Banana Micropropagation System in Taiwan

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Abstract

Banana meristem culture was first reported in Taiwan in 1972 and applied to the mass propagation of plantlets in 1983. Adventitious buds were induced from decapitated shoot apex of sucker in a modified MS medium (containing 5 ppm BA, 2 ppm IAA and 160 ppm adenine sulfate). Suckers used in micropropagation were indexed for BBTV using ELISA test. Clusters of adventitious buds were dissected repeatedly to increase bud number. Elongation and rooting of adventitious buds resulted in plantlets which after acclimatized in the screened nursery for 2 to 3 months can be supplied for planting in the field. This micropropagation system is a cooperation between Taiwan Banana Research Institute (TBRI) and the Taiwan Provincial Fruit and Marketing Cooperative. Rooted plantlets are delivered to the screened nurseries located in the major banana growing regions managed by the Fruit Cooperative. Plantlets are transplanted in plastic pots for 2 to 3 months and supplied to growers for field establishment between February and June. Micropropagated banana plantlets have higher survival rate, more vigorous and uniform growth compared to suckers. The disease-free plantlets can check the spread of Fusarium wilt and other systemic diseases (caused by BBTV or CMV) carried by the suckers. Within the last decade, a total of 15 million plantlets have been distributed for field planting. The occurrence of off-types was observed to be 2 to 3%. The cultivars propagated are Pei Chiao (Giant Cavendish), Tai Chiao No. 1 (resistant to Fusarium wilt) and Cavendish B.F. (a selected semi-dwarf cultivar). On going research is aimed at improving the quality of plantlets and to reduce production cost.

Introduction

Edible bananas do not form seeds and are propagated vegetatively. In Taiwan, farmers traditionally use sword suckers as planting material. Each mother plant can supply 1 to 2 suckers during the planting season between February and May. Thus, planting materials are in great demand during this period when most growers replant their orchards every season to adjust the harvesting period for export. However, in recent years, Fusarium wilt of banana caused by *Fusarium oxysporum* f.sp. *cubense* (race 4) has reached crisis proportions in the major banana production areas in Taiwan (Su et al., 1986). Since suckers obtained from wilt-infested orchards are important sources of inoculum, suckers used for planting must be obtained from disease-free areas. This is becoming increasingly difficult because of the widespread nature of the disease. A meristem culture technique first reported by Ma and Shii (1972) was employed to induce the formation of adventitious buds from the decapitated shoot apex of sucker and was consequently developed for the mass propagation of disease-free plantlets for commercial planting scale since 1983 (Hwang *et al.*, 1984).

Procedure for the Micropropagation of Banana Plantlets

The procedure for commercial micropropagation of banana plantlets consists of four stages: culture initiation, multiplication of adventitious buds, regeneration of plantlets and acclimatization in the nursery (Fig. 1).

Stage 1: Culture Initiation

Young suckers selected from healthy and true-to-type mother plants were checked for symptoms of fusarium wilt and indexed for banana bunchy top virus (BBTV) by the ELISA test according to the method of Wu and Su (1990).

The surface leaves were removed with a sharp knife and wiped clean with 95% alcohol. The next layers of leaves and excess corn tissue were removed to obtain a block measuring 10 to 15 cm long, 6 to 8 cm in diameter. Under aseptic conditions, superfluous tissue was removed by trimming away the tightly overlapping leaf sheaths and leaf bases, exposing the meristemic cells in between the leaf bases. The shoot tip was decapitated and a block of tissue about 1.5 cm^3 was excised and inoculated onto multiplication medium. The multiplication medium contained Murashige and Skoog (MS) salts supplemented with 0.4 mg/1 thiamine-HC1, myo-inositol and L-tyrosine both at 100 mg/1, 30 g/1 sugar and 5 g/1 agar. The amount of plant growth regulators used were 5 mg/1 BA, 2 mg/1 IAA, 160 mg/1 adenine sulfate. The pH was adjusted to 5.8 before autoclaving. The explant was incubated at 26° - 28°C with 12 hours light/dark cycle. After 4 to 5 weeks, about 15 to 25 buds would be induced.

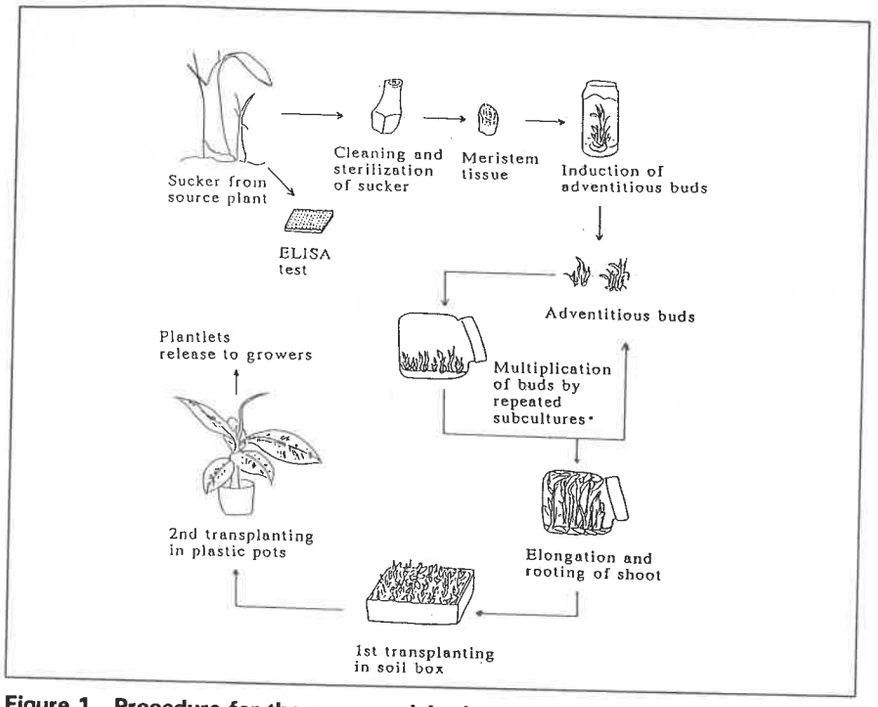

Figure 1. Procedure for the commercial micropropagation of banana plantlets comprises: culture initiation, multiplication of adventitious buds, regeneration of plantlets, and nursery stages.

Stage 2: Multiplication of Adventitious buds

The cluster of buds formed on the explant was dissected into smaller pieces and inoculated onto fresh multiplication medium. Clusters of adventitious buds were subcultured repeatedly by dividing into groups of 1 to 2 buds and the outermost overarching leaf removed to expose the meristem. Each individual bud would produce 4 to 6 buds within 4 to 5 weeks.

Stage 3: Regeneration of Plantlets

Elongation and rooting of adventitious buds was achieved by adding 60 ml of liquid regeneration medium (MS salts plus 20 g/1 sugar and 1.6 g/1 activated charcoal) directly to the established culture of adventitious buds in plastic culture flasks measuring 10 cm x 10 cm x 10 cm. Alternatively, plantlets could be regenerated by dividing the cluster of buds into pieces containing 2 to 3 buds and inoculate in agar regeneration medium containing MS salts supplemented with 0.4 mg/1

thiamine-HC1, 100 mg/1 each of myo-inositol and L-tyrosine, 2.5 mg/1 BA, 1.0 mg/1 IAA, 30 g/1 sugar, 5 g/1 agar and 0.5 g/1 activated charcoal. When plantlets have reached 5 to 6 cm tall, the culture vessel was placed in 50% shade for 2 to 3 weeks for plantlets to acclimatize to outdoor conditions prior to transplanting.

Stage 4: Acclimatization in nursery

Plantlets were removed from the culture vessel under water to minimize injury and to remove agar attached to the roots. Clusters of plantlets were separated by forceps into individuals and sorted according to size: large > 5 cm, medium 3-5 cm, small < 3 cm. Plantlets were dipped in fungicide solution (0.2% Dithane M-45 W.P.) for 5 seconds and then transplanted in soil mix at a density of 126 plantlets/box (58 x 36 x 14 cm). The potting medium used must be free from diseases or pests. Plantlets were covered with a plastic sheet for 7 to 10 days to preserve moisture. After the first new leaf and roots have emerged (about 2 to 3 weeks), the plantlets were transplanted in plastic pots (9 cm high, 10 cm in diameter). After transplanting, 3 g of Nutricote (14N-14P-14K) was added to each pot. Plantlets were maintained in the screened nursery for 1.5 to 2 months reaching 15 cm tall before planting in the field.

Discussion

Micropropagated banana plantlets are well accepted by banana growers for the following reasons. The traditional planting materials have a low survival rate at field establishment thus causing difficulties in field management. Suckers may carry pathogens which may spread diseases such as Fusarium wilt and banana bunchy top disease. Micropropagated plantlets are more uniform in plant height, convenient to handle, has a higher survival rate, and retain more healthy leaves than suckers. Orchards established with micropropagated plantlets exhibit more vigorous and uniform growth and normal fruit yield. The harvesting period was shortened from the usual 3 months to 1.5 months. Moreover, plantlets are cheaper and easier to propagate and transport than suckers. A comparison of micropagated plantlets and suckers is given in Table 1.

In the management of microprogated plantlets in the nursery, strict hygienic conditions are essential to prevent the entrance of pathogens such as fusarium wilt and aphids which may carry BBTV and CMV. Fertilizers and insecticides should be applied periodically. Variants observed at this stage should be rogued.

Table 1. Comparison of micropropagated plantlets and suckers.

Item	Plantlets	Suckers
1. Survival rate in the field	95%	70-80%
2. Labor cost for planting	NT$2,000/ha	NT$8,000/ha
3. Cost of disease and pest control	NT$5,000/ha	NT$10,000/ha
4. Percentage of fruits qualified for export	90%	70-80%
5. Yield/ha	30-40 tons	30-40 tons
6. Off-types (variants)	2-3%	none

At field establishment, plantlets are sensitive to herbicides so that the use of herbicides should be avoided. Plantlets should not be planted next to cucumber or beans to avoid the chances of infection by cucumber mosaic virus carried by aphids.

With the support and guidance from the Council of Agriculture, Taiwan Banana Research Institute (TBRI) and the Taiwan Provincial Fruit and Marketing Cooperative have jointly established the micropropagationand extension system of disease-free plantlets. TBRI is responsible for the *in vitro* micropropagation while the Fruit Cooperative manages the nurseries located in the major banana growing areas. The Fruit Cooperative will survey the market demand for the coming season and TBRI will schedule the mass propagation by collecting suckers from January to April as explant material and then increase the number of adventitious buds by subculturing 5 to 6 times and supply the plantlets to growers from February to June the following year. Members of the Cooperative can pre-register at the local offices for the number of plantlets, cultivar and desired time for field planting. About 60%-70% of the total supply falls in March and April. This uneven distribution of plantlets supplied to growers creates certain stress on the system.

The banana micropropagation system in Taiwan is established as a service to the growers in order to promote the banana industry and export trade. The disease-free plantlets are supplied to growers at a minimal fee to cover the production cost.

Within the last 10 years, a total of 15 million plantlets have been propagated for field planting (Table 2). The system is now producing over 2 million plantlets per year. The major cultivars being propagated are Pei Chiao (Giant Cavendish), Tai Chiao No. 1 (GCTCV-215-1, a tissue culture variant resistant to Fusarium wilt) and a semi-dwarf variety Cavendish B.F. The micropropagation system has been used for the rapid clonal propagation of disease resistant cultivars. In the last 3 years, a total of 4.5 million plantlets of Tai Chiao No. 1 has been propagated for planting in orchards heavily infested with Fusarium wilt to replace the susceptible cultivars. It is also used in the promotion and field trial of semi-dwarf cultivars.

Table 2. Number of banana plantlets propagated for commercial planting between 1983 and 1992.

Year	Number of plantlets	Hectarage
1983	540,000	270
1984	600,000	300
1985	280,000	140
1986	920,000	460
1987	2,000,000	1,000
1988	1,850,000	925
1989	1,412,000	706
1990	2,000,000	1,000
1991	2,800,000	1,400
1992	2,300,000	1,150
Total	14,702,000	7,351

Current research aims at improving the quality of plantlets and efficiency of the system from several approaches. With the occurrence of variants at 2 to 3% (Hwang and Ko, 1987), the amount and types of growth regulators used in the culture media will be further investigated to reduce the number of variants. Regeneration of plantlets from adventitious buds is a key step in the micropropagation system. The media and methods used in this process will be improved and adjusted to maximize the percentage of plantlets regenerated. In the nursery stages, effort will be made to search for a better potting medium to enhance the growth and development of the root system in the winter months. It is also important to devise ways and means to trap solar energy during the colder months to prevent cold damage and to accelerate the growth rate of plantlets. With the uneven distribution of work load (60 to 70% of plantlets supplied in 2 months), short term *in*

vitro storage methods will be explored to alleviate the excessive work load. Due to the shortage of manual labor and soaring wages in Taiwan, it is urgent to pursue certain degree of mechanization or automatization in the system to reduce production cost.

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