmission of the banana viruses, can be found in the book *Technical Guidelines* for the *Movement* of *Musa Germplasm*, published by FAO/IPGRI in 1995 (Dickmann and Putter 1995).

A range of techniques can be used to detect and identify banana viruses. Methods include observation of symptoms, examination of tissues by electron microscope, the use of indicator plants, serology and nucleic acid hybridization. Each method has certain advantages and disadvantages. Modern serological detection techniques can be highly sensitive in detecting known viruses, but are also highly specific, so that they may not be very reliable if a virus has a wide range of serological diversity. For example, a recent study has shown the BBTV polymerase chain reaction (PCR) technique applied to banana bunchy top (BBTV) was able to detect six strains of BBTV isolates collected from Taiwan and Malaysia. However, another two strains, both latent strains from Malaysia, could not be detected by enzyme-linked immunosorbent assay (ELISA) (Tsao 1998).

Three banana viruses have so far been reported from Taiwan. Banana bunchy top and cucumber mosaic virus are widespread in banana production areas, and occasionally cause disease outbreaks of epidemic proportions. More recently, banana streak virus was found in a few plants of Mysore (AAB) grown in the germplasm collection farm at the Taiwan Banana Research Institute (Su et al. 1997). Although banana streak has not yet been found in Cavendish cultivars, further studies have indicated that Cavendish cultivars are even more susceptible to this virus than Mysore. Banana streak thus poses a great threat to

Taiwan's banana industry. In Taiwan, the ELISA method was developed for the detection of banana bunchy top and cucumber mosaic virus, while the PCR method is used to detect banana bunchy top and banana streak (Tsao 1998, Wu and Su 1990).

Sampling techniques are an important consideration when assaying for banana viruses. Virus concentrations and symptom expression can vary considerably from one plant to another, and even in different leaves of the same plant. Studies in Taiwan demonstrated that the concentration of banana bunchy top virus was higher in young leaves, and decreased with increasing age of the leaf (Tsao 1998). In contrast, banana streak virus was present at higher concentrations in old leaves, and less common in young ones (Fan 1998). Accordingly, samples for banana bunchy top detection should be taken from young leaves, while samples to detect banana streak virus should be taken from old leaves.

Production of Virus-Free TC Banana Plantlets in Taiwan

Since the tissue culture program began in 1983, a total of 26 million banana plantlets have been produced for commercial planting in Taiwan. The Cavendish cultivars used for propagation are "Giant Cavendish", "Tai-Chiao No. 1" and "Tai-Chiao No. 2". Tai-Chiao No. 1 is a somaclone derived from "Giant Cavendish" which has resistance to race 4 of Fusarium wilt. It was released in 1991 for commercial planting in infected areas in order to control this disease (Hwang et al. 1994). "Tai-Chiao No. 2", an introduced semi-dwarf cultivar, was released for planting in 1992. The use of TC techniques to produce banana plantlets benefits banana growers in two ways. Not only does it produce disease-free planting materials, but it can also be used for the rapid multiplication of new varieties.

The procedure for commercial micropropagation of banana plantlets consists of four stages: Culture initiation, bud multiplication, plantlet regeneration and acclimatization in the nursery. A production program producing millions of plantlets annually needs hundreds of thousands of suckers for culture initiation each year. If suckers are obtained directly from the field, they should be subject to virus indexing. Only those shown to be free of virus should be used for culture initiation. However, virus indexing such a large number of samples is time-consuming and costly. The solution was to establish virus-free foundation stock which could supply virus-free material for culture initiation. The establishment and management of the virus-free foundation stock at the Taiwan Banana Research Institute is described below.

Facility

A 32-mesh screenhouse measuring 40 m in length, 40 m in width and 3.5 m in height has double doors with an airlock at the entrance. The screenhouse is insect-proof and vector-free.

Stock Preparation

For each variety, 10-20 plants showing vigorous growth and producing excellent bunches, each an excellent true-to-type example of the variety, are selected from commercial orchards. Each plant is regarded as a "line".

For each line, 1-2 suckers are taken to the tissue culture laboratory and subjected to virus indexing for the three viruses known to be present in Taiwan. ELISA and PCR

double tests are used for BBTV and BSV, and the ELISA alone for CMV. Suckers which show a negative reaction to all three viruses are further multiplied by tissue culture method to produce 5 plantlets/sucker. Those showing a positive reaction to any one of the three viruses are discarded.

All the plantlets produced for each line (about 5-10 plantlets/line) are subject to virus indexing for a second time. Plantlets of virus-free lines are used as stock plants for the foundation screenhouse.

Management

- Plantlets are planted in a growth medium of sawdust and compost (4:1 v/v) which is free of Fusarium wilt pathogens. Irrigation is carried out with a drip system, drawing water from underground to make sure it remains free of pathogens.
- Stock plants in the screenhouse are regularly observed for symptoms of virus and Fusarium wilt, and virus indexing is conducted at three-month intervals. Any infected plant which is found is eradicated immediately. Chemical sprays to control banana diseases and insects are applied regularly. The plants receive adequate fertilizer to promote the formation of numberous suckers.

For culture initiation, only suckers from the foundation stock are used. At this stage, virus indexing is done for only one sucker from each line. Virus indexing is not necessary at the bud multiplication and plantlet regeneration stage, but should be carried out again when the plantlets reach the final nursery stage.

Commercial nurseries should be established in an area where bananas are not grown. Construction and management of a nursery is basically much the same as for the foundation stock mentioned above, except that indexing for viruses is necessary only for a certain number of plantlets. The nursery should be insect-proof and vector-free. Sanitary procedures should be strictly followed to prevent virus reinfection or the introduction of other pathogens. Plantlets should be inspected regularly to detect any off-type mutants, or any abnormal plantlets showing possible symptoms of virus infection.

Table 1 Comparison of CMV Incidence in Suckers and TC Plantlets

Variety	Kind of planting material	No. of plants surveyed	CMV (%)
Giant Cavendish	TC Plantlets	16,087	2.9
	Suckers	1,660	0
Tai-Chiao No.1	TC Plantlets	3,072	12.0
	Suckers	5,554	0.2
Tai-Chiao No. 2	TC Plantlets	13,350	0.4
	Suckers	2,340	0

1. The survey was carried out in July 1995, 2-3 months after planting

CULTIVATION OF VIRUS-FREE BANANA TC PLANTLETS

Compared to suckers, the use of plantlets grown by tissue culture (TC plantlets) has many advantages. TC plantlets are cheaper and easier to propagate and transport. They have a higher survival rate in the field. They reduce the cost of controlling foliar diseases by 50%. Their uniformity of growth makes it possible to control the time of flowering and harvesting, and give a significant increase in yield and fruit quality (Hwang *et al*.1984).

However, TC plantlets also bring some new problems. There is strong evidence that TC plantlets are more susceptible to CMV than suckers (Table 1). In some plantations where TC plantlets were grown, outbreaks of CMV with infection rates of up to 65% were observed. Outbreaks of CMV are usually associated with poor weed control, or occur in the neighborhood of vegetable crops such as bean, cucumber, and pepper which are alternative hosts of CMV.

At an early stage of growth, TC plantlets are more sensitive to herbicide than suckers. Because labor costs in Taiwan are high, weed control by hoeing is impossible. Over-use of herbicides inevitably causes some damage to the plants. Another problem is that mature plants grown from TC plantlets tend to develop "floating mat"*, which makes them likely to topple over after shooting.

To overcome these problems, we recommend the following guidelines when growers use virus-free plantlets. These guidelines are designed to keep the plantlets healthy, with a special emphasis on virus control.

Deep Planting

To prevent the "floating mat" problem, planting holes should be fairly deep (about 10-15 cm below the soil surface).

Plastic Mulch

To reduce the herbicide damage at early growth stages, a plastic mulch should be used. Plastic mulch also promotes the growth of banana plants, probably because of the higher soil moisture underneath the plastic.

Fertilization

The fertilization program for plantlets can follow the standard recommendations for commercial banana plantations, except that the first application of fertilizer should be made earlier for plantlets than for suckers. While suckers receive fertilizer about one month after planting, plantlets should be fertilized about ten days after planting. Heart rot of TC plantlets caused by boron and calcium deficiency is occasionally observed in Taiwan (Ko *et al*. 1997). In plantations where the soil is sandy and acidic, the application of borax (2 kg/ha) and lime is recommended to control heart rot disease.

INTEGRATED CONTROL OF VIRAL DISEASE

Avoid Alternate Hosts of CMV

CMV is transmitted by aphids to banana plants from weeds, and from many vegetables such as bean, cucumber, pepper, and tomato. TC plantlets should not be grown near these vegetables, and this combined with good weed control should reduce aphid populations. In vegetable production areas known to have high CMV infection potential, growers should use suckers rather than TC plantlets, since suckers are more resistant to virus infection. Suckers must be obtained from a plantation free of any symptoms of virus disease.

Use Large Plants

Larger plantlets should be used where virus is a problem. Greenhouse studies have shown that tolerance to BBTV and BSV is correlated with the size of banana plantlets (Fan 1998, Tsao 1998). For example, when plantlets 5 - 10 cm tall were inoculated with BSV, the infection rate reached 75%, while the average incubation period was 22 days. When plantlets 30 cm tall were inoculated with BSV, the corresponding figures were 50% infected, with an incubation period of 80 days. For plantlets 50 cm tall, only 25% were infected after an incubation period of 135 days.

Selection of Planting Time

In Taiwan, most banana plantlets are planted out in the field during the period from March to May. The fruit are harvested during the same period the following year. Field surveys over the past three years revealed that the incidence of CMV was consistently highest in March (8.0%), moderate in April (3.3%) and lowest in May (1.9%) (Chao, unpublished). To escape virus infection, farmers are recommended to plant TC plantlets after mid-April.

Shiny Plastic

Mulching the soil with shiny plastic fabric to repel aphids has proved to be effective in controlling virus diseases in many crops. Experiments conducted at the Taiwan Banana Research Institute (TBRI) showed that the incidence of CMV fell from 52% in control plots to 11.2% in plots with shiny plastic mulch (Chao, unpublished). Mulching is done before planting, and also serves for weed control.

Sanitation

The most fundamental rule for virus disease control is early detection and removal of diseased plants. Diseased banana plants should be sprayed with kerosene to kill aphids before the plant itself is killed with herbicide.

Conclusion

Banana production is suffering heavy losses from virus diseases in many countries. To date, five virus infecting Musa spp. have been reported: AbMV, BBMV, BBTV, CMV and BSV. These viruses can be transmitted in vegetative planting material. Successful control of virus diseases should begin with virus-free planting materials. The solution is to develop cheap, efficient production of "clean" planting material through tissue culture.

In Taiwan, Fusarium wilt used to be the most serious disease affecting banana production. As a systemic disease, Fusarium wilt pathogen can be spread through suckers, the conventional planting material used by banana growers in Taiwan. A tissue culture program for mass production of disease-free banana plantlets for commercial planting was developed in 1983. A total of 26 million TC plantlets were released to banana growers between 1983 and 1998.

Advances in biotechnological studies of banana viruses in the last decade in Taiwan led to the development of sensitive, reliable methods of virus detection. Three banana viruses are known to be present in Taiwan, BBTV, CMV and BSV. ELISA is used to detect BBTV and CMV, and PCR is used to detect BBTV and BSV. These methods were implemented recently in the banana TC plantlet production system, so that seedlings can be even more reliably certified as virus-free.

For reliable detection of banana viruses using ELISA and PCR, knowledge of the serological divrsity of a virus is important. For BBTV and CMV, a few distinctive strains have been reported, while little is known about the serological properties of BBMV and BSV (Tsao 1998). Further studies are needed.

Although tissue culture is believed to eliminate any risk that plantlets might be carrying fungal, bacterial or nematode pathogens or insect pests of banana, TC plantlets could still be carring virus pathogens if the source plants used for culture initiation are infected with virus. In a country where the banana viruses are very widespread, suckers should not be taken from commercial plantations and used directly for tissue culture. In Taiwan, a virus-free foundation stock was set up to supply the large number of suckers needed each year for tissue culture. This foundation stock is kept protected in an insect-proof, vector-free screenhouse. Plants are inspected frequently for virus symptoms, and are subject to regular virus indexing. At the final stage, when the seedlings are almost ready for distribution, the nursery used to acclimatize them should also be insect-proof and vector-free, and sanitation measures should be enforced. Regular inspection for off-type mutants, and virus indexing for those plantlets showing possible symptoms of virus

infection, are necessary before plantlets are released to growers.

After planting in the field, TC plantlets at an early stage of growth were found to be more vulnerable to herbicide damage than suckers, and more susceptible to virus infection, especially CMV. TC plantlets also have a tendency to develop a "floating mat" root system when mature. To overcome these problems, TC seedlings should be planted deeper than suckers, in a planting hole 10 - 15 cm deep, and the soil should be mulched with plastic for weed control. When TC plantlets are grown, fertilizer applications should begin early, at about ten days after planting. Integrated management practices recommended to control virus diseases include use of larger plantlets for planting, selection of a suitable planting time to excape virus infection, the use of shiny plastic mulch to repel aphids and control weeds, avoiding planting seedlings near vegetable crops known to be hosts of CMV, and early detection and removal of diseased plants.

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DISCUSSION

Participants discussed the use of TC plantlets and virus infection. It was pointed out that all banana and plantain species contain segments of the DNA of banana streak virus. The process of tissue culture can activate the integrated DNA of the virus in the DNA of the plant, so that the new plant becomes infected with virus. However, banana streak virus tends to show severe symptoms only in plants that are poorly managed and under stress. Generally, the more the plants are stressed, the more severe the symptoms and the greater the effect on yield.

There was also discussion of the difficulty of persuading farmers in the Philippines to destroy infected banana plants. Dr. Hwang suggested that farmers might benefit greatly if the Philippine government were to establish demonstration farms in the various banana producing regions. The land could be well prepared, and clean planting material used. Since banana bunchytop spreads slowly in the field, the use of tissue culture for virus-free plants is effective. The government could provide transport to local farmers so they could visit the demonstration farm. Such a scheme might be attractive to growers who rely on banana as their sole source of income. It would be less interesting for growers who have only a few plants and regard banana as a secondary crop.