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Cover photo: Professor Edmond De Langhe, Director of INIBAP inspecting tissue-cultured bananas in a greenhouse at Tully, North Queensland.

Post-Flask Management of Tissue-Cultured Bananas

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Foreword

The banana is a very important basic food and revenue source for countries throughout the tropical world including the South Pacific.

Banana production in many of the Pacific Island countries is threatened by the fungal leaf disease black Sigatoka.

In response to this problem ACIAR commissioned a project which included a plant improvement program for the region. A selection, importation and redistribution scheme has provided banana germplasm from a range of sources including international collections and breeding programs.

The project has a high tissue culture component to enhance multiplication, progeny improvement, quarantine and distribution activities.

The results of work on methods of hardening-off tissue cultured plantlets, conducted as part of the project, are reported in this ACIAR Technical Report to enable practitioners to maximise the advantages of using the new technologies such as in vitro rapid multiplication.

G.H.L. Rothschild
Director, ACIAR

Post-Flask Management of Tissue-Cultured Bananas

Jeff Daniells and Mike Smith

TISSUE culture as a means of banana propagation has been increasing in importance since its inception about 10 years ago. However, widescale adoption of this method of propagation has been hampered by high levels (0–90%) of somatic mutations in populations derived from tissue culture and to a lesser extent by the extra care that young tissue cultured plants require compared to conventional sucker and bit material. Tissue culture holds great potential to supply uniform pest and disease free planting material for field plantings of bananas, but this will not come to fruition until the somatic mutation problem is greatly reduced. In the meantime tissue culture is playing a vital role in the transfer of clean planting material between and within countries. This booklet describes the ingredients for success for handling tissue cultured bananas from flask to field and beyond.

Deflasking Procedure

In vitro plants can be deflasked at various sizes. However, best results are usually achieved by allowing plants to grow to 50–70mm (Plate 1) with an active root system. Deflasking should preferably be done out of full sunlight in a sheltered, shaded area with high humidity.

Step 1 Flasks should be first filled with water and gently shaken to loosen the agar and plantlets from the bottom of the flask.

Step 2 The flask contents can then be carefully tipped out and washed under running water to remove the agar. If any agar remains on the plant it can promote infection with pathogens. Older plantlets need to have their longer root systems carefully disentangled.

Plate 1 *In vitro* plants ready for deflasking.

Step 3 A sterilized potting mix is required for planting into. One part peat to two parts sharp, washed sand is a good potting mix. Good drainage is the major characteristic sought in the potting mix. Sterilization of the potting mix at this stage is critical as the tissue culture plantlets are at their most vulnerable stage to infection by pathogens. The potting mix should be either steam pasteurized or treated with methyl bromide. Small pots (5 cm diameter) should be half filled with potting mix and the small plantlets put in and backfilled with further potting mix (Plate 2).

Step 4 During the potting up procedure batches of plants need to be watered in soon after deflasking and prevented from desiccating by regular misting (hand spray pack) before transferring to a humid enclosure (humidicrib). The cuticle of tissue

Plate 2 Recently deflasked tissue culture plants in 5 cm pot.

cultured plants is usually poorly developed and the stomata are not functioning properly hence the need for special attention. Also the roots developed by tissue-cultured plants are inefficient at taking up water and remain so for several days after transfer. For these reasons recently transferred plants can become water stressed when the relative humidity remains below 90%.

Step 5 Trays of plants can then be transferred to a humidicrib (Plate 3). This can be constructed by enclosing a frame in strong clear plastic with moist sand in the bottom to maintain high relative humidity. Plants need to be out of full sunlight at this stage. About 60-70% shade over the structure above the humidicrib would be satisfactory. The impact of heat build-up inside a plastic enclosure can be minimized by having a high ceiling (≈1m) to the enclosure. Tubs with plastic

Plate 3 Humidicrib capable of holding about 800 deflasked plants in 5 cm pots.

over the top can be used to handle small quantities of plants. Mist beds have generally proven unsatisfactory for establishment of banana plants with overwetting being a problem, again because of poor cuticular development. If cool nights are a problem, heated beds may be used.

Deflasking Summary

Plant tissue culture can produce large numbers of healthy, actively growing, clonal plants. Establishing these plants requires some care and specialised environmental conditions. Remember, tissue-cultured plants of any species are not like normally propagated material from seed, cuttings, rhizomes etc. and cannot be treated as such. Tissue-cultured plants are raised from a closed, sterile environment. They have been supplied with nutrients and controlled conditions to ensure maximum growth. When removed from this environment they must adjust to varying light levels, changes in temperature, reduced humidity, changes in availability of nutrients and attack by pathogens. Because they are subject to so many changes, the procedures outlines above are recommended for optimal establishment.

Greenhouse Management

Water: Plants should remain in the humidicrib for one to two weeks giving them sufficient time to have their root system established and formation of new leaves before removal from the humidicrib. Once removed from the humidicrib plants will usually require watering daily because of the restricted root system. Careful attention to watering is required at all stages.

Temperature: Generally air temperatures between 18–38°C, with an optimum 25–32°C, should be maintained.

Sanitation: Remember that pathogens thrive in a warm, moist environment that is necessary in establishing tissue-cultured plants. A high standard of hygiene is necessary when handling young tissue-cultured plants.

Lighting: Plants can be exposed steadily to greater sunlight while in the greenhouse. It is desirable for them to be adjusted to full sunlight prior to potting on (to larger pots). This can be achieved by progressively moving shadecloth or moving plants progressively to fuller sun exposure.

Fertilizer: A complete liquid fertilizer at half strength is best applied weekly while plants are in the 5 cm pot stage (stage 1 potting). After potting up to a larger pot (stage 2) a small quantity (about 1g) of controlled release complete fertilizer will give best results. The fertilizer should be kept well away from the stem of the plant to avoid 'burns' that can damage the young plant.

Potting Mix: The most important attribute of the mix should be that it is free draining. A 30 : 70 peat sand mix plus basal fertilizer is the recommended combination.

Potting Up: Potting up can proceed once the plant's root system has filled the soil in the 5 cm pot. Transfer to a 12 cm pot will give satisfactory field results later. However, larger pots or bags, while more costly and bulkier to handle, will grow a plant that requires less frequent watering in the greenhouse and during initial field establishment.

A new alternative system of deflasking directly into long tubes is quite satisfactory. One less transfer means a big saving in labour costs but more sterilized potting mix and humidicrib space is required.

Field Planting and Management

Plants are ready for field planting when about 20 to 30 cm tall (Plate 4). Smaller plants can be successfully established in the field but require ideal water management to achieve this. Mechanisation of planting is possible using machinery adapted from that used for transplanting vegetable seedlings. This is better suited to the smaller plants in 5cm pots. Tissue culture plants should be planted in a furrow to prevent the plant's corm from developing too near the surface (Plate 5). Shallow rooted plants are more subject to blowing over. As the plants grow the furrow can be filled

Plate 4 Tissue cultured plant ready for field planting.

Plate 5 Tissue cultured plants should be planted deeply in the field to prevent shallow corm formation.

mechanically as part of the weed control procedures. Further weed control is best obtained with contact herbicides using hoods on the spray wand to prevent leaf burn. If mechanisation is not possible, plants can be planted in a deep hole and

then backfilled to cover lower leaves. Shallow corms also lead to problems later in selecting followers for subsequent ratoon crops.

Young tissue culture plants do not resist dry conditions as well as plants from suckers or bits so provision needs to be made for irrigation or plantings scheduled to coincide with moister soil conditions.

Tissue culture plants have more prolific early even suckering behaviour than conventional planting material. Special attention needs to be paid to sucker management because if too many suckers are killed after bunch emergence the plant will become unstable and topple easily with the weight of the bunch. It is best to progressively kill unwanted suckers while they are still small (<30 cm high). This type of suckering behaviour does not persist into the ratoon crops. Tissue culture plants are useful for establishing planting material nurseries as they generally produce more planting pieces than from conventional material in the same time.

Tissue culture plants usually grow taller and have bigger heavier bunches than from conventional planting material. Time of harvest may be earlier or later compared to conventional planting material and will depend upon the type of conventional material used (suckers being earlier than bits), size of material (larger planting pieces being earlier) and depth of placement. Generally the larger that tissue culture plants are at planting the earlier they will be harvested.

Somatic Mutations (offtypes)

The percentage of offtypes present in a planting can vary from 0 to 100%. If a new variety has been acquired via tissue culture, offtypes can easily go unnoticed. This is particularly so with the case of untrained observers.

Detection: Offtypes can take many forms. Some of those found to date are listed in Table 1. Usually the offtype is stable but sometimes reverts to normal in the first ratoon or is unstable in its expression from crop to crop suggesting the presence of a tissue chimera. The most commonly occurring offtype is the dwarf (compare Plates 6 and 7) which is characterised by shorter height, shorter leaf stalks (petioles), a lower ratio of leaf length:breadth; retention of flowers and bracts on the stalk below the bunch (Plate 8). Also they often suffer from choke throat and have shorter fingers. The dwarf offypes are not identical to the cultivar Dwarf Cavendish – the offtypes generally suffering worse from choke throat and having shorter fingers. Various other offtypes are shown in Plates 9 to 15.

It is possible to eliminate more than half of the dwarfs present while still in the 12 cm pot stage based on the leaf length:breadth, petiole and internode lengths (Plate 17). Dwarf offtypes are more obvious if left to grow larger in big pots.

Table 1. The range of offtypes derived from tissue cultured Giant Cavendish clones in north Queensland.

Variant Characteristics
1. Dwarf approximately 2m in height similar to Dwarf Cavendish.
2. The same as 1. but with greenish pseudostem, petioles and midribs.
3. Dwarf approximately 1.8m in height with thinner pseudostem.
4. Dwarf approximately 1.8m in height with thinner pseudostem and narrow leaves.
5. The same as 1. but with upper surface of leaves having a golden sheen.
6. The same as 1. but irregular arrow-shaped leaves and fruit that do not fully fill.
7. Intermediate stature, short petioles, erect leaves;
8. Giant >3m.
9. Normal stature with irregular arrow-shaped leaves. Susceptible to *Erwinia* sp.
10. Same as 7. but with white and black spots on leaves.
11. Intermediate stature with thin pseudostem, narrow leaves and fruit with pointed tips.
12. Variegated leaf.
13. Very weak pseudostem which collapses before bunch emergence.
14. Brown streaks on pseudostem.
15. Black streaks on pseudostem and fruit.
16. Black blotches on pseudostem with irregular arrow-shaped leaves.
17. Black blotches on pseudostem.
18. Black pseudostem
19. Red blotches on pseudostem.
20. Greenish pseudostem, petioles and midribs.
21. Shiny green-yellow bracts on emerging bunch.
22. Whitish/maroon male bud.
23. Long peduncle.
24. Short fingers approximately half normal.
25. Long fingers 2 - 3 cm longer than usual.
26. Top 2 hands fail to fill out.

Good offtypes with improvement on the normal are rare but worth watching for. One offtype has been found with fruit fingers 3-5 cm longer than the normal.

Management: Most offtypes are of poor quality with limited marketing potential. Most offtypes are readily noticeable following bunch emergence, some earlier. If tissue cultures have been planted for a planting material nursery the offtypes (if undesirable) should be identified and killed before planting material digging/preparation so that they are not multiplied further. To kill unwanted plants cut and inject with kerosene. If a field planting has been established directly from tissue culture then gaps formed by culling of offtypes can be partially filled by retaining more suckers on nearby plants. It is seldom worthwhile to replant misses because competition from older neighbouring plants greatly suppresses their growth. Where offtypes are minimal the crop uniformity possible with tissue culture exceeds that generally possible with conventional planting material (Plate 18).

Plate 7 Dwarf offtype.

Plate 6 Normal plant.

Plate 8 Dwarf offtypes aften exhibit choke throat and retain flowers and bracts.

Plate 9 Offtype with golden leaves.

Plate 10 Offtype with long peduncle.

Plate 11 Short-fingered offtype.

Plate 12 Offtype of TU8 with bicolour petioles and leaf bases.

Plate 13 Offtype in which the top 2 hands fail to fill.

Plate 15 Naturally occurring offtype with severe scarring on the fruit peel.

Plate 14 Offtype with black stem and petioles.

Plate 16 Naturally occurring offtype with an incomplete bunch.

Plate 17 Tissue cultured plants ready for field planting. The dwarf offtype is on the left. Note the shorter petioles and shorter internodes and lower leaf ratio of the dwarf.

Plate 18 Very uniform bunching is possible with tissue culture.

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