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IN VITRO RESPONSES OF OIL PALM (*ELAEIS GUINEENSIS* JACQ.) APICAL MERISTEM AND ZYGOTIC EMBRYO TO GROWTH REGULATORS AND CULTURE MEDIA

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ABSTRACT

The most obvious *in vitro* plant propagation pathway is by apical meristem and zygotic embryos culture (direct organogenesis system). However, the apical meristem procedure has limited use in oil palm cultivation because excision of explants kills the source plant and produces one plantlet ultimately. Furthermore, generated offsprings from zygotic embryo culture are not true to type. Somatic embryogenesis is the alternative approach for obtaining normal oil palm plantlets but with inconsistencies. Consequently, studies are necessary to resolve such issues and the adoption of the knowledge gathered from direct organogenesis is likely to proffer solution. It was a 2 x 2 x 3 x 9 factorial experiment which involved: light regime, oil palm types, growth media and plant growth regulator concentrations. Explants were incubated in either uninterrupted light or in uninterrupted darkness. For apical meristems, one set each of the three media; (Murashige and Skoog (MS) 1962), modified MS and Eeuweens (1976) were supplemented with either 30, 50, 70 and 90 mg l^{-1} NAA or with 5, 10, 15 and 20 mg l^{-1} 2,4-D. Control treatments were without plant growth regulator. For zygotic embryos, a set of the three media were supplemented with 35, 70, 105 and 140 mg l^{-1} NAA (Naphthalene Acetic Acid) and another set with 4, 8, 12 and 16 mg l^{-1} 2,4-D. Control units of the three media were without either NAA) or 2,4-D. The plantlet initiation rates from apical meristems and zygotic embryos were 100 % respectively, but the procedure is of limited use in oil palm because it results in loss of germplasm. In any case, the technique has provided a better understanding of *in vitro* oil palm culture and has subsequently facilitated the development of somatic embryogenesis, the preferred system for oil palm plantlets production.

Keywords: Adoption, Embryogenesis, Organogenesis, Factorial, Oil palm

INTRODUCTION

The most obvious way to propagate plants *in vitro* is by apical meristem and zygotic embryos culture. Apical meristem culture involves the isolation of buds and shoot tip whose outer leaves are carefully extracted until the shoot apical meristem is encountered leaving one primordial leaf and cultured in a growth medium under sterile condition. Success in the use of this method of culture requires knowledge of how to break the bud dormancy. Dormancy may be broken by providing either long-day treatment (16-hour daylight/day) or cold treatment (10 to 15 ^{o}C) or both Gibberellic acid or other gibberellins, and cytokinins may stimulate formation of shoots. In any case, some plants will develop shoots without any pre-treatment.

An immature or mature zygotic embryo can also be isolated and cultured in a medium under sterile conditions (zygotic embryo culture) with the aim of producing viable plants. The direct organogenesis when compared with somatic embryogenesis is less complex and has quicker responses. Despite the relative success with direct organogenesis the procedure is of limited utility in oil palm because excision of apical meristems for use as explants kills the source plant and only one plantlet is obtained. Furthermore, there is no certainty of the generated offspring being true to type in those generated from zygotic embryos because of the cross-pollinating nature of oil palm unlike in meristem culture and to some extent in somatic embryogenesis, which is most widely utilised for the rapid multiplication of elite plant materials.

In any case, somatic embryogenesis is the preferred approach *in vitro* procedure for plant regeneration in the oil palm industry because of the much potential benefits it presents but slow progress is known to be its challenge since discovery. Attempt to improve this system will entail the adoption of the knowledge gained from direct organogenesis system of culture. It is expected that a thorough understanding of the development of the explants cultured, using one of the methods can create for more insight and a better knowledge of the *in vitro* principles that can be useful to same explants when

cultured/incubated with the other method. Therefore a hands-on trial on apical meristem and zygotic embryos culture of the oil palm tissues was necessitated as it is likely to reveal the important tips that will cause breakthrough/improvement in oil palm somatic embryogenesis. These will eventually enable the researcher conduct for more experiment in the entire *in vitro* culture task in the long run.

MATERIALS AND METHOD

Culture Media Preparation for Apical meristem culture: Three culture media: Murashige and Skoog (1962) (MS), Modified MS after Hyndman *et al*. (1982) and Eeuween's (1976) were prepared. They were each supplemented with 0.7 % (w/v) plant agar, 0.25 % charcoal, 0.1 g l^{-1} Meso-inositol, 0.005 g l^{-1} thiamine-HCl, 0.0005 g l^{-1} Pyridoxine-HCl, 0.005 g l^{-1} nicotinic acid and 0.04 g l^{-1} adenine sulphate respectively. However, the Eeuween's medium was supplemented with 4.5 % sucrose while the other two were supplemented with 3 % sucrose (Street, 1973). Each of the three culture media was supplemented with either 30, 50, 70 and 90 mg l^{-1} NAA or with 5, 10, 15 and 20 mg l^{-1} 2,4-D. Control treatments of the three different culture media were without the plant growth regulator supplements. The pH was adjusted and media sterilized with the autoclave at a steam pressure of 15 psi at 121 ^{o}C for 15 min.

Explants selection/Preparation: One-year old seedlings of dura and tenera oil palms were selected from an oil palm nursery. Pisifera oil palm was not included because it is not reliably identifiable at any time below mature fruiting age which is about seven years. Furthermore, at that age and above, explants of oil palms are not significantly responsive to *in vitro* culture. A scalpel was used to remove the leaves and roots from the harvested seedlings. The trimmed explants were then placed in clean beakers according to type and covered with glass Petri plate as lid. Three hundred and sixty (360) millilitres Sodium hypochlorite (NaOCl) solution (2.6 %) was prepared and two drops of 'Teepol' (detergent solution) were added in order to improve the penetration of the tissues of the explants by the sterilant. The NaOCl solution was shared equally into the two beakers, each of which contained one explants only, representing any one of the oil palm types of dura and tenera. The beakers were placed within the laminar hood until the explants were fully submerged in the solution. The explants were allowed to soak in the NaOCl solution for 15 min. The beakers containing the explants were regularly agitated to ensure complete wetting of the explants. The NaOCl solution was drained off and the explants in the beakers were rinsed three times with distilled water.

Explant inoculation/Incubation: Each explant had its outer leaf fronds carefully extracted in the laminar hood until the meristem was encountered leaving one primordial leaf. Each meristem explant was cultured on the medium. The experiment was a 2 x 2 x 3 x 9 factorial and the factors involved were: light regimes, oil palm types, growth media, and plant growth regulator concentrations (Table 1). Half of the experimental units were incubated at 27 ± 1 ^{o}C under uninterrupted light with intensity of 1000 lux which was provided by warm white fluorescent lamps (Narayanaswamy, 1977). The other experimental units were incubated at the same temperature but in uninterrupted darkness (24 hours).

Experimental design/Layout: The explants were cultured on the media with one hundred and eight treatment combinations (Table 1). There were ten explant units in each of the treatments which were replicated five times. The test tubes were arranged in a completely randomised design within the growth chamber. The meristem explants were re-cultured unto freshly prepared culture media of the same compositions regularly at four weeks interval.

Data collection: The shoot emergence percentage of the cultured meristems were calculated and recorded at the end of the experiment .
Thus, shoot emergence (%) = No. of explants with emerging shoot 100 --- x --- Total no. of explant units cultured 1
Photographs of the emerging shoots were taken as they developed in the treatments. Contaminated explants were recorded and removed. Data were collected for a period of 12 weeks.

Root initiation medium preparation: The sprouted meristems constituted the materials for this stage of the experiment. Initiation of roots in meristems with shoots required a root initiation medium. It consisted of modified MS medium to which organic supplements were added as culture media preparation above. Then 60 g l^{-1} sucrose was also added according to Eeuweens (1976). The media were supplemented with NAA (20, 40 and 60 mg l^{-1}) as well as 2,4-D (5, 10 and 15 mg l^{-1}) (Rohani *et al.*, 2003). There were also control units without the auxin supplements. Adjustment of the pH of the culture media, media sterilisation and addition of agar were as in culture media preparation above. For explants sterilisation and inoculation, the shoot tips were treated the same way as the oil palm leaf explants.

The 10 sprouted meristems were then cultured per treament while each treatment was replicated five times. A daily inspection was carried out on all the experimental units and record of percent root initiation were taken every two weeks.

Zygotic embryo culture media preparation: One set each of the three media, namelyMS (1962), modified MS (1962), and Eeuweens (1976), were supplemented with 35, 70, 105 and 140 mg l^{-1} NAA and another set with 4, 8, 12 and 16 mg l^{-1} 2,4-D. Control units of the three media were set up with non-addition of either NAA or 2,4-D as supplement. Adjustment of pH, media sterilisation, and addition of agar were as in experiment 1.

Zygotic embryos explant selection/preparation: Ripe fruits from 12-year old dura and tenera were collected. At this age, pisifera oil palm type can be identified but its fruits do not have viable seeds. This is why it was not included in the experiment. For explant sterilisation and inoculation, the ripe palm fruits drawn from dura and tenera types were placed in a beaker, washed with detergent and then rinsed thrice with distilled water. They were immediately soaked in absolute ethanol for one hour and air dried in a laminar air-flow cabinet. Each sterilised fruit was then cut in half with secateurs and mature embryos were eased out of the seeds. The extracted embryos were then soaked in 2.6% NaOCl for five minutes and rinsed thrice with distilled water. Each mature embryo was cultured on the appropriate nutrient medium.

Zygotic embryos explant inoculation/incubation: One set of the experimental units was incubated in uninterrupted light and the other in uninterrupted darkness until the first leaf appeared. Subsequently, germinated embryos were re-cultured on media with same composition. Contaminated tubes were identified and culled after recording.

Experimental design/layout: The explants were cultured on the media with one hundred and eight treatment combinations. The test tubes containing the cultured explants treatment combinations (Table 2) were arranged in a completely randomised design (RCD) within the growth

Chamber. There were 15 zygotic embryo explants per treatment with five replications.

Data collection: The shoot initiation percentage of the cultured zygotic embryos were recorded.

Thus, shoot initiation (%) = No. of zygotic explants with emerging shoot $\frac{100}{1}$ x Total no. of zygotic explant units cultured

Photographs of the emerging shoots were taken as they developed in the treatments. Contaminated explants were recorded and removed. Data were collected for a period of eight weeks.

RESULTS

Initiation of viable shoots in apical meristems: Under uninterrupted light, the excised apical meristems initiated shoots (Plate 1) three to four weeks after inoculation in the three plant growth regulator-free culture media ranging from 70 to 80%. There was also shoot initiation in media supplemented with 30 mg l^{-1} NAA. Each of the responding treatments initiated one shoot, only. There was no viable shoot initiation in the other treatments with higher plant growth regulator concentrations i.e. 50 to 90 mg l^{-1}. Instead, the initiated shoot gradually developed calli. The calli initiated had stunted growth and gradually withered. The percentage of normal shoot initiation in meristems cultured in media supplemented with 30 mg l^{-1} NAA ranged from 80 % for tenera in MS and modified MS to 100 % in Eeuweens (1976) medium for both dura and tenera types (Table 3). In media supplemented with 2,4-D, the excised apical meristems also initiated shoots within three to four weeks after inoculation in the three culture media which were plant growth regulator-free, at a rate ranging from 70 to 80 %. As with NAA the lowest value was for tenera oil palm in MS media supplemented with 5 mg l^{-1} 2,4-D also initiated normal shoots with each responding treatment initiating one shoot as with NAA. There was no viable shoot initiation in the other treatments with increase in plant growth regulator concentration such that only a few calli developed in media supplemented with 20 mg l^{-1} 2,4-D. The calli exhibited stunted growth and withered gradually. The rate of shoot initiation in meristems cultured in media supplemented with 5 mg l^{-1} 2,4-D ranged from 70 % for tenera in MS (1962) medium to 80 % in the remaining treatments for dura and tenera oil palm types. Although there was shoot initiation in the media without supplements, there was slight improvement in shoot initiation

X 0.5

Plate 1. Oil palm meristem initiating shoot.

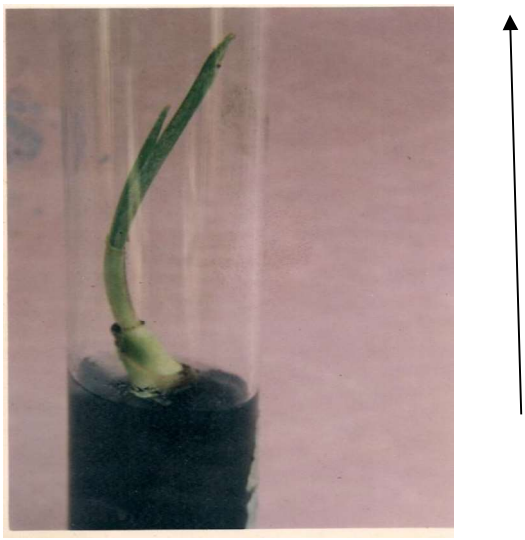
with supplements but only at the lowest levels i.e. 30 mg l-1 of NAA and 5 mg l-1 of 2,4-D, with similar rates of shoot initiation but no shoot initiation at the higher levels. Under uninterrupted darkness, the cultured excised apical meristems initiated shoots from three to four weeks in the three plant growth regulator-free culture media. Shoot initiation rate was 60 % for dura meristems in MS medium and 70 % in the remaining treatments. As in light, there was viable shoot initiation only in media supplemented with 30 mg l-1 NAA with the meristems initiating single shoots. At the higher concentrations no shoot was initiated, also as in light. There was minimal calli development instead of shoot growth in 50, 70 and 90 mg l-1 NAA. However, the calli gradually withered. The rate of viable shoot initiation in meristems cultured in media supplemented with 30 mg l-1 NAA ranged from 60 % for tenera in MS mediu m to 90 % for dura in Eeuweens (1976) medium (Table 3). In media supplemented with 2,4-D there was shoot initiation with 5 mg l-1 2,4-D with each responding meristem initiating one shoot. At higher concentrations of 2,4-D there were a few poorly formed calli instead of shoot initiation. This was similar to the response of NAA, at concentrations.

Higher than 30 mg l^{-1}. The rate of shoot initiation in meristems cultured in media supplemented with 2,4-D ranged from 60 % by tenera in MS (1962) to 70 % in the remaining treatments.

Initiation of roots in shoot-sprouting apical meristems: Under uninterrupted light, the apical meristems which sprouted shoots were cultured in root initiation media. With NAA as culture medium supplement, only 40 mg l^{-1} NAA promoted the initiation of simple roots in the shoot-sprouting meristems and thus produced plantlets (Plate 2, Table 4). Roots were initiated after three to four weeks of explant culture. No roots were initiated with the other concentrations of NAA. The proportion of meristem explants that initiated roots in culture ranged from 60 % by tenera type oil palm in MS and modified MS to 100 % by dura type in Eeuweens media (Table 4). With 2,4-D as culture media supplement, initiation of simple roots in the shoot-sprouting meristems occurred only with 10 mg l^{-1} concentration in five to six weeks. No roots were initiated in the other concentrations of 2,4-D. The proportion of meristem explants that initiated roots ranged from 50 % by tenera type in the two MS culture media to 70 % by dura type in the three culture media.

Under uninterrupted darkness, the concentration of NAA that supported initiation of simple roots, the time of roots initiation after culture and the initiation of roots in other concentrations of NAA were as reported above for uninterrupted light also with NAA as culture media supplement. The proportion of meristem explants that initiated roots in culture ranged from 60 % by tenera in MS (1962) to 100 % by dura in Eeuweens (1976) media. With 2,4-D as culture media supplement, the initiation of roots in the shoot-sprouting meristems occurred as reported for under uninterrupted light in terms of the concentration that supported the initiation of roots i.e. 10 mg l^{-1}, time of root initiation after culture and the initiation of roots in other concentrations. The percentage of meristem explants that initiated roots in culture ranged from 50 % by tenera in MS and modified MS to 80 % by dura in Eeuweens media (Table 4).

It is noteworthy that unlike shoot initiation which occurred only in the absence of the plant growth regulators, NAA and 2,4-D, as reported in subsection 3.1.0 no roots were initiated in the absence of the plant growth regulators.



X 2

Plate 2. Initiated plantlet from oil palm meristem

Initiation of plantlets from oil palm zygotic embryos: With NAA as culture supplement, the oil palm zygotic embryos initiated plantlets successfully in the three culture media three to four weeks after inoculation, under uninterrupted light. However, only concentrations of 35 and 70 mg l^{-1} supported plantlet initiation in the zygotic embryos with all cultured zygotic embryos initiating plantlets (Plates 3, 4, 5, 6 and Table 5). Root initiation also occurred along with shoot initiation. The plantlets initiated in culture media supplemented with 35 mg l^{-1} NAA were more vigorous in growth and all the initiated plantlets were identical within oil palm types. With 2,4-D as culture supplement, the oil palm zygotic embryos also initiated plantlets successfully in the three culture media from three to four weeks with only

concentrations of 4 and 8 mg l^{-1} supporting plantlets initiation. Root initiation also occurred along with shoot initiation as with NAA. The culture media supplemented with 4 mg l^{-1} 2,4-D produced the more vigorous plantlets.

x 1

Plate 3. Excised zygotic embryos cultured on sub-cultured on nutrient medium

x 1

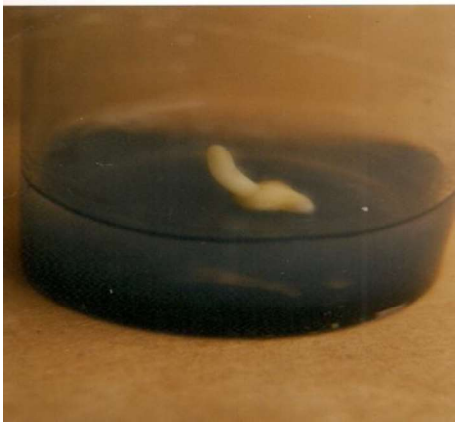
Plate 4. Excised zygotic embryo nutrient medium

x 2

Plate 5. Initiation of plantlet from oil palm

NAA (Table 5). All the same, the initiation of roots

Under uninterrupted darkness, with NAA as culture media supplement, the oil palm zygotic embryos initiated plantlets successfully in the three culture media from three to four weeks. However, only 35 mg l^{-1} NAA supported normal plantlets initiation in the zygotic embryos. Stunted shoots were initiated with 70 mg l^{-1} occurred in both normal and stunted plantlets. The oil palm zygotic embryos also initiated plantlets successfully in the three growth media from three to four weeks under uninterrupted darkness with only 4 mg l^{-1} concentration of 2,4.D supporting normal plantlets initiation. With 8 mg l^{-1} 2,4-D stunted shoots were initiated. However, as with NAA above, initiation of roots occurred in both normal and stunted plantlet



x 1

Plate 6. Initiation of plantlets from oil palm zygotic zygotic embryo in nutrient medium embryo in nutrient medium

DISCUSSION

Plantlets are more easily initiated through direct organogenesis than somatic embryogenesis. However, this advantage is not widely exploited in

oil palm rapid multiplication and cloning because of the significant variation that has been found in plants produced through culturing zygotic embryos that are pollinated by natural forces. On the other hand, meristem culture is not a useful method for cloning oil palm, a monocotyledonous plant. This is because harvesting of its shoot tip, as explants for culture, results in death and thus loss of germplasm. In any case, despite the disadvantages of the technique, direct organogenesis has led to a better understanding of oil palm *in vitro* culture which has subsequently facilitated the development of other pathways such as somatic embryogenesis for regeneration of oil palm plantlets.

In the present study, all the three culture media, namely MS, modified MS, and Eeuweens (1976) supported plantlet initiation in apical meristems, and zygotic embryos. Initiation took place in all three media from three to four weeks. A vigorous growth of explants was obtained in nutrient media which were plant growth regulator-free. Under both light and darkness, there was 100 % plantlet initiation in media without supplement and plantlets exhibited moderate growth, with root initiation. Growth is retarded in the treatments having either NAA or 2,4-D plant growth regulator supplement (subsection 3.1.0). Growth retardation became pronounced as the plant growth regulator concentration increased,. The growth retardation was due to the fact that increasing concentration of plant growth regulator in nutrient media resulted in herbicidal action instead of growth stimulation (Lemaux,1999). Furthermore, the requirement for plant growth regulator varies with types of plant tissue and it is likely that the endogenous auxin i.e. NAA or 2,4-D in oil palm was initially high enough to support growth before the additional supply which eventually accumulated to toxic levels (Bhojwani and Razdan, 1992), as may occur in tissue culture generally (Pierik, 1989) thereby retarding plant tissue growth.

Initiation of roots in the sprouting apical meristems under uninterrupted light and darkness was accomplished with 40 mg l^{-1} NAA as nutrient supplement in combination with 60 g l^{-1} sucrose, a higher concentration than the usual 30 g l^{-1} applied in somatic embryogenesis. On the other hand, 10 mg l^{-1} 2,4-D employed as nutrient media supplement in combination with 60 g l^{-1} sucrose initiated roots in the sprouting apical meristems. These responses conform to the reports of Horgan (1984) and Rohani *et al.* (2003) also with oil palm. Initiation of roots with NAA was possible in three to four weeks whereas it took five to six weeks with 2,4-D.

CONCLUSION AND RECOMMENDATION

The plantlet initiation rates from apical meristems and zygotic embryos were 100 % respectively, but the procedure is of limited use in oil palm because it results in loss of germplasm. In any case, the technique has provided a better understanding of *in vitro* oil palm culture and has subsequently facilitated the development of somatic embryogenesis, the preferred system for oil palm plantlets production.

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Table 1: Apical meristem culture treatments in respect of two oil palm types in two light regimes

			Oil palm types					
			Dura			Tenera		
Light regime	**Plant regulator**	**growth**	**MS (1962) medium**	**Modified MS medium**	**Euweens (1976) medium**	**MS (1962) medium**	**Modified MS medium**	**Euweens (1976) medium**
		mg l^{-1}						
Uninterrupted light	NAA	30	T_1	T_{10}	T_{19}	T_{28}	T_{37}	T_{46}
		50	T_2	T_{11}	T_{20}	T_{29}	T_{38}	T_{47}
		70	T_3	T_{12}	T_{21}	T_{30}	T_{39}	T_{48}
		90	T_4	T_{13}	T_{22}	T_{31}	T_{40}	T_{49}
	2,4-D	5	T_5	T_{14}	T_{23}	T_{32}	T_{41}	T_{50}
		10	T_6	T_{15}	T_{24}	T_{33}	T_{42}	T_{51}
		15	T_7	T_{16}	T_{25}	T_{34}	T_{43}	T_{52}
		20	T_8	T_{17}	T_{26}	T_{35}	T_{44}	T_{53}
	Control	0	T_9	T_{18}	T_{27}	T_{36}	T_{45}	T_{54}
Uninterrupted darkness	NAA	30	T_{55}	T_{64}	T_{73}	T_{82}	T_{91}	T_{100}
		50	T_{56}	T_{65}	T_{74}	T_{83}	T_{92}	T_{101}
		70	T_{57}	T_{66}	T_{75}	T_{84}	T_{93}	T_{102}
		90	T_{58}	T_{67}	T_{76}	T_{85}	T_{94}	T_{103}
	2,4-D	5	T_{59}	T_{68}	T_{77}	T_{86}	T_{95}	T_{10}
		10	T_{60}	T_{69}	T_{78}	T_{87}	T_{96}	T_{105}
		15	T_{61}	T_{70}	T_{79}	T_{88}	T_{97}	T_{106}
		20	T_{62}	T_{71}	T_{80}	T_{89}	T_{98}	T_{107}
	Control	0	T_{63}	T_{72}	T_{81}	T_{90}	T_{99}	T_{108}

$T_1 - T_{108}$ represent the treatments/treatment combinations.

Table 2: Treatment combinations in zygotic embryo culture

			Oil palm types					
			Dura			Tenera		
Light regime	Plant growth regulator.	mg l^{-1}	MS (1962) medium	Modified MS (1962) medium	Euweens (1976) medium	MS (1962) medium	Modified MS (1962) medium	Euweens (1976) medium
Uninterrupted light	NAA	35	T_1	T_{10}	T_{19}	T_{28}	T_{37}	T_{46}
		70	T_2	T_{11}	T_{20}	T_{29}	T_{38}	T_{47}
		105	T_3	T_{12}	T_{21}	T_{30}	T_{39}	T_{48}
		140	T_4	T_{13}	T_{22}	T_{31}	T_{40}	T_{49}
	2,4-D	4	T_5	T_{14}	T_{23}	T_{32}	T_{41}	T_{50}
		8	T_6	T_{15}	T_{24}	T_{33}	T_{42}	T_{51}
		12	T_7	T_{16}	T_{25}	T_{34}	T_{43}	T_{52}
		16	T_8	T_{17}	T_{26}	T_{35}	T_{44}	T_{53}
	Control	0	T_9	T_{18}	T_{27}	T_{36}	T_{45}	T_{54}
Uninterrupted darkness	NAA	35	T_{55}	T_{64}	T_{73}	T_{82}	T_{91}	T_{100}
		70	T_{56}	T_{65}	T_{74}	T_{83}	T_{92}	T_{101}
		105	T_{57}	T_{66}	T_{75}	T_{84}	T_{93}	T_{102}
		140	T_{58}	T_{67}	T_{76}	T_{85}	T_{94}	T_{103}
	2,4-D	4	T_{59}	T_{68}	T_{77}	T_{86}	T_{95}	T_{104}
		8	T_{60}	T_{69}	T_{78}	T_{87}	T_{96}	T_{105}
		12	T_{61}	T_{70}	T_{79}	T_{88}	T_{97}	T_{106}
		16	T_{62}	T_{71}	T_{80}	T_{89}	T_{98}	T_{107}
	Control	0	T_{63}	T_{72}	T_{81}	T_{90}	T_{99}	T_{108}

T_1– T_{108} represent treatments/treatment combinations.

Table 3: Shoot initiation rates (%) in cultured apical meristems of dura and tenera oil palm types.

			Plant growth regulator concentration (mg l^{-1})									
			NAA					2, 4-D				
Light regime	Oil palm type	[1]Culture medium	0	30	50	70	90	0	5	10	15	20
			----------%----------									
L	Dura	MS	80	90	0	0	0	80	90	0	0	0
		Mod. MS	80	90	0	0	0	80	90	0	0	0

		Ee	80	100	0	0	0	80	100	0	0	0
	Tenera	MS	70	80	0	0	0	70	80	0	0	0
		Mod. MS	80	80	0	0	0	80	80	0	0	0
		Ee	80	100	0	0	0	80	100	0	0	0
D	Dura	MS	60	80	0	0	0	60	70	0	0	0
		Mod. MS	70	80	0	0	0	70	70	0	0	0
		Ee	70	90	0	0	0	70	70	0	0	0
	Tenera	MS	70	60	0	0	0	70	60	0	0	0
		Mod. MS	70	70	0	0	0	70	70	0	0	0
		Ee	70	80	0	0	0	70	70	0	0	0

L = uninterrupted light, D = uninterrupted darkness.
1 MS, Murashige and Skoog (1962); Mod. MS, ModifiedMurashige and Skoog (1962); Ee, Eeuweens (1976).
Number of meristem units cultured per treatment = 10.
NAA = Naphthalene acetic acid, 2,4-D = 2,4-Dichlorophenoxy acetic acid.

Table 4: Root initiation rates (%) in cultured shoot-sprouting oil palm apical meristems.

			Plant growth regulator concentration (mg l^{-1})									
			NAA					2, 4-D				
Light regime	Oil palm type	[1]Culture medium	0	20	40	60	80	0	5	10	15	20
			----------%----------									
L	Dura	MS	0	0	80	0	0	0	0	70	0	0
		Mod. MS	0	0	80	0	0	0	0	70	0	0
		Ee	0	0	10 0	0	0	0	0	70	0	0
	Tenera	MS	0	0	60	0	0	0	0	50	0	0
		Mod. MS	0	0	60	0	0	0	0	50	0	0
		Ee	0	0	80	0	0	0	0	60	0	0
D	Dura	MS	0	0	80	0	0	0	0	70	0	0
		Mod. MS	0	0	90	0	0	0	0	70	0	0
		Ee	0	0	10 0	0	0	0	0	80	0	0
	Tenera	MS	0	0	60	0	0	0	0	50	0	0
		Mod. MS	0	0	70	0	0	0	0	50	0	0
		Ee	0	0	80	0	0	0	0	60	0	0

L = uninterrupted light, D = uninterrupted darkness.
1 MS, Murashige and Skoog (1962); Mod. MS, ModifiedMurashige and Skoog (1962); Ee, Eeuweens (1976).
Number of meristem units cultured per treatment = 10.
NAA = Naphthalene acetic acid, 2,4-D = 2,4-Dichlorophenoxy acetic acid.

Table 5: Plantlet initiation rates (%) in cultured oil palm zygotic embryos.

Light regime	Oil palm type	[1] Culture medium	Plant growth regulator concentration (mg l^{-1}) NAA 0	NAA 35	NAA 70	NAA 105	NAA 140	2,4-D 0	2,4-D 4	2,4-D 8	2,4-D 12	2,4-D 16
			----------%----------									
L	Dura	MS	100	100 +	100	0	0	100	100 +	100	0	0
		Mod. MS	100	100 +	100	0	0	100	100 +	100	0	0
		Ee	100	100 +	100	0	0	100	100 +	100	0	0
	Tenera	MS	100	100 +	100	0	0	100	100 +	100	0	0
		Mod. MS	100	100 +	100	0	0	100	100 +	100	0	0
		Ee	100	100 +	100	0	0	100	100 +	100	0	0
D	Dura	MS	100	100	100 x	0	0	100	100	100 x	0	0
		Mod. MS	100	100	100 x	0	0	100	100	100 x	0	0
		Ee	100	100	100 x	0	0	100	100	100 x	0	0
	Tenera	MS	100	100	100 x	0	0	100	100	100 x	0	0
		Mod. MS	100	100	100 x	0	0	100	100	100 x	0	0
		Ee	100	100	100 x	0	0	100	100	100 x	0	0

L= uninterrupted light, D = uninterrupted darkness.
1 MS, Murashige and Skoog (1962); Mod. MS, Modified Murashige and Skoog (1962); Ee, Eeuweens (1976).
Number of meristem units cultured per treatment = 15. + Vigorous plantlets produced, x stunted plantlets, in comparison with other plantlets of moderate vigour.

NAA = Naphthalene acetic acid, 2,4-D = 2,4-Dichlorophenoxy acetic acid.