Plantlet regeneration from leaf explants of oil palm seedlings

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Plantlet development was achieved from leaf explants of 18-month-old *dura* and 6-month-old *tenera* oil palm seedlings. Callus induction was noticed after 100–120 days in *dura* and 150–180 days in *tenera* on culturing in half strength Murashige–Skoog basal medium supplemented with 25 mg l^{-1} 2,4-dichlorophenoxyacetic acid. Seven per cent of the cultured explants in *dura* and 10% in *tenera* produced embryogenic calli. These were transferred to regeneration medium containing 1 mg l^{-1} zeatin riboside. Plantlet development from calli was achieved through both somatic embryogenesis and organogenesis. Histological studies on developmental stages were also reported.

CLONAL propagation of oil palm has commercial importance[1–3]. Being a recalcitrant tree crop, the success of micropropagation of oil palm is chiefly related with the choice of explants; tissues from juvenile trees are found to be more responsive[4]. The tender leaf tissues of oil palm seedlings offer good source of explants for reasons of easy availability and sampling. Micropropagation will enable large-scale production of *tenera** hybrids from the performance tested *Dura* × *Pisifera* crosses. Here we describe the various stages of plantlet regeneration by using tender leaf explants of 18-month-old *dura* and 6-month-old *tenera* seedlings.

The sprouts of *dura* and *tenera* were collected from the Central Plantation Crops Research Institute, Regional Centre, Palode and maintained as pot cultures at CPCRI, Kasaragod. The *dura* seedling sampled for the present study is from the cross between 3D × 266D and the *tenera* hybrid from 333D × 609P. The seedlings were sampled destructively by removing the outermost leaves and retaining a few interior leaves with the middle column and surface sterilized by alcohol flaming inside a laminar flow. Subsequently, the outer leaf whorls were removed and only the central portion with meristematic region was used for culturing. Leaf lamina and leaf base were cut into small pieces (0.5 to 1 cm). A total of 60 leaf explants of *dura* and 30 leaf explants of *tenera* were initially inoculated into callus induction medium.

The details of the culture media are given in Table 1. The cultures in the callus induction medium were first incubated in controlled conditions (temp. 27 ± 2°C; relative humidity 55–60%) in dark till callus induction was noticed. Subsequently, the cultures were maintained in illuminated conditions with 16 h photoperiod (2500 lux) for further differentiation. Subculturing was done at monthly intervals.

The specimens selected for histological studies included developmental stages of somatic embryos and meristemoids. These were fixed in Carnoy's B fixative (60% absolute alcohol; 30% chloroform; 10% acetic acid) for 24 h and were dehydrated in alcohol–butanol series before embedding in paraffin wax. Serial sections of 10 μm were taken using a microtome. After deparaffinization, they were stained with 0.1% toluidine blue (TB), periodic acid Schiff's reagent (PAS) and mercuric bromophenol blue (MBB) respectively for the detection of total nucleic acids, total insoluble polysaccharides and total proteins[5,6].

Nodular calli were produced from leaf veins after 100–120 days of inoculation in *dura* (Figure 1) (4/60 cultures), while it took 150–180 days in *tenera* (3/30 cultures). No direct embryogenesis was observed. The actively dividing cells in culture medium are dominated by callus-forming habits[7] and so was the case with the leaf explants excised from the basal parts. On transfer to the somatic embryogenesis/organogenesis medium the primary calli regenerated into somatic embryos and fast growing calli (Figures 2 and 3). The frequency of various developmental stages is shown in Table 2. Compared to *dura*, the regeneration process was slow in *tenera*. In this case the supplementation of zeatin riboside to the medium was delayed for 50–60 days and might have slowed down the regeneration process. The quantity of calli and the regeneration capacity were found to reduce on increasing the interval between subculturing (45–50 days). Somatic embryos developed into complete plantlets in the regeneration medium (Figures 4 and 5) whereas, fast-growing calli directly gave rise to a large number of meristemoids (Figures 6 and 7). Shoot development from meristemoids took 90 to 100 days. It may be seen that a larger share of plantlets was derived through organogenesis than somatic embryogenesis. However, plantlet regeneration through somatic embryogenesis with a minimum callus phase was also reported in oil palm[8]. Shoots with 3–4 leaves and having a height of 10–12 cm were subsequently transferred to rhizogenesis medium. Sufficient roots were produced (Figure 8) within 3–4 weeks. In some cases, filter paper bridges were provided to prevent the complete immersion of shoot for a long time. Plantlets with balanced roots and shoot were obtained within 60–80 days of culturing in rhizogenesis medium (Figures 9 and 10) and were transferred to pots.

At the time of transferring to pots, the plantlets were treated with Bavistin (1%) and thereafter with IBA solution (1000 ppm) for an hour. The potting mixture

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*Based on the fruit structure, oil palm is classified as *Dura* (thick shell; less mesocarp), *Pisifera* (shell less; embryos rarely formed) and the commercially cultivated *Tenera*, the D × P hybrid (thin shell; more mesocarp (60–95%), with high oil content).

Table 1. Culture media for callus induction, somatic embryogenesis/organogenesis and rhizogenesis

	Callus induction	Somatic embryogenesis/ organogenesis	Rhizogenesis
Macro nutrients	$\frac{1}{2}$ MS	$\frac{1}{2}$ MS	$\frac{1}{4}$ Y3
Micro nutrient	$\frac{1}{2}$ MS	$\frac{1}{2}$ MS	$\frac{1}{4}$ Y3
Vitamins	$\frac{1}{2}$ MS	$\frac{1}{2}$ MS	$\frac{1}{4}$ Y3
Hormones			
2,4-D	25 mg/l	0.1 mg/l	–
2-iP	3 mg/l	3 mg/l	–
Zeatin riboside	–	1 mg/l	–
IBA	–	–	5 mg/l
NAA	–	–	1 mg/l
Ads	40 mg/l	40 mg/l	–
Thiamine	2 mg/l	2 mg/l	–
Casein, enzymatic hydrolysate	500 mg/l	500 mg/l	–
Sucrose	3%	3%	2%
Phytagel	0.2%	0.2%	–
Charcoal	0.25%	0.15%	0.1%
pH	5.7	5.7	5.7

IBA: Indole-3-butyric acid; 2,4-D: 2,4-dichlorophenoxyacetic acid; NAA: α-naphthaleneacetic acid; MS basal medium[14]; 2-iP: 6-γ-γ-dimethylallylamino purine; Ads: Adenine sulphate; Y3 medium[15].

Table 2. Frequency of various plantlet regeneration stages at different periods of time (one out of 4 cultures in *dura* (D) and one out of 3 cultures in *tenera* (T)) in the regeneration medium

Days after initial inoculation	Somatic embryogenesis: No. of embryos formed D	T	Total output of plantlets (cumulative no.) D	T	Organogenesis: Fast-growing calli subcultures D	T	Shoots separated D	T	Total output of plantlets (cumulative no.) D	T
200	14				25					
230	31		7		77					
250	8	3	7		178	25	175			
270	19	17	36	2	232	158	196	55		
300	88	30	42	15	344	243	416	162	98	40
400	192	46	121	24	754	280	792	324	560	74

used was sterilized with autoclave soil, sand and coir dust in equal portions. Initially, high humidity was provided to the plantlets by covering them with polythene bag; humidity was then gradually reduced by providing perforation to the bags and later by removing the bags during night. After 4 weeks, the bags were removed completely. The *ex vitro* establishment of plantlets was observed to be satisfactory (Figure 11). After 18 months from the start of the experiments, there were 175 plantlets established in pots. Comparable results were observed on repeating the experiments in three *dura* seedlings and two *tenera* seedlings.

Histological studies were done in *dura* palm cultures and the following observations were made. The primary calli were nodular and originated from the perivascular region of the vein, similar to the type obtained by Schwendiman *et al.*[9] in oil palm. These adventitious nodules could be easily separated from each other at the fragmentation lines (Figure 13). This physical isolation of calli from the explants is a prerequisite for somatic embryogenesis[10]. The fragmentation lines that demarcate the meristematic locus were also reported in the case of date palm[11] and oil palm[12]. The nodular structures derived out of pale yellow coloured calli that tend to differentiate were found to be rich in nucleic acids (Figure 13). Cell differentiation was found to be very rapid in these fast-growing calli. In this stage, a combination of increased divisions and decreased differentiation confers a meristematic state on the cell nodules, which is an essential prerequisite for embryogenic competence of its cells[13]. It was observed in the present study that the origin of somatic embryos/mer-


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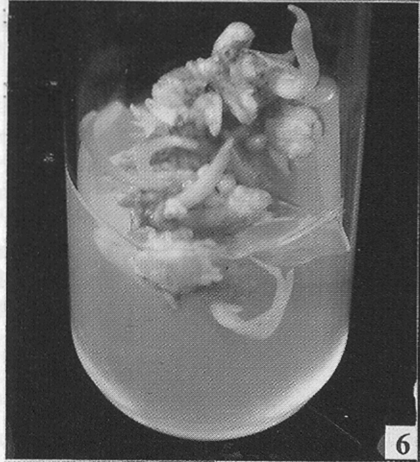
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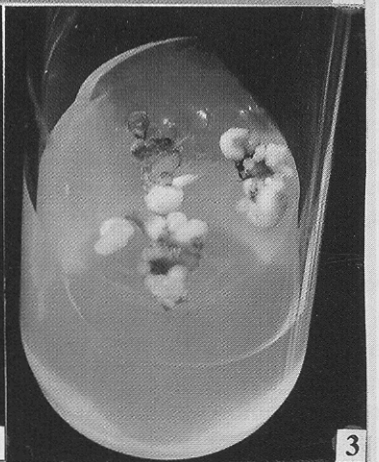
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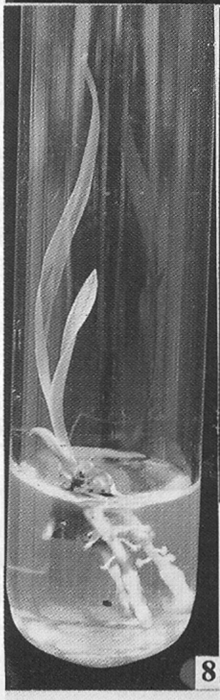
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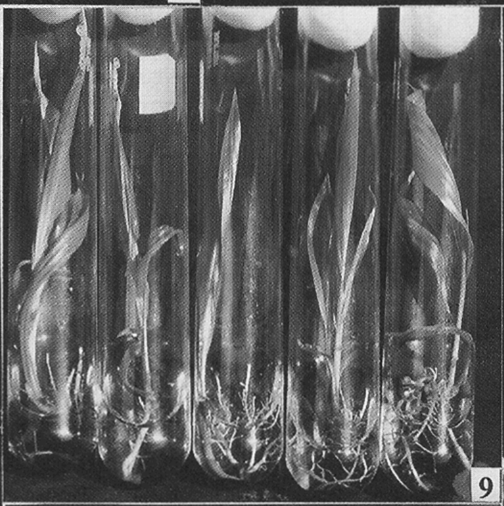
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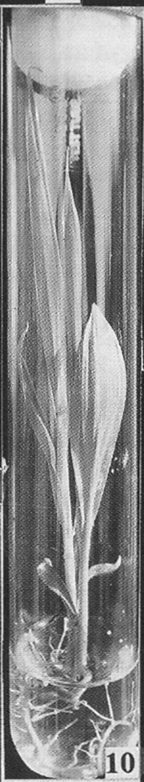
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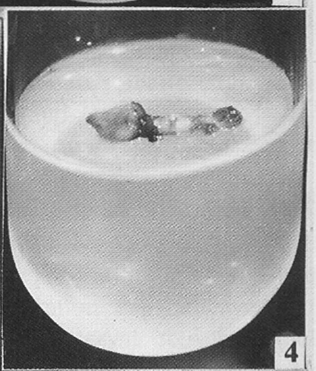
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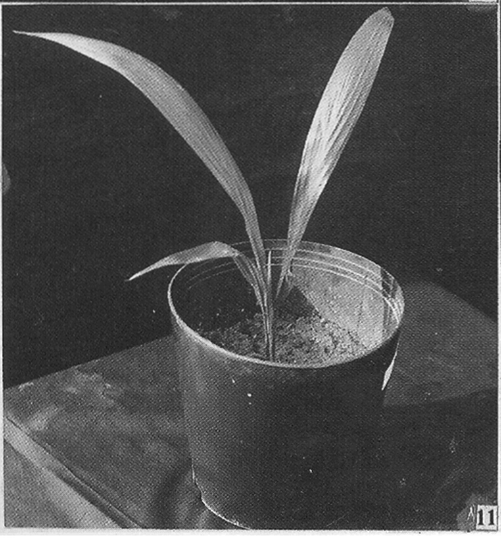
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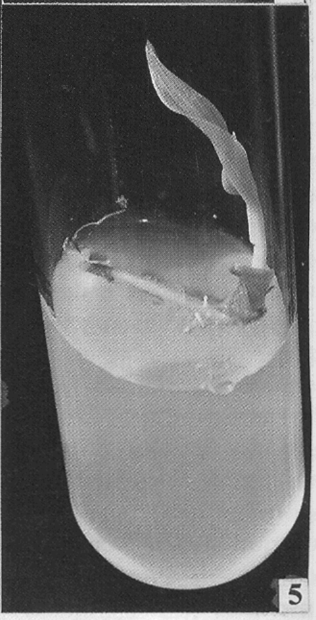
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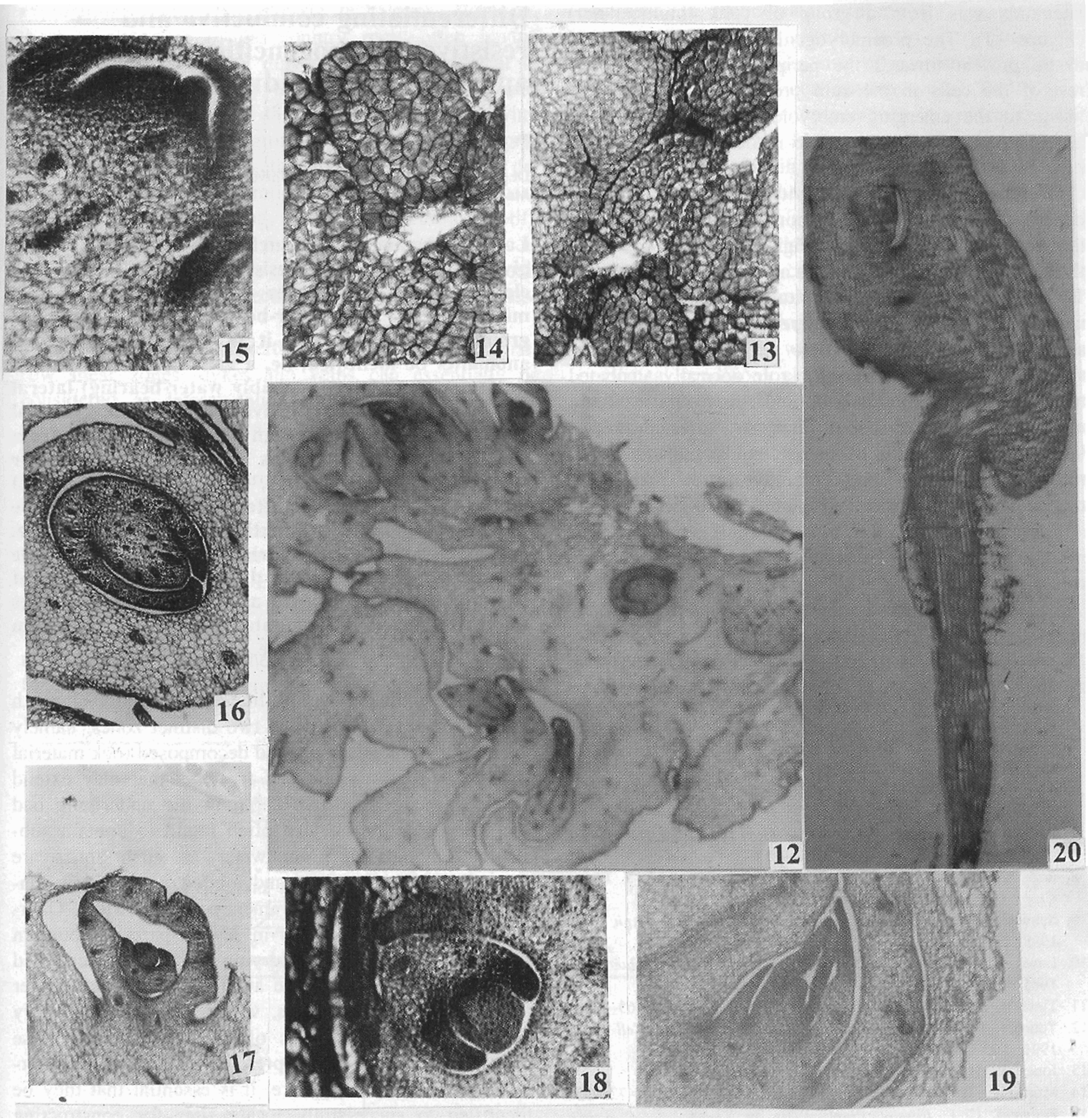

Figures 12–20. Ontogeny of organogenesis/somatic embryogenesis from *dura* oil palm leaf explant. 12. Section of 5 mm sized fast-growing calli showing 5 shoot meristems (100 ×). 13, Section of embryogenic calli showing few embryos cut in oblique section (400 ×). 14, An enlarged globular embryo with actively dividing large sized, dense cytoplasmic cells with small haustorium (400 ×). 15–18, Serial sections of meristemoid and shoot development from fast-growing calli. 19. LS of shoot meristem with 4 leaf whorls (400 ×). 20, LS of germinated somatic embryo with shoot and root (PAS) (100 x).

Figures 1–11. Somatic embryogenesis/organogenesis from *dura* (3D × 266D) oil palm leaf explant. 1, Callus induction after 100–120 days in 25 mg/l 2,4-D, 3 mg/l 2-iP, and 40 mg/l adenine sulphate on half strength MS medium. 2, Friable calli separated from the leaf explant in regeneration medium with zeatin riboside 1 mg/l. 3, Formation of somatic embryos as well as fast growing calli in regeneration medium. 4, Germination of separated somatic embryo. 5, Somatic embryo developed into plantlet. 6 & 7. Fast-growing calli after 4 weeks in regeneration media gave large number of meristemoids and multiple shoots. 8, Formation of roots in rhizogenesis medium. 9 & 10, Plantlets with balanced roots and shoot are ready for transferring to pots. 11, Established plantlet in pot.

istemoids was from a group of cells (multicellular) (Figure 13). The proembryogenic nodules were found to be present towards the periphery of the calli, the rest of the cells in the calli probably acting as nurse tissue to the emerging embryoids. These observations are in confirmation with those made by Schwendiman *et al.*[9].

Continued meristematic activity was observed in the calli which led to the formation of a central zone of the embryonic shoot apex (Figure 14). Subsequently, the apex became well defined as a rounded dome (Figures 15–17). After 6 weeks of culture in the regeneration medium, green-coloured structures were observed on the surface of the calli. All these structures were highly vascularized and they play the role normally attributed to the haustorium by taking up nutrients from the culture medium. Cross sections of these structures showed a large number (10–25) of meristemoids and shoot primordia (Figure 12). A representative series of developmental stages is shown in Figures 14–20.

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Differentiating conductive and resistive inhomogeneities: A new approach in groundwater exploration

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Lateral inhomogeneities, such as dykes or shear zones give rise to false low-resistivity layers in vertical electrical sounding curves which are likely to be misinterpreted as a water-bearing zone at depth. In groundwater exploration it is necessary that such anomalies be identified, i.e. a near-horizontal discontinuity, a conductive (probably water-bearing) lateral inhomogeneity or a resistive (e.g. a dyke) vertical feature. Lateral effects can be distinguished from those due to depth effects by offset soundings or crossed azimuth soundings, while conductive lateral inhomogeneities can be differentiated from resistive ones by a modified azimuthal sounding technique. Under favourable geoelectric conditions it may be possible to determine the direction and amount of dip of the vertical feature as well so that well sites could be located at a suitable distance in the down dip direction of the feature.

IN the predominantly crystalline rock terrains of south India, groundwater occurs in two distinct zones, namely the near-surface weathered and decomposed rock material (regolith) and the joints and fractures that may extend to a few hundred metres depth in the underlying bed rock. Shear zones are also often found to contain substantial amounts of groundwater, as such zones are generally highly fractured and jointed, a condition conducive for storage and movement of groundwater. Overexploitation of groundwater in several parts of southern India has resulted in the drying up of the weathered rock horizon, restricting the availability of groundwater to the fractured rock zones, which extend to relatively deeper levels. The success of a borewell under these conditions depends on the presence of such deep water-bearing zones, and therefore, it is essential that they be identified to successfully locate sites for constructing water wells.

Availability of groundwater in these fractures, joints, shear zones, etc. is facilitated by weathering of the parent rock along them, increasing their porosity, and hence, water holding and transmitting capacity. It must be emphasized here that below a certain depth the weathering of the parent rock is confined mainly to the joint planes of the fracture and shear zones, leaving the bulk of the rock body un-altered and relatively fresh. The weathering renders such zones electrically more conductive in comparison to the country rock, and hence, at first glance, it may appear to be an easy target for