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LITERATURE CITED

1. Arnold Bik, R. and Boertje, G.A. 1975. Fertilizing standards for potting composts based on the 1:1½ volume extraction method of soil testing. *Acta Hort.* 50: 153-6.
2. Barrow, N.J. 1967. Studies on extraction and on the availability to plants of adsorbed plus soluble sulfate. *Soil Sci. 104*: 242-9.
3. Cartwright, B., K.G. Tiller, B.A. Zarcinas, and L.R. Spouncer 1983. The chemical assessment of the boron status of soils. *Aust. Jour. Soil Res. 21*: 321-32.
4. Handreck, K.A. 1984. Quality of potting mixes in retail packs. *Austral. Hort. 82*: 9-24.
5. Lindsay, W.L. and W.A. Norvell 1978. Development of a DTPA soil test for zinc, iron, manganese and copper. *Jour. Amer. Soc. Soil Sci. 42*: 421-8.

THE USE OF TISSUE CULTURE IN THE SEARCH FOR PANAMA DISEASE RESISTANT CLONES OF BANANA

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The banana is one of the most important fruit crops in Queensland with a gross annual value of $45M in 1984. According to Simmonds (20) all current banana cultivars have been derived from two species. They are *Musa acuminata*, which is the source of the "A" genome, and *Musa balbisiana*, which is the source of the "B" genome. Commercial cultivars are usually seedless triploids and tetraploids comprising various combinations of these two genomes.

Panama disease, also known as fusarium wilt, is caused by *Fusarium oxysporum* Schlecht ex Fr. f. sp. cubense (E.F. Smith) Snyd. & Hans. This disease has been known for a long time in Queensland where it is the major limiting factor in the production of the 'Lady Finger' (AAB group) banana.

The first world recording of fusarium wilt in bananas was made by Bancroft in Queensland in 1874 (1). He found the disease to be prevalent in the Brisbane district and noted that the 'Sugar' (AAB group) banana was most susceptible and the 'Dwarf Cavendish' was not affected. Tryon (26) when describing wilt of 'Sugar' and 'Gros Michel' (AAA group) (the latter being introduced into Queensland in 1910) stated that the Cavendish banana either "escapes its onslaught altogether" or is highly resistant. Subsequently 'Lady Finger' was found to be much less susceptible than 'Sugar' (Purss, unpublished); however, the disease has been devastating in 'Lady Finger' and has forced many growers to replant with resistant 'Cavendish' (AAA group) cultivars.

Purss (18) found a disease resembling Panama disease on three 'Williams' ('Giant Cavendish') plants at Woongoolba in southern Queensland. An unidentified *Fusarium* sp. was isolated and proved to be pathogenic to 'Lady Finger' and 'Williams' but, in the same experiment, Purss found that *F. oxysporum* f. sp. *cubense*, isolated from 'Lady Finger', did not attack 'Williams' but produced symptoms typical of Panama disease in 'Lady Finger' plants.

In a field experiment at Nambour in southern Queensland (17) a number of cultivars were planted into a site where 'Lady Finger' plants had been devastated by Panama disease. 'Lady Finger', 'Mysore' (AAB group), and 'IC2' (AAAA group) were susceptible, but 'Mons Mari' ('Giant Cavendish'), '2390-2' (AAAA group), and 'Bodles Altafort' (AAAA group) were not affected.

During 1976, symptoms resembling Panama disease were detected in 'Mons Mari' plants growing at Wamuran in southern Queensland. In 1977 Peterson (pers. comm.) isolated *F. oxysporum* from these plants and found, in limited pathogenicity tests using suckers, that the isolate did not attack 'Williams' bananas. He concluded that a new race of the fungus had not evolved and suggested that the resistance in the 'Mons Mari' plants had broken down under unfavourable soil conditions. During 1981, Mayers (pers. comm.) provided conclusive evidence that a new race of *F. oxysporum* f. sp. *cubense*, capable of attacking Cavendish cultivars, had evolved in Queensland. Fusarium wilt of these cultivars has now appeared in fifty plantations between Caboolture and Eumundi in southern Queensland. As Cavendish cultivars represent the majority of commercial plantings in Australia, it now appears that the Australian banana industry is threatened by Panama disease. There are reports of Cavendish cultivars succumbing to fusarium wilt in Canary Islands, Taiwan, Natal, Jamaica, and Central America (21, 22, 27), but these outbreaks were

considered to be due to adverse growing conditions or excessively high inoculum levels in the soil overcoming host resistance, rather than a new race of *Fusarium*. However, the outbreak of Panama disease in Cavendish cultivars in southern Taiwan has subsequently been attributed to Race 4 of *F. oxysporum* f. sp. *cubense* (23). There are also indications that a new race capable of attacking Cavendish cultivars has evolved in South Africa (B.Q. Manicom, pers. comm.) and Philippines (D. Littman, pers. comm.) Four races of the pathogen have been defined. Race 1 attacks AAA triploids such as 'Gros Michel'; race 2 is pathogenic to certain ABB triploids such as 'Bluggoe'; race 3 causes wilt of Heliconia spp.; and race 4 attacks Cavendish cultivars.

Thus there is a need to produce Cavendish cultivars that are resistant to the new race of fusarium wilt. As plant breeding is limited by the scarcity of seeds in edible bananas (13) we are studying the potential of tissue culture for the production of natural and induced variation, and subsequent selection of disease-resistant plants.

There have been a number of reports on tissue culture of banana and successful regeneration of plantlets. Berg and Bustamante (2) and Bower and Fraser (3) have described techniques for meristem culture of lateral bud apices for removal of pathogens from Cavendish cultivars. The first report of rapid multiplication of banana cultivars using tissue culture was that of Ma and Shii (11, 12). They cultured shoot apices from suckers on both agar-based media and liquid media. Subsequently, various techniques and media for the rapid multiplication of bananas via shoot-tip culture from suckers have been described by de Gusmán and Tolentino (7), Cronauer and Krikorian (5), Hwang, Chen, Lin and Lin (10) and Swamy, Sriniv and Chacko (24). Hwang *et al.* (10) reported the production of one million pathogen-free plants in 1983 to prevent the spread of fusarium wilt in commercial plantations in Taiwan.

Callus cultures have been produced from sections of banana fruit (6, 15, 25); however there have been no reports of organogenesis or production of plants from this callus. Cronauer and Krikorian (4) have reported somatic embryogenesis using 2,4,5-T in their growth medium.

Genetic variability has been observed in banana plants produced by rapid multiplication of shoot-tip cultures *in vitro*. Reuveni, Israeli, Degani and Eshdat (19) noted three common mutants in Israel. They were dwarfed plants; plants with thick curled leaves with streaks similar to mosaic virus infection; and a mutant characterised by reddish colour of the leaves

and petioles. In plantings of 'Grand Naine', 7.2% of plants were identified as off-types and, with the cultivar 'Williams', 9.3% of the plants were mutants. In Taiwan, where multiplication is via culture of shoot apices of banana suckers, 3% of plants in field plantings have been identified as off-types (28). In Alstonville, Australia, plants were produced from inflorescence-section cultures. Ten percent of the resultant plants were observed as off-types in large field plantings (Turner, pers. comm.). Off-types observed were variations in leaf type and thickness, variegated and chlorophyll deficient leaves, and dwarf plants.

There have been some reports on the use of mutagenic agents to produce variability in bananas. Menéndez (14) used ethyl methane sulphonate on seeds of *M. acuminata*. De Guzmán, Decena and Ubalde (8) treated shoot-tip explants of 'Lacatan' with gamma radiation. Low dosage (1.0 Kr/hr) was stimulatory to bud formation and high dosage (10.0 kr/hr) was lethal. A highly proliferating tissue strain was isolated from a culture of an irradiated explant. Epp (pers. comm.) has added fusaric acid and fungal filtrates to tissue cultures of Cavendish clones in an attempt to distinguish levels of resistance between clones.

We are using plant tissue culture in an attempt to produce Cavendish clones resistant to race 4 *Fusarium* wilt. This involves the development of screening techniques to identify resistant clones. We are also assessing other banana cultivars for possible sources of resistance to Panama disease. Our approach to this work is presented in the summaries below.

Multiplication of Shoots from Cultures of Apical Tips of Inflorescences in Cavendish Clones. Explants were taken from inflorescences because similar cultures produced more variation in field-grown plants than those cultured from sucker explants in Alstonville (Turner pers. comm.). Initial explants consisted of apical tips from inflorescences of cultivar 'New Guinea Cavendish', removed when the fruits were mature green and ready for harvest. Apical tips (1 cm in length), with bracts and flowers removed, were cultured in 250 ml Erlenmeyer flasks on a horizontal orbital shaker at 120 rpm. They were transferred monthly into a fresh solution containing Murashige and Skoog (MS) salts and vitamins (1972) plus (per litre) 5 mg BAP and 20 g sucrose. After six months, the explants had doubled in size and had developed small lateral buds. At this stage, they were bisected longitudinally and placed either into liquid medium on a roller drum at 4 rpm or onto agar-based medium (8 g/l) of similar composition as used previously.

Multiplication rates were higher in the liquid medium than on agar-based medium. For the next 18 months, resultant multiplying shoots were sub-cultured monthly onto fresh medium. Rooted plantlets were then produced on a medium containing MS salts, 0.1% activated carbon, and (per litre) 20 g sucrose and 8 g agar, before being transferred to a steam-sterilized potting mix of peat and perlite.

Of the first 800 plants produced 8 have survived two root dip inoculations in race 4 Fusarium at an inoculum density of 1 million conidia per ml. These, and other surviving plants, will be planted in a field where outbreaks of race 4 *Fusarium* have occurred.

This work is now being repeated with cultivar 'Williams' (Figure 1).

Figure 1. Rooted plantlets of 'Williams' bananas on a medium containing M.S. salts and vitamins plus (per litre) 20g sucrose and 8g agar.

In vitro Propagation of Other Banana Cultivars from Suckers. Twenty-two cultivars representing six genomic types have been cultured from shoot tips isolated from suckers on a modified de Guzman, *et al.* (8) medium containing (per litre) 5 mg BAP and 0.1 mg IBA: The survival rate for these explants in culture was much higher (with all cultivars) when the apical dome was not removed. Cultivars varied widely in their response to different growth regulators in terms of multiplication rates, shoot elongation, root growth and development. These results have been discussed more fully in a paper by Wong (in press).

Plantlets of the diploid SH-3362 and the tetraploid SH-3436 have been obtained from Dr. P. Rowe in Honduras and are being multiplied in tissue culture. SH-3142, the parent of both these cultivars, is reported to be resistant to race 4 *Fusarium* and we are hopeful that this resistance will be found in its progeny.

All these cultivars are being screened for resistance to Fusarium.

Callus Culture of Banana. Callused leaves and stems were produced on banana shoots when 2μM 2,4-D was added to the multiplication medium of freshly sub-cultured shoots of cultivar 'New Guinea Cavendish', (Figure 2). Sections of callused leaf and stem were removed and grown on a number of media. Mineral formulations (B5 and M5) of Gamborg (1982) have been compared with MS and half-strength MS salts. Various concentrations of BAP, kinetin, and 2,4-D have been used in these media. Best growth of this tissue has been obtained on a medium containing MS salts and vitamins plus (per litre) 20 μM BAP and 2 μM 2,4-D. Over 6 to 12 weeks, these cultures produced compact nodular masses of tissue, (Figure 3). A major limiting factor to the growth of this tissue was the production of a black phenolic exudate which often prevented growth after a few weeks. A number of additives have been used in an attempt to overcome this problem. Best results have been obtained with citric acid (75 mg/l) and ascorbic acid (50 mg/l), PVP (0.01%) and activated charcoal (0.5%); however, only a partial reduction of exudate has been achieved. Further reductions of exudate have been achieved when the tissue has been emersed in stationary liquid culture medium, and when the cultures have been incubated in darkness.

Small green nodules which subsequently developed into shoots have been formed on this tissue when placed on a roller drum at 4 rpm in solution containing MS salts and vitamins and 20 g/l sucrose. Rapid initiation and growth of roots has occurred in these cultures with shoots when 0.5% activated carbon has been added to the solution.

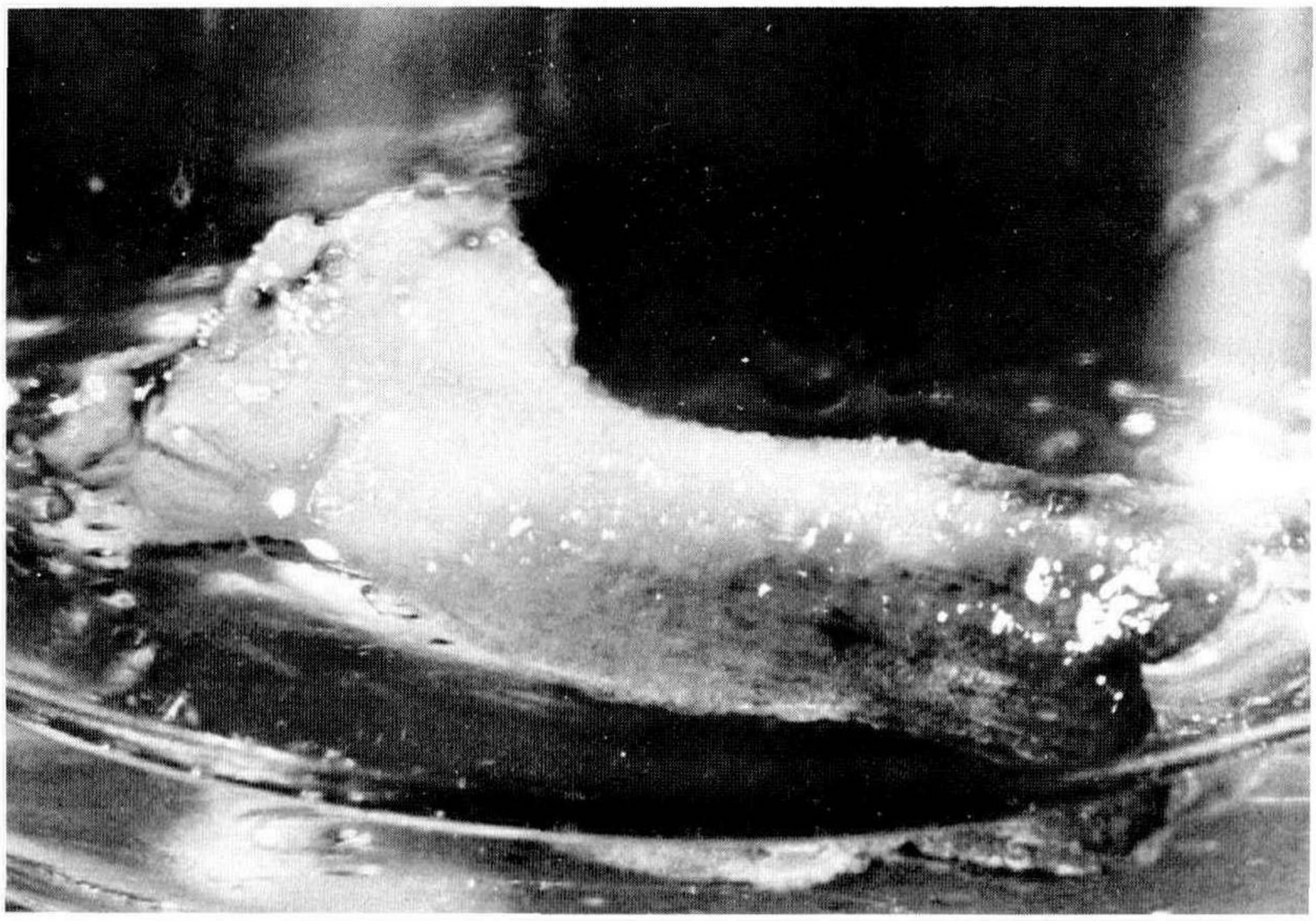

Figure 2. Callused leaf of 'New Guinea Cavendish' produced when 2μM 2,4-D was added to the multiplication medium.

Figure 3. Compact nodular masses of tissue on medium containing M.S. salts and vitamins plus (per litre) 2μM 2,4-D and 20μM BAP.

The Use of Culture Filtrates and Gamma Radiation. *Fusarium oxysporum* f. sp *cubense* was cultured on liquid medium and its metabolites (principally fusaric acid) used *in vitro* to select for resistance to these toxins. The supernatant from fungal filtrates was passed through a millipore filter and was then incorporated in or used to flood the multiplication medium. Surviving plants were grown for three generations on the toxin-amended multiplication medium prior to being tested in the glasshouse and field. More than 95% of adventitious buds which were developed from a 'Williams' plantlet, died when cultured on this medium. Although we do not have any evidence that *Fusarium* overcomes host resistance through the actions of these toxins on host tissue, and very weak evidence that toxins contribute to the fusarium wilt syndrome, we are hopeful that surviving plants will be resistant to the wilt pathogen.

Multiplying shoot cultures have been treated with low dose gamma radiation in an attempt to produce useful off-types.

Screening Techniques. In banana, *F. oxysporum* f. sp *cubense* gains entry into the xylem elements of the adventitious roots. It then spreads into the rhizome stele and invades the elements of the pseudostem. In resistant banana cultivars the infection is checked within the roots or rootlets or at the root bases. With tissue-culture plantlets the juvenile roots are unable to preclude the fungus from the rhizome stele and any defence reaction has to take place in the rhizome. This may not occur if undifferentiated vascular elements are present in the rhizome. Therefore, with these plantlets inoculum density greatly influences symptom development. Theoretically these plantlets should be able to express resistance if a very low inoculum density is used. In our experiments we have been using a density of 300,000 conidia per ml, but we plan to experiment with a series of inoculum dilutions. Research workers in other countries believe that Panama disease resistance testing can be done only in the field and that glasshouse testing does not give a true indication. In field screening, a susceptible cultivar is usually grown for a full growth cycle on the trial site as a means of building up the inoculum level in the soil, to fully test plants for resistance. Thus we intend to use a disease nursery to field test any plants which survive initial screening in the glasshouse.

LITERATURE CITED

1. Bancroft, J. 1876. Report of the board appointed to enquire into the causes of disease affecting livestock and plants. Queensland, 1876. In: Votes and Proceedings 1877. 3:1011-1038.

2. Berg, L.A. and Bustamante, 1974. Heat treatment and meristem culture for the production of virus-free bananas. *Phytopathology* 64:320-322.
3. Bower, J.P. and Fraser, C. 1982. Shoot tip culture of Williams bananas. *Subtropica*. 3:13-14.
4. Cronauer, S.S. and Krikorian, A.D. 1983. Somatic embryos from cultured tissues of triploid plantains (Musa 'ABB') *Plant Cell reports*. 2:289-291.
5. Cronauer, S.S. and Krikorian, A.D. 1984. Rapid multiplication of bananas and plantains by in vitro shoot tip culture. *HortScience* 19:234-235.
6. de Guzmán, E.V. 1975. Project on production of mutants by irradiation of in vitro cultured tissues of coconut and bananas and their mass propagation by the tissue culture technique. In: Improvement of Vegetatively Propagated Plants Through Induced Mutations. Int. At. En. Agen., Tech. Doc., 173, Vienna. 53-74.
7. de Guzmán, E.V. and Tolentino, E.G. 1978. Hormonal control of growth and shoot formation in abaca tissue culture. In: Proc. of the Sci. Meet. of Crop Sci. Soc. of the Philippines May 11-13. 1-6:
8. de Guzmán, E.V., Decena, A.C. and Ubalde, E.M. 1980. Plantlet regeneration from unirradiated and irradiated banana shoot tip tissues cultured in vitro. *Phil. Agr.* 63:140-146.
9. Gamborg, O.L. 1982. Callus and cell culture. In: Plant Tissue Culture Methods. Ed. Wetter L.R. and Constabel F. 1-9.
10. Hwang, S.C., Chen., C.L., Lin, J.C. and Lin, H.L. 1984. Cultivation of banana using plantlets from meristem culture. *HortScience* 19 (2):231-233.
11. Ma, S.S. and Shii, C.T. 1972. *In vitro* formation of adventitious buds in banana shoot apex following decapitation. *Jour. Hort. Soc. China*. 18(3):135-142.
12. Ma, S.S. and Shii, C.T. 1974. Growing banana plantlets from adventitious buds. *Jour. Chinese Soc. Hort. Sci.* 20(1):6-12.
13. Menéndez, T. 1973a. Application of mutation methods to banana breeding. In: Induced mutations in vegetatively propagated plants. Int. At. En. Agen., Vienna. 75-83.
14. Menéndez, T. 1973b. A note on the effect of ethyl methane sulphonate on *Musa acuminata* seeds. In: Induced mutations in vegetatively propagated plants. Int. At. En. Agen., Vienna. 85-90.
15. Mohan Ram, H.Y. and Steward, F.C. 1964. The Induction of growth in explanted tissue of the banana fruit. *Can. Jour. Bot.* 42:1559-1579.
16. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15:473-497.
17. Nicolson, M.E. 1969. Clonal testing for panama. Proc. Pacific-Asian Banana Cultural Exchange. Aust. Ag. Council.
18. Purss, G.S. 1953. A disease in Williams Hybrid banana produced by *Fusarium* sp. *Qld. Jour. Ag. Sci.* 10:126.
19. Reuveni, O., Israeli, Y., Degani, H. and Eshdut, Y. 1984. Genetic variability in banana plants multiplied via in vitro techniques. Int. Board for Pl. Gen. Res. Meet., Leuren University, Belgium. 1-8.
20. Simmonds, N.W. 1962. The evolution of the edible bananas. In: The Evolution of the Bananas, by N.S. Simmonds. Longmans, London.
21. Stover, R.H. 1972. Banana, plantain, and abaca diseases. Comm. Mycol. Inst., Kew, Surrey, England. 316.

22. Stover, R.H. and Malo, S.E. 1972. The occurrence of Fusarium wilt in normally resistant 'Dwarf Cavendish' banana. *Plant Dis. Rep.* 56:1000-1003.

23. Sun, E.T., Su, H.T. and Ko, W.H. 1978. Identification of *Fusarium oxysporum* f. sp. *cubense* race 4 from soil on host tissue by cultural characters. *Phytopathology* 68:1672-1673.

24. Swamy, R.D., Sriniv, A.R. and Chacko, E.K. 1983. Tissue culture propagation of banana. *Sci. Hort.* 18:247-252.

25. Tongdee, S.C. and Boon-Long, S. 1973. Proliferation of banana fruit tissue grown in vitro. *Thia Jour. Agric. Sci.* 6:29-33.

26. Tryon, H. 1912. Natural enemies of bananas in Queensland. In: The Banana in Queensland, by A.J. Boyd. Govt. Printer, Brisbane.

27. Waite, B.H. 1977. Inoculation studies and natural infection of banana varieties with race 1 and race 2 of *Fusarium oxysporum* f. sp. *cubense*. *Plant Dis. Rep.* 61:15-19.

28. Wong, W.C. 1985. Report on visit to Taiwan, 16 January-23 January 1985. Report to Qld. Banana Board.

PROPAGATION OF ORNAMENTAL RAINFOREST PLANTS

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Any discussion about rainforest plants generally leads to some disagreement about which plants are truly rainforest species and which are not. The distinction is not as clear as one might imagine because some species, for instance *Lophostemon confertus* (better known as *Tristania conferta*), are prominent in some rainforests and can be equally prominent in some eucalypt forests.

The "Language of Botany" defines rainforest as "a closed community dominated by trees which form a two or more layered dense canopy in which lianes and epiphytes are usually conspicuous with a lower sparse assemblage of small trees, shrubs and herbs, including ferns".

Other definitions also include orchids, palms, wide-leaved forbs such as philodendron relatives, ginger relatives and bananas, special plant modifications such as trunk buttresses and leaf drip tips, and an absence of grasses, annual herbs, eucalypts, and acacias.

Rainforests are widespread in tropical and sub-tropical lands or parts of those lands which receive a fairly continuous and high rainfall. Rainfall is more important to the development of rainforest than soil type or soil fertility, although good soil drainage is usually an important factor. Provided topogra-