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Plant regeneration from embryogenic suspension cultures of oil palm (*Elaeis guineensis* Jacq.)

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Abstract. Suspension cultures of oil palm (*Elaeis guineensis* Jacq.) were established in a medium with 80 or 100 mg.l^{-1} 2,4-dichlorophenoxyacetic acid and 1 g.l^{-1} activated charcoal, from calli producing embryogenic

The first results confirm theoretical expectations in terms of both the value and homogeneity of the clones (Durand Gasselin *et al.* 1990).

The micropropagation technique is carried out on agar for all stages except rooting. This involves constraints

12:12-h, agitation 90 rpm. The medium was renewed every 4 to 6 weeks. At each transfer, biomass was weighed and residual callus fragments were removed until a suspension was obtained. Different inoculum densities (0.05 to 0.5 g) and 2,4-D concentrations 25 $mg.l^{-1}$ (= M 25), 50 mg.l-1 (= M 50) or M 100 were tested to monitor proliferation.

Regeneration : Either nodules were transferred to basal medium supplemented with 3% sucrose, for early development (1 or 2 cycles), then plated on the same medium with 0.8 % agar onto Petri dishes (9 cm diameter), or the suspension was directly plated. Embryos were subcultured on the same medium until conversion. Rooted plants were sent to Côte d'Ivoire for soil transfer.

Histology : The technique used was described by Schwendiman *et al.* (1990). The sections (3.5 µm) were double-stained by the Schiff-periodic acid reaction and naphtol blue black.

Results and discussion

1) Initiation of the suspension

Description of the initial calli

Friable embryogenic callus with small (< 1 mm) white nodules, slightly connected to the rest of the beige callus was used to initiate the suspension. (Fig. 1).

Histological examination showed that the nodules were small compact aggregates located at the periphery of the parenchymatous callus tissue, with highly vacuolated cells (Fig. 2).

The aggregates were composed of typical meristematic

Table 1. Embryogenic masses released (EM) from callus cultured in M 80, M 100, M 150 and M 200 and suspension establishment (S).

Cycle	1		2		3		4		5		6	
	EM	S	EM	S	EM	S	EM	S	EM	S	EM	S
M 80	4/10	0/10	4/8	0/8	3/7	3/7	2/7	4/7	1/7	5/7	0/8	6/7
M 100	4/10	0/10	6/9	0/9	6/9	2/9	4/9	3/9	2/9	5/9	0/8	7/8
M 150	3/10	0/10	4/7	0/7	0/6	0/6	0/6	0/6	•			
M 200	2/10	0/10	0/7	0/7	0/5	0/5	0/5	0/5	•			

Each value represents the ratio : competent calli/calli conserved.
Callus elimination was due to browning or infection.
* calli grown in M 150 and M 200 were eliminated after 4 cycles.

Ebert and Taylor (1990) reported that 2,4-D was adsorbed at about 98% in a liquid medium very similar to M 100. We presume that 2,4-D adsorption is not linear, and free 2,4-D was probably much higher in M 150 and M 200 than in M 100.

High concentrations of 2,4-D have already been reported to slow down the proliferation of white poplar suspension (Park and Son 1988), to inhibit proliferation of soybean suspension (Finer and Nagasawa 1988), or to promote non-embryogenic tissue growth on Chinese yam suspension (Nagasawa and Finer 1989).

2) Proliferation of the suspension

Histology

Histological studies showed that the proliferating

The best inoculum weight was between 0.1 of and 0.3 g, with a weight increase by a factor of around 4 after 1 month.

Concentrations of 2,4-D in M 25 and M 50 were insufficient to maintain nodules at this stage (data not shown). Nodules tended towards differentiation ; weight and size increased and clumps turned yellow.

Sometimes they looked like the haustorial structures developed in hormone-free liquid medium (see below).

On the other hand, in M 100 the suspension established during early experiments has been maintained for over 18 months with no decline of embryogenic potential.

3) Embryo development

In liquid medium

The most advanced form obtained in hormone-free liquid medium, was an embryo with a gemmule and a radicle, which did not develop further and became necrotized after transfer to agar medium.

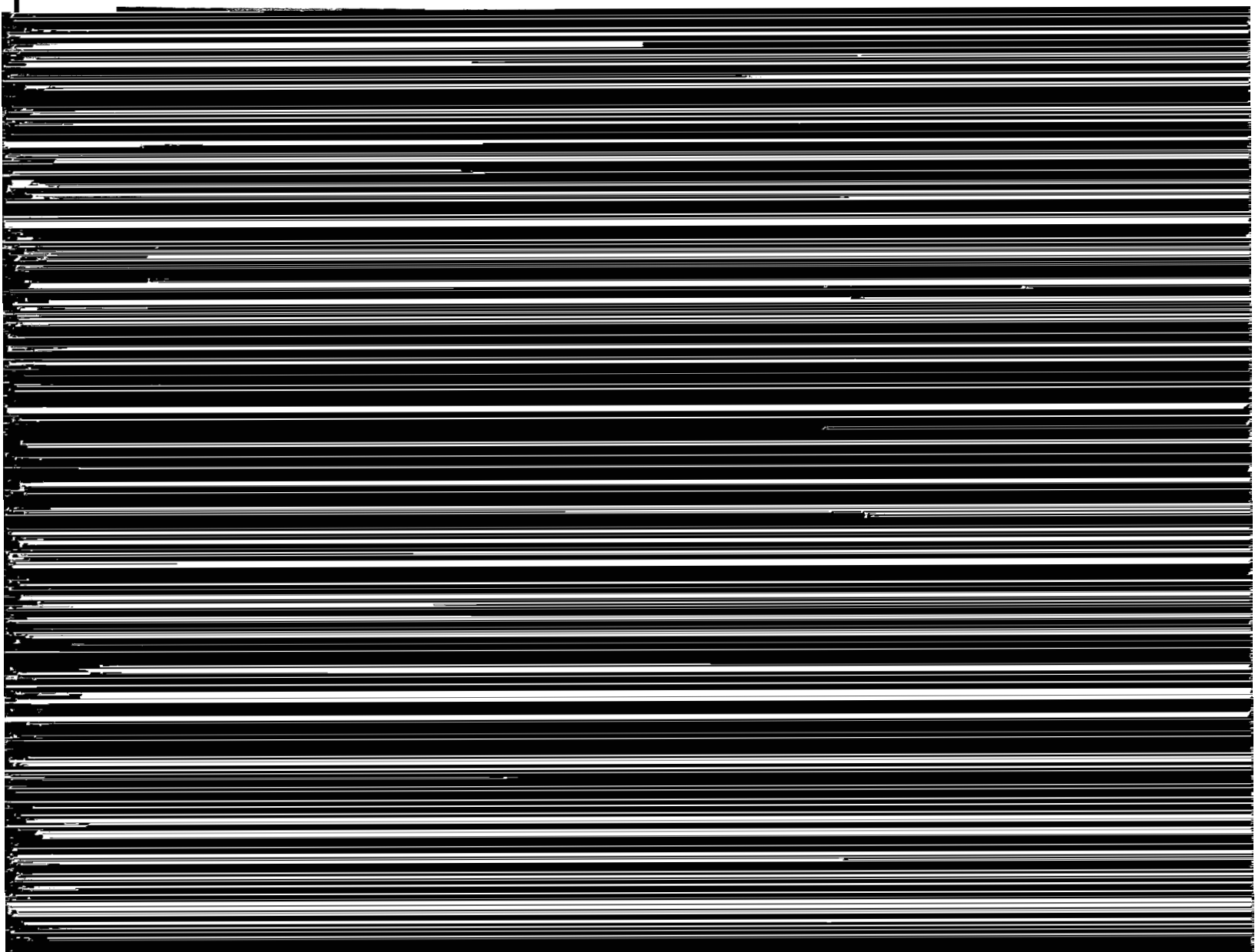
If conserved for a long time in liquid medium without (at least 1 cycle) or with low auxin concentration (M 50 or M 25: at least 2 cycles), the swollen nodules tended towards haustorium-like structures, sometimes with roots, and remained at this stage.

Histological studies revealed they had lost their embryogenic potential. They showed no cell division, and had enormous amyloplast accumulation around the edges. The centres were vascularized and an epidermis was sometimes visible.

In solid medium

Embryos were obtained:

- either, by direct spreading of proliferating nodules from M 80 or M 100. After a latent period (1 week to 1 month), a stage of multiplication was observed, prior to obtaining embryos. When plating isolated embryos, adventitious embryogenesis was noticed, as in the current process. It was probably due to the remaning effect of the 2,4-D.



- or from synchronously maturing nodules, at an early stage of differentiation, in a liquid medium deprived of hormone. The stage was determined visually before plating : clumps were pale yellow or white, maximum 3 mm, with one or several lobes (Fig 5). Under these conditions, multiplication was greatly reduced, and one nodule usually produced one to three embryos.

4) Plant Regeneration

Embryos developed in one of two ways:

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