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RECENT DEVELOPMENTS IN CELL AND TISSUE CULTURE OF OIL BEARING PALMS

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CONTENTS

Clonal Propagation	2
Genetic Stability	4
Protoplasts	5
Embryo Culture	5
Cryopreservation	6
Anther Culture	6
Biochemical Studies	7
Concluding Remarks	7

RECENT DEVELOPMENTS IN CELL AND TISSUE CULTURE OF OIL BEARING PALMS*

ABSTRACT

About a dozen plant species account for more than 90% of the world's production of vegetable oils and fats. The important oil producing species include two palms: coconut and oil palm. Their yields are much higher than other oil producing species, averaging 2.6 and 5 tonnes/hectare/year respectively. Both these crops are not amenable to vegetative propagation by conventional horticultural methods. Furthermore, in common with other perennials, crop improvement in these crops is a slow process. Novel methods of crop improvement could clearly be useful adjuncts to conventional methods in palms.

This paper primarily highlights recent achievements in cell and tissue culture of palms. Potentially far-reaching consequences of clonal propagation are discussed. Besides clonal propagation, the prospects of utilising embryo culture, protoplast, cryopreservation and anther culture methods in crop improvement of palm are also considered.

More than 90% of the world's production of edible vegetable oils and fats is obtained from about a dozen plant species. Most of the oil producing plants cultivated in the temperate regions are annuals. In the tropics, the important oil producing crops are two palms: oil palm *(Elaeis guineensis* Jacq.*)* and coconut (*Cocos nucifera* L.)

World production of the major edible oils in 1984 is summarised in *Table 1*. The annuals account for about 66% of world production whilst oil palm and coconut together account for 22%. In terms of yield, however, oil palm is the most productive (5 tonnes/hectare/year), followed by coconut (2.6 tonnes/hectare/year) and the annuals (all less than 1 tonne/hectare/year).

Breeding progress in the annuals has been rapid, largely because of the short generation intervals and limited land requirements for trials. Nevertheless, significant improvements in yield through breeding and selection have been made in both oil palm and coconut. In this respect, the discovery of the mode of inheritance of shell-thickness in oil palm and the introduction of coconut hybrids might be considered as major developments.

Indeed palm oil and palm kernel oil are expected to become the world's major fat and oil export, estimated to reach 44% of world production by the year 2000 (Mielke, 1985). The projected increase is expected to be due to (a) the high profitability of oil palm leading to increased cultiva-

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tion in Malaysia, Indonesia, South and Central America and West Africa and (b) continuing rise in palm oil yields due to improved cultivation techniques, and the introduction of new genotypes including *in vitro* propagated clones from 1990 onwards. In coconut it is very likely that replanting of old stands with higher yielding hybrids currently in progress in several countries will contribute significantly in maintaining its status as a major oil crop.

The application of modern methods of biotechnology to crop improvement will seem particularly relevant and even realistic in the context of progress in tissue culture of palms that is now evident. This paper briefly summarises progress in tissue culture research in oil palm and coconut and examines areas of research that may provide a basis for improvement of both palms.

CLONAL PROPAGATION

An overview

Intensive research programmes on tissue culture of palms of economic importance were started in the sixties. The first successes were reported in oil palm around the mid-seventies by two groups: Uniliver in England (Jones, 1974) and IRHO in France (Rabechault and Martin, 1976). Being commercially oriented both groups have not freely published their research findings pertaining to cloning of oil palm. Nevertheless, several other groups have now independently developed methods for propagation of oil palm and there are probably a dozen laboratories around the world now capable of cloning oil palm. Current production of plantlets is estimated at 0.5 million a year, against current world requirements of 100 million for newplantings and replantings (Rajanaidu, 1985).

Coconut tissue culture research has been intensivey studied at Wye College and in the Philippines and more actively in recent times in India. Progress has been less spectacular in relation to oil palm, though reports of plantlet regeneration have recently appeared (*e.g.* Branton and Blake, 1983; Raju *et. al.*, 1984; Bhaskaran, 1985).

Current planting materials

Present day oil palm and coconut planting materials are largely if not entirely raised from hybrid seeds. Oil palm seeds are obtained from crosses between dura palms, the fruits of which have thick shells surrounding the seeds and pisifera palms, in the fruits of which the shell is absent. Shell thickness is controlled by a single gene, both the dura and pisifera genotypes being homozygous respectively for the dominant and recessive alleles (Beirnaert and Vanderweyen, 1941). Crosses between the two genotypes result in the thin-shelled tenera hybrid which has been the only genotype planted commercially since the sixties.

The individual tenera palms, including palms of the best progenies differ from one another in yield, oil quality and vegetative characteristics. This variation is due to genetic differences between the individual palms but environmental influences also contribute to the variation. The variation due to genetic differences can be overcome if oil palm could be propagated clonally, like rubber and cocoa, for example. More importantly higher yields and superior oil quality can be obtained by cloning elite palms with the desired characteristics.

The need for clonal propagation in coconut is evident for rather similar reasons. Coconut is propagated only by seed. Though rare phenomena such as branching, offshoots and reversions of inflorescences or flowers into vegetative shoot structures have been reported, routine vegetative propagation methods have not been worked out.

Methods

Numerous plants are now routinely multiplied by *in vitro* culture of apices and axillary buds. These explants proliferate in culture to form numerous shoots which can then be rooted or further multiplied. In both coconut and oil palm and indeed most palms, all axillary buds develop into inflorescences. The single apical bud in each palm offers limited scope for development of suitable methods. *In vitro* vegetative multiplication of palms in almost all successful attempts in contrast involves the production of embryoids from callus, the latter being developed from actively growing parts of the palm such as roots, leaves and inflorescences. Direct embryogenesis from leaf explants in coconuts has recently been reported (Raju *et. al.*, 1984). Nevertheless, unlike many other callus systems, large-scale *in vitro* vegetative multiplication in palms (at least in oil palm) is dependent on continuous adventive proliferation of embryoids. Shoots regenerated from embryoids are individually developed and rooted before establishment in soil. *Figure 1* summarises the various stages involved in vegetative propagation of oil palm. Several reviews summarise in detail the protocols that have been used for palm tissue culture (Tisserat, 1981; Pannetier and Buffard Morel, 1983; Paranjothy, 1984; Wooi, 1984). The main features will be outlined here.

Callus initiation. Seed embryos and aseptically grown seedlings developed by seed or embryo culture have been used as a source of explants (Jones, 1974; Nwankwo and Krikorian, 1982). Their use involves minimal loss of cultures through contamination and the absence of inhibitory or toxic effects of sterilants on explants.

When it is possible to select palms for characters with high heritability, it would clearly be advantageous to use explants from palms where such characters have been recorded. For fruit characters, for example, it is clearly desirable to use mature palms. Leaf material close to the apex is uncontaminated and can be excised without damage to the apex. Roots can be easily removed from the soil, though sampling must ensure that roots from neighbouring palms are avoided. Inflorescence tissue remains uncontaminated until the enclosing spathes separate and is therefore another convenient source (Paranjothy, 1984).

Browning is a frequently encountered problem when palm explants are cultured. Browning is frequently encountered in leaf and inflorescence explants. The inclusion of activated charcoal or pretreatment of explants with ascorbic acid has been reported to be advantageous (Paranjothy and Rohani, 1982).

An auxin is clearly essential for callus initiation. Cytokinins tend to inhibit callus formation (Smith and Thomas, 1973) and in this respect there may be similarities between palm explants and those of grasses and cereals. Commonly used auxins include 2,4-D and NAA. Standard nutrient formulations such as that of Murashige and Skoog seem to be adequate for callus initiation (Paranjothy and Rohani, 1982). Coconut explants have been reported to require higher levels of iodine than that found in Murashige and Skoog's medium (Eeuwens, 1976) but oil palm callus does not appear to have a requirement for the element (Smith and Thomas, 1973).

Callus subculture and embryogenesis. Callus from all explants is amenable to subculture, but is usually slow in growth. Callus grown on media containing NAA tends to produce pneumathodes and is generally whitish in appearance when compared with callus grown on 2,4-D media (Smith and Thomas, 1973). Embryogenesis has been observed in callus derived from all explants, but is more frequent in callus derived from explants from juvenile palms (Hanower and Pannetier, 1982).

The embryoids resemble zygotic embryos in appearance and indeed sometimes produce haustoria and cotyledonary sheaths typically found in germinating seed embryos cultured *in vitro* (Paranjothy and Rohani, 1982). The embryoids usually proliferate rapidly, forming clusters of embryoids. Development of embryoids in not always normal and when roots emerge from embryoids first, shoot growth is rarely seen.

Polyembryogenic structures. On repeated subculture these embryoids continue to proliferate new embryoids adventively, but loose their typical whitish appearance and become greenish nodular structures. Anatomical sections of these latter structures reveal the presence of numerous meristemoids from which new nodules or shoots emerge (Nwankwo and Krikorian, 1982). The shoots can be individually isolated for further development whilst the nodular polyembryogenic stuctures are recultured for further proliferation.

Rooting and establishment in soil. Shoots that have attained a height of 5 to 10 cm are rooted in auxin media before transfer to soil. Successful establishment in soil is obviously dependent on a well developed root system. Additionally, hardening under high-humidiy is essential (Wooi, 1984).

GENETIC STABILITY

The successful utilisation of *in vitro* cell cultures for clonal propagation of palms will obviously be dependent on the genetic stability of the cultures. Chromosome counts of oil palm plantlets produced *in vitro* have been reported to be normal (Corley *et. al.*, 1981; Jones *et. al.*, 1982). This

is further corroborated by reports that variability in clonal populations is less than that in seedling populations (Corley *et. al.*, 1981).

The stability of the cultures, on the other hand, also means that somaclonal variation will have to be induced, if such variation is seen as a useful source of variation for breeding purposes. No attempts in this direction have been evident to-date.

PROTOPLASTS

Vouyouklis (1981) reported isolation of viable protoplasts from oil palm root and leaf tissues. The protoplasts, however, did not survive to regenerate cell walls. Bass and Hughes (1984) prepared protoplasts from oil palm cell suspension cultures. The protoplasts were cultured on a "nurse" medium containing oil palm cells in the presence of which protoplasts formed cell walls and divided to form cell cultures. At the Palm Oil Research Institute of Malaysia (PORIM) protoplasts are isolated routinely from leaf and inflorescence tissues. These have regenerated cell walls and divided to form microcolonies (Ismail and Ali, 1985). Protoplasts have also been successfully isolated from embryoids and mesocarp tissue and utilised for studies on fatty acid metabolism (Sambanthamurthi *et. al.*, 1985).

EMBRYO CULTURE

Embryo culture of coconut and oil palm has been extensively studied. Though the interest in these studies is mainly of a fundamental nature, several potential uses are evident.

Oil palm fruits are normally harvested when mature and depericarped before transfer to intermediate quarantine stations. Sampling is thus restricted to palms bearing mature fruits at the time of prospection. In oil palm, seeds are normally fully developed 20 - 24 weeks after anthesis but embryos from 10 - 12 weeks old immature fruits can be isolated, cultured and developed into rooted plantlets. This would hence enable sampling of more palms at a particular site.

Fertile pisifera palms and partially fertile pisifera palms are important in oil palm breeding. Their frequency in natural populations is extremely low and the seeds from these palms, being shell-less, remain viable for only short periods. For these reasons past prospections have scarcely included pisifera germplasm. Embyros from seeds of pisifera palms can be cultured *in vitro* and developed into rooted plantlets with a high degree of success. Indeed this approach is being used routinely in PORIM as an alternative to conventional seed germination for pisifera seeds (Paranjothy *et. al.*, 1985). Embryo culture could likewise be utilised for regeneration of seedlings from accessions of pisifera origin.

Very thin-shelled seeds from tenera palms also tend to germinate poorly. Embryo culture has already been used successfully in PORIM to renegerate seedlings from such accessions obtained in a recent prospection to West Africa (Paranjothy *et. al.*, 1985).

In coconut, embryo culture appears to be potentially useful for propagation of the non-germinating macapuno mutant (Guzman, 1969). Coconuts of this variety, which are much in demand as a delicacy, do not normally germinate and are believed to carry the recessive macapuno gene in the homozygous condition, the natural propagation of the gene being dependent on phenotypically normal nuts carrying the gene heterozygously (Torres, 1973). It has also been suggested that collection of coconut embryos instead of seednuts will facilitate cheap exchange of germplasm on a large-scale, overcoming at the same time restrictions imposed by phytosanitary regulations (Guzman, 1975).

CRYOPRESERVATION

Germplasm conservation in palms like most perennial crops is largely restricted to field genebanks. Whilst germplasm can only be studied and evaluated in the field, any method that would enable prolonged seed storage will serve as a useful logistic tool in germplasm management — the advantages of minimal space and attention being obvious.

Isolated oil palm seed embryos have been shown to be amenable to storage in liquid nitrogen (Grout, 1983). Whole seeds (*i.e.* kernels) and nuts of oil palm can also be stored in liquid nitrogen without damage to the embryo. Embryos excised from these propagules stored in liquid nitrogen germinate normally *in vitro*, thus providing a simple method for long-term storage of oil palm seed germplasm (Paranjothy *et. al.*, 1985).

With the advent of clonal propagation, cryopreservation of embryoids and callus cultures might well play a role in future germplasm conservation. Embryogenic callus cultures of date palm subjected to − 196°C in the presence of cryoprotective mixtures have been successfully revived with subsequent development of plantlets (Tisserat *et. al.*, 1981). Oil palm somatic embryos have been successfully revived after storage in liquid nitrogen (Engelmann *et. al.*, 1985). Revived embryoids formed plantlets, though at a low frequency.

ANTHER CULTURE

The value of homozygous diploids in breeding, especially of perennials, is potentially great. Most palms, however, are outbreeders and it is quite likely that generation of haploids, if successful, will be low in frequency. Reports of the development of multicellular bodies and embryoids from coconut pollen (Tuyen and Guzman 1983; Monfort, 1985) are therefore encouraging, though no plantlet formation has yet been observed.

BIOCHEMICAL STUDIES

Plant cell cultures provide several advantages over intact plants for metabolic studies. These include continuous availability, ease of handling, storage and extraction and response to changes in culture conditions. Radwan and Mangold (1980) have reviewed in depth the biochemistry of lipids in plant cell cultures.

Oil palm cultures have been used to-date primarily in attempts to explore the biochemical events underlying embryogenesis. Turnham and Northcote (1982) noted that one of the initial biochemical events that occurs just before the embryoid can been seen is the accumulation of fat droplets within the cells. This accumulation of lipid was found to correlate with an increase in acetyl-CoA carboxylase activity. The carboxylase was considered to be a probable rate-limiting factor in fatty acid synthesis and it was suggested that the enzyme could be used as a quantitative marker of somatic embryogenesis.

CONCLUDING REMARKS

To-date the most important development emerging from palm tissue culture research with immediate commercial potential is obviously clonal propagation. Clones producing higher yields than average, clones with widely differing oil composition and mesocarp carotenoid contents have emerged in field evaluations (Corley *et. al.*, 1981; Jones, 1983; James, 1984) and by the end of this decade it is very likely that planters will begin exploiting the advantages of not only higher yields but also premium oil quality.

The laboratory methodology now requires improvement, primarily towards more rapid propagation and reduced costs. In this respect any form of automation that can be applied to minimise the slow and laborious manual tasks in large-scale micropropagation could be a major step forward. It would be necessary, however, to develop cultures that are synchronous, for example, in shoot initiation, shoot development and rooting before any form of automation is considered. Embryogenesis in liquid media from single cells might also be expected to improve production rates and amenability to automation. These are therefore potentially useful areas of research. Costs could also be saved if embryoids could be manipulated in a manner that would enable them to germinate with simultaneous production of roots and shoots.

The next major development that is being anticipated of course is the commercialisation of recent successes in clonal multiplication of coconut. Other less known, but potentially economic oil producing palms such as babassu (see also Balick, 1979) may be commercialised through cloning of elite individuals.

In common with most perennials, callus initiation and subsequent embryogenesis is poorer in explants from mature palms (Hanower and Pannetier, 1982). These are fundamental problems

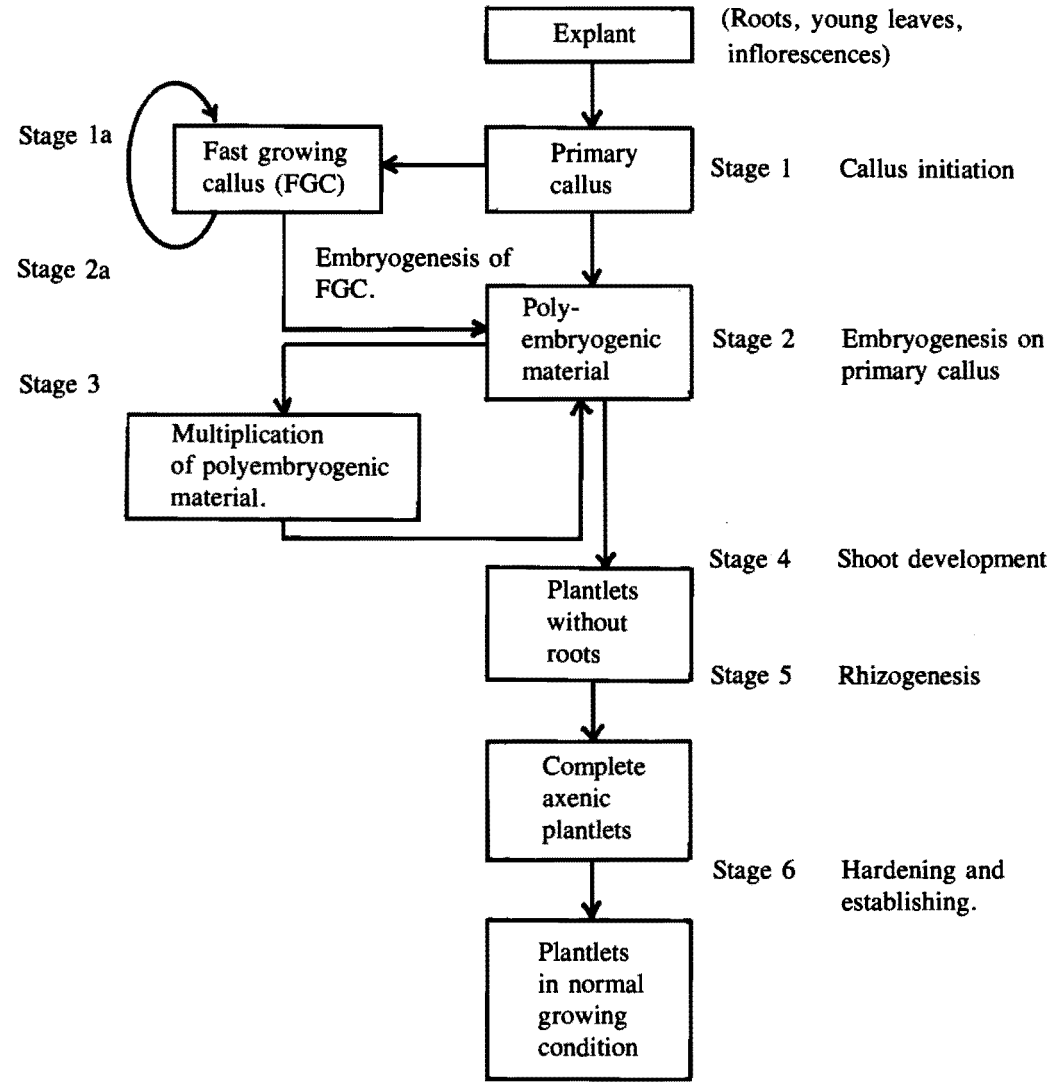
in plant tissue culture that require studies of a basic nature. Of particular importance, indeed as in most crops, is the need to improve the speed and frequency of embryoid formation.

The wide genetic variation in outbreeding species such as coconut (except inbreeding dwarf varieties) and oil palm has certainly not been fully exploited and it is obvious that novel methods such as utilisation of somaclonal variation will probably be useful as adjuncts to conventional methods for a long time. Nevertheless current concepts of "genetic engineering" transcend those of conventional breeding methods. Clearly it is important that cell and tissue culture of palms be developed to a state where these novel methods can be applied successfully. In this respect, the utilisation of palm cell cultures for manipulation will very likely be dependent on progress in the generation of plantlets from protoplasts. Indeed this is an important pre-requisite for the aplication of techniques for somatic cell hybridisation, recombinant DNA and other "genetic engineering" methods.

TABLE 1. WORLD PRODUCTION OF MAJOR EDIBLE VEGETABLE OILS ('000 TONNES).

Soyabean oil	13424.6
Sunflower seed oil	5827.6
Rape seed oil	5349.1
Cotton seed oil	3400.6
Ground nut oil	2899.0
Olive oil	1655.2
Coconut oil	2094.0
Palm oil	6296.7
Palm kernel oil	822.9

Source : Oil World, 1985.



Adapted from Lioret, 1981.

Figure 1. Propagation of oil palm by tissue culture

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