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TISSUE CULTURE OF INDUSTRIAL CROPS

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I have chosen to speak on tea, coffee, oil palm, black pepper, and cocoa. Plantek has commercial tissue culture experience in all these crops. The economic importance of these plants to Asia and the Pacific region can be seen in Table 1 which shows that three quarters or more of world production of oil palm, tea, and black pepper come from this region.

Table 1: Some Important Industrial Crops in Asia and the Pacific Region.

	Production (1000 t)			Export (million US$)	
Crop	World	Asia-Pacific		World	Asia-Pacific
Coffee	5897	798 (14%)		9639	930 (10%)
		Indonesia	327 (41%)		
		India	170 (21%)		
Tea	2247	1641 (73%)		1844	1230 (67%)
		India	656 (40%)		
		China	451 (27%)		
Palm (oil)	7420	5642 (76%)		294	216 (74%)
		Malaysia	4000 (71%)		
		Indonesia	1148 (20%)		
Black Pepper	163	122 (75%)		215	159 (74%)
		Indonesia	45 (37%)		
		India	28 (23%)		
Cocoa	1739	155 (9%)		2051	188 (9%)
		Malaysia	101 (65%)		
		Papua	36 (23%)		

Production data from: Regional Office for Asia and the Pacific (RAPA), FAO Bangkok, Publication: 1986/14;

Export data from: United Nations Yearbook of International Commodity Statistics, 1985.

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Cultivation on a plantation scale requires a stable supply of a large numbers of uniform and healthy planting material. Clonal propagation is therefore one of the most direct applications of tissue culture to plantation agriculture. As an example, I would like to quote from our experience with tea. We have estimated that starting with 500 axillary buds, a total of three to four hundred thousand plants could be ready in less than two years for 20 ha of planting, by means of tissue culture. The traditional method of single-node leaf cutting would take seven years to produce two hundred thousand rooted cuttings sufficient for planting 15 ha. Despite such potential benefit, however, plant tissue culture has, so far, been under-utilized in the production of tropical industrial crops. A survey was made by Dr. Irwin Chu (2) on 205 commercial tissue culture laboratories world-wide. He found only 6% of these to be working on tropical plantation crops as compared to 58% on orchids. Obviously, there can and will be wider commercial applications of tissue culture techniques to the tropical industrial crops.

TISSUE CULTURE OF FIVE TROPICAL INDUSTRIAL CROPS

I will first discuss four industrial crops that are relatively amenable to tissue culture methods. They are: oil palm, coffee, tea, and black pepper. The rationale for tissue culture and the basic methods will be looked at. I will then discuss cocoa as an example of a difficult crop to tissue culture.

Oil Palm—*Elaeis guineensis*

Oil palm is the most productive crop for edible oil yielding 5 t/ha/yr compared to less than 1 t/ha/yr of the annuals (6). At present, oil palm planting material is all raised from "hybrid" seeds; vegetative propagation is unknown. Success in clonal production was first reported by Unilever in 1974 and by IRHO in France in 1976. Current plantlet production by tissue culture is about 0.5 million against current world demand of about 100 million (6). To date, all successful work has been achieved by the process of somatic embryogenesis from calli derived from various explants such as roots and young leaves.

Tea—*Camellia sinensis*

Tea is a natural outbreeder. Traditionally, large scale planting is based on single leaf bud cuttings obtained from selected bushes. However, research in Kenya shows that elite tea bushes are very rare—only one bush in 400,000 combines plant vigour and the right tea making properties (8). Therefore, *in vitro* clonal propagation as a potential means to multiply elite cultivars is now receiving considerable attention.

Two methods are used at Plantek to micropropagate tea plants. These are bud enhancement and somatic embryogeny. Bud enhancement involves increased axillary branching using lateral

and terminal buds. In somatic embryogeny, embryoids are obtained from calli originating from various explants such as cotyledons from mature seed and shoot tips, or leaf lamina from *in vitro* shoot culture.

Coffee—*Coffea* spp.

The genus *Coffea* comprises about 70 species of which *C. arabica* accounts for 70% of the world coffee trade. Two of the main breeding objectives to improve this species are, (1): to introduce rust resistance from *C. canephora* and; (2) to obtain beans without caffeine from *C. bengalensis*. However, of all the species *C. arabica* happens to be the only self-pollinator, which makes it difficult to introduce new traits by sexual means. As an alternative, somatic hybridization through protoplast fusion is being seriously considered. In the case of the coffee plant then, *in vitro* culture is not only a method for propagation but also a possible means for plant improvement.

At Plantek, the two main *in vitro* methods used are shoot multiplication and somatic embryogenesis. In shoot culture, excised shoot tips and nodes were cultured on Murashige-Skoog (MS) medium supplemented with BA. Microcuttings from these explants were harvested and subcultured at regular intervals. In somatic embryogenesis, embryoids were obtained after eight weeks on MS medium supplemented with IBA and BA.

Black Pepper—*Piper nigrum*

Black pepper is a tropical climber. Traditionally it is vegetatively propagated by stem cuttings with six nodes from vines. Current cultivars are all susceptible to *Phytophthora* spp. foot rot. Very active breeding programmes for foot rot resistance are now taking place. Tissue culture will be a very useful tool for rapid clonal propagation of resistant cultivars for replacement planting when they become available.

At Plantek, pepper has been successfully multiplied by adventitious shoot formation when seedlings and embryos are cultured on a full MS medium supplemented with cytokinin. Using mature plants as an explant source has proved to be more difficult. We are now trying to overcome excessive production of phenolic compounds and of mucilages by the explants.

Cocoa—*Theobroma cacao*

Research on cocoa since the early 1950s has shown that this species is difficult to tissue culture as, indeed, are many other woody plants. Although abundant calli could be formed from most explants, organized development from them has not been possible. Recently, Litz (4), showed that axillary buds could be induced to proliferate from shoot tips and nodes but further growth could not be sustained. He also showed that callus derived from leaf discs could form somatic embryoids at low frequency on media having

high levels of cytokinin and activated charcoal. These embryoids grew from globular stage to the late heart stage but not any further. At Plantek, we are testing if micrografting could rejuvenate the scion to make them more amenable to tissue culture handling.

COMMERCIAL PRODUCTION BY TISSUE CULTURE

Commercially, tissue culture has been widely accepted as a viable means to achieve rapid propagation of desirable clones in large numbers, to produce uniform and disease-indexed planting materials, to carry out unseasonal production, to maintain and to move clean germplasm, and to manipulate phenotypes such as the production of juvenile and compact growth form. Tissue culture is also now considered to have commercial potential as a delivery system for genetically engineered products and for heterozygous products for hybrid seed companies.

As a result of economy of scale, automation, and other improvements in efficiency, production cost in tissue culture has not gone up as much as the cost of conventional production (7). Although this conclusion is drawn from large scale tissue culture production for horticultural crops in the United States, we can reasonably expect this to also hold true for tissue culture production of the tropical industrial plants.

Commercial application of tissue culture is not without its fair share of problems. Some of the difficulties are: production scheduling, seasonality of demand, high labour cost, and product variations. The most commercially undesirable of these problems is perhaps variation. Although it is unclear what causes genetic variation in tissue-cultured plants, it is still possible to contain the problem as the two following examples from tropical plantation crops will show.

The first example comes from the banana plant. Tissue-cultured plants grown in Jamaica resulted in as much as 30% off-types. In this case no explanation could be given since the plantlets were obtained from adventitious buds arising from corm tissue, in a similar way to plants produced conventionally from suckers (5). On the other hand, variation has not been a problem to other growers (Mohamed Aaouine, personal communication). It is now considered that variation in banana tissue culture may be controlled by keeping a low ratio of the number of plantlets to be produced to the number of explants used.

The second example comes from oil palm tissue culture laboratories in Malaysia using root tips as explants. In this case, sterile fruit bunches were produced by some clones (3). Since oil palm trees do not bear fruits until they are three years old, this problem has serious economic consequences. It now appears that this problem may be related to the type of explants used because tissue culture systems using young leaves as explants have had no

problem with sterility according to Indonesian scientists (1). Recently in Singapore, at a plant biotechnology conference, Dr. Eeuwens of Unilever showed that the problem of abnormal flower development in the tissue culture system using root tips could be epigenetic, i.e. non-hereditory and reversible. Some of the initially sterile clones are now reverting back to fertility. The problem of variation may also be related to the duration of callus in culture. In general, if a tissue culture procedure involves the callus stage, this phase is preferably maintained as short a time as possible to reduce the chances of variation.

CONCLUSIONS

I would like to say that at Plantek, we are also interested in other industrial crops. The following plants may be of common interest to Australia. First, macadamia nut, which is indigenous to Australia. Second, cashew nut, of which you have maintained a good germplasm collection at Darwin and Cairns. Third, *Calamus* spp., collectively known as the rattans, which must be abundant in your tropical rainforests and, lastly, tree species of *Acacia* and *Eucalyptus* which are now regarded as important plants for fuel-wood and agroforestry.

Abbreviations:

BA: 6-benzylaminopurine
GA_3: gibberellic acid
IBA: indole-3-butyric acid
MS: Murashige and Skoog (1962).

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