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SOMATIC EMBRYOGENESIS AND PLANTLET REGENERATION FROM OIL PALM (*ELAEIS GUINEENSIS* JACQ.) CALLUS

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ABSTRACT

Callus cultures were initiated from mature embryos of oil palm (Elaeis guineensis *Jacq.) on half strength MS (Murashige and Skoog, 1962) medium enriched with either 30 mg/l NAA (α-naphthaleneacetic acid) or 3 mg/l 2,4-D (2,4-dichlorophenoxyacetic acid), 3% sucrose and 0.05% (w/v) activated charcoal. Induction of embryogenic, fast-growing calli were obtained when calli were transferred to similar medium with an increase of NAA and 2,4-D to 70 mg/l and 5 mg/l, respectively. These embryogenic calli produced numerous embryoids in these media after several subcultures. Somatic embryo maturation was obtained on medium devoid of NAA or 2,4-D and supplemented with 15% (v/v) coconut water to produce young plantlets. Histological study of embryoids at different stages of development revealed the morphological organization resembled that of zygotic embryo.*

INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) is native to Africa and now widely cultivated across the Southeast Asia. It is a valuable resource for vegetable oil and accounts for about 15% of the world's total production of vegetable oil[1]. In Thailand oil palm is a major plantation crop in the South. The rate of increase in plantation area is very high thus causing a lack of good quality seedlings. Oil palm is propagated by seeds and the most commonly used seedling at present is the *tenera* hybrid[2]. Since it is a hybrid, it does not breed true and no natural means of vegetative propagation is available for oil palm[3]. Under this circumstance, there is a high potential for tissue culture application in rapid clonal propagation of an elite oil palm tree. The culture of oil palm *in vitro* has been reported previously by several authors[4-12]. However, information concerning details of media and growth regulator amendments is still a fundamental requirement of the intense commercial production.

In this paper, we describe the production of embryoids and their development into plantlets from callus culture of oil palm.

MATERIALS AND METHODS

Plant material

Mature *tenera* seeds were collected from a garden in Changwat Krabi, Southern Thailand. The kernels were immersed in 70% ethanol and then cut into small cubes. These cubes were then surface sterilized in 40% Clorox solution containing 1 drop of Tween-20 emulsifier per 100 ml solution for 20 minutes. The sterile cubes containing intact embryos were transferred to sterile distilled water and kept for 24 hours prior to excision.

Nutrient media

Embryos were initially cultured on a half basal nutrient medium composed of Murashige and Skoog[13] (MS) salts, 3% (w/v) sucrose, 0.05% (w/v) activated charcoal (AC), and 0.2% Gelrite. NAA was added in the nutrient medium at the concentration of 30 mg/l, or 2,4-D was supplemented at 3 mg/l concentration. For embryoid production, calli initiated from medium containing NAA or 2,4-D were transferred to 70 and 5 mg/l respectively. For plantlet development, all embryoids were transferred to the same medium without NAA or 2,4-D but supplemented with 15% (v/v) coconut water (CW). The pH of all nutrient media was adjusted to 5.6 with 0.1 N NaOH or HCl before Gelrite was added.

Environmental conditions

Cultures were incubated in a temperature-controlled room at 25 ± 1 °C with a 16-hours photoperiod daily exposure to 2000 lux Gro-Lux light. The initial cultures and subsequent recultures were incubated for periods of eight weeks.

Histological studies of embryoids

Embryoids at various stages of development were fixed in FAA solution of 90 ml 50% ethyl alcohol, 5 ml glacial acetic acid and 5 ml formalin solution. Materials were embedded in paraffin. Sections were cut at 10 μm and stained with safranin and fast green.

RESULTS

Development of callus was visible during the first 8 weeks after planting embryos on half strength MS medium. Two types of callus were easily induced but better callus growth was achieved on medium containing 2,4-D than an medium containing NAA. With NAA in the medium callus tended to produce fibrous roots and was generally friable and whitish in appearance. On media containing 2,4-D calli were yellow creamy and compact in texture.

Following experiments which involved callus formation, these calli were subdivided and placed in other media to promote the formation of fast-growing callus tissues since most of the calli grew very slowly. Thus it is necessary to increase the rate of callus growth for further study. Optimal growth of the friable-to-nodular callus occurred when callus was transferred from NAA containing medium to half strength MS medium with an increase of NAA to 70 mg/l and the fast-growing calli were easily subcultured providing a mass of calli. They could be distinguished from the primary callus by the presence of tiny nodular structures of varying sizes linked to one another (Fig. 1). These nodules contained provascular strand and regions of meristematic cells. Their outer surface was smooth with a clearly defined epidermal layer. They were polarized and became dense at the distal and proximal ends (Fig. 2). After being cultured in this medium for 2-3 months, the embryoids showed no further development. However, small localized intense green spots appeared on the embryoids which suggested partial differentiation. Transfer of these embryoids to similar medium devoid of NAA and supplemented with 15% CW allowed the differentiation of shoot-like growth structure with a height of 1-1.5 cm (Fig. 3) and subsequent complete plantlets (Fig. 4).

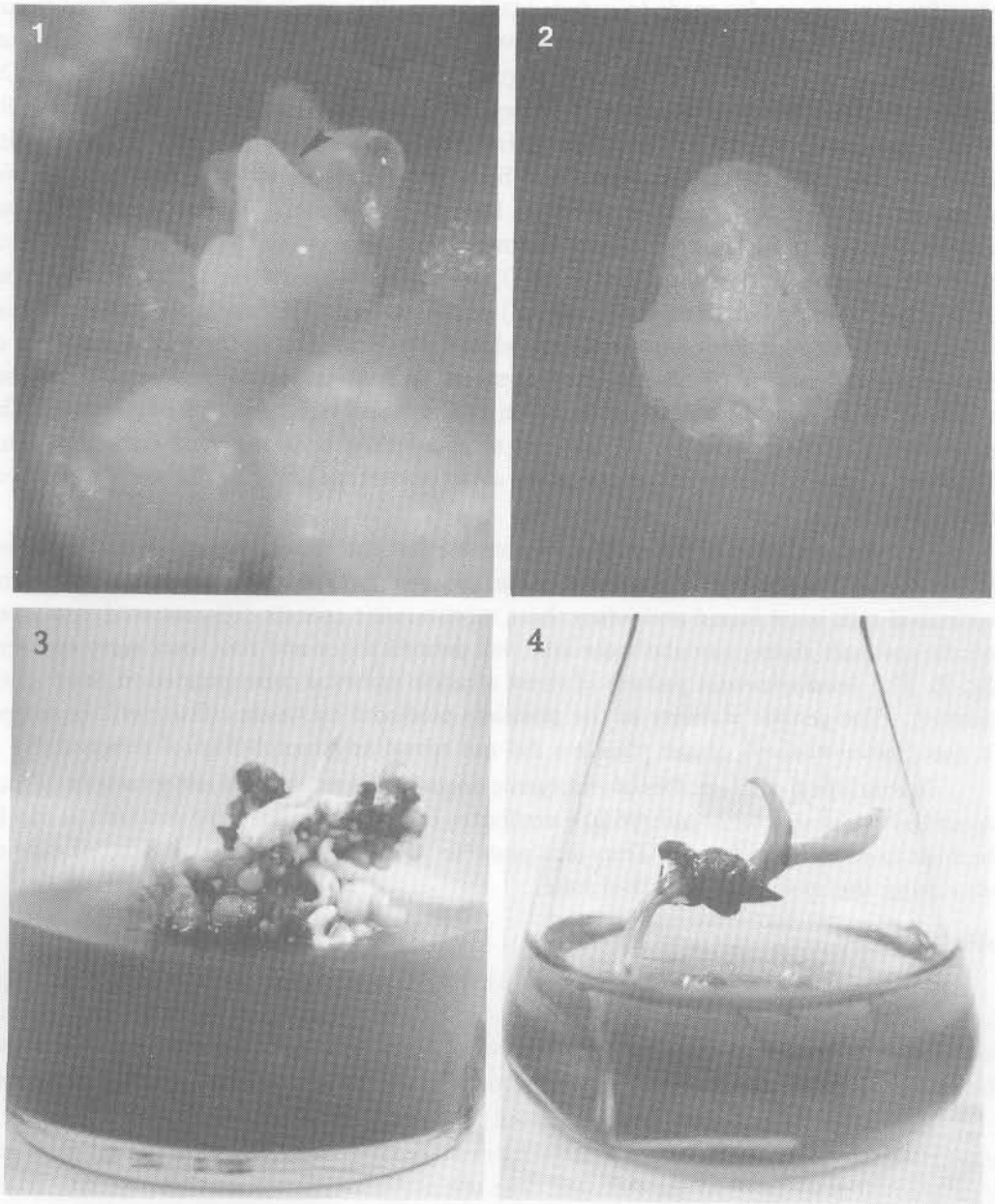

Fig. 1. Embryoid induction derived from fast-growing callus after being transferred from 1/2 MS medium supplemented with 30 mg/l NAA to similar medium with an increase of NAA to 70 mg/l. Compact nodular structure (arrow) was evident.

Fig. 2. Embryoid with cauline and radicular pole.

Fig. 3. Development of shoot-like growth emerged from embryoid in 1/2 MS medium devoid of NAA with 15% CW.

Fig. 4. Complete oil palm plantlet in 1/2 MS medium devoid of NAA with 15% CW.

Attempts were also made to induce maximum callus production through application of 2,4-D. The rate of multiplication of yellow creamy callus was 3-4 fold the original size in 2 months when transferred to half strength MS medium containing 5 mg/l 2,4-D. On this medium fast- growing calli were obtained and the newly formed calli were extremely granular and friable and could be separated from each other easily (Fig. 5). Opaque white formation with clear borders appeared and became greenish rapidly (Fig.6). Nevertheless the growth of these embryoids showed no further development if cultured in the same medium without reculture or changing the medium composition. When embryoids were allowed to develop in medium lacking 2,4-D and supplemented with 15% CW, embryoid differentiated into organized structures (Fig.7) which, upon reculturing in the same medium, readily gave rise to bipolar embryos and produced plantlets (Fig. 8) through a sequence of development reminiscent of zygotic embryogenesis. In both cases plant regeneration appears to have taken place as a result of an omission of auxin and replacement by cytokinin. The period for the whole processes from callusing to plantlet regeneration was 5-6 months. This phenomenon was still infrequent since only a small percentage (27%) of the somatic embryos was formed.

Anatomical studies of the embryoids revealed that the structure of embryoid resembled that of zygotic embryo. At the early stage of development, meristematic centers and procambial cells were found and when these meristematic centers differentiated into green nodular compact shape, vascular trace and well defined shoot and root apex were apparent (Fig. 9). The developmental pattern of these somatic embryos corresponded to the zygotic situation. The genetic stability of the plantlets produced by tissue culture will be tested. To date, visual analysis of the plantlets did not reveal any morphological abberations.

In conclusion callus tissues of embryonic origin can be established and grown in culture. Organized structures with embryo like attributes can develop. Plantlet production can be obtained from callus cultures. Thus it is possible to propagate large numbers of elite oil palm using the procedure described here.

DISCUSSION

Many sources of explants have been used for tissue culture propagation of oil palm. Embryo culture by Rebechault *et al.*[14] produced callus in the presence of 2,4-D and then produced nodules which they termed embryoids. Nwankwo and Krikorian[15] also reported the clonal propagation of *pisifera via* callus derived from embryos and seedling explants.

A number of common features found in oil palm tissue culture were the same as other palms[16-19]. Browning is a major problem associated with the culture of oil palm and usually resulted in death of the cultures. The use of AC can eliminate this problem. Wang and Huang[20] indicated that favorable effects of addition of AC are probably due to the adsorption of certain unidentified active agents rather than darkening of the medium. Our media also employed a small amount of AC to remove the effects of those substances. The inclusion of AC reduced not only growth-inhibiting substances but also the availability of growth regulators, therefore it was necessary to supply high concentrations of NAA and 2,4-D. However, this is not the case of Christmas palm since embryogenic callus was induced from mature zygotic embryos when they were cultured on charcoal-free MS medium[19]. It seems clear that a high level of auxin is required for callus initiation of cultured palm

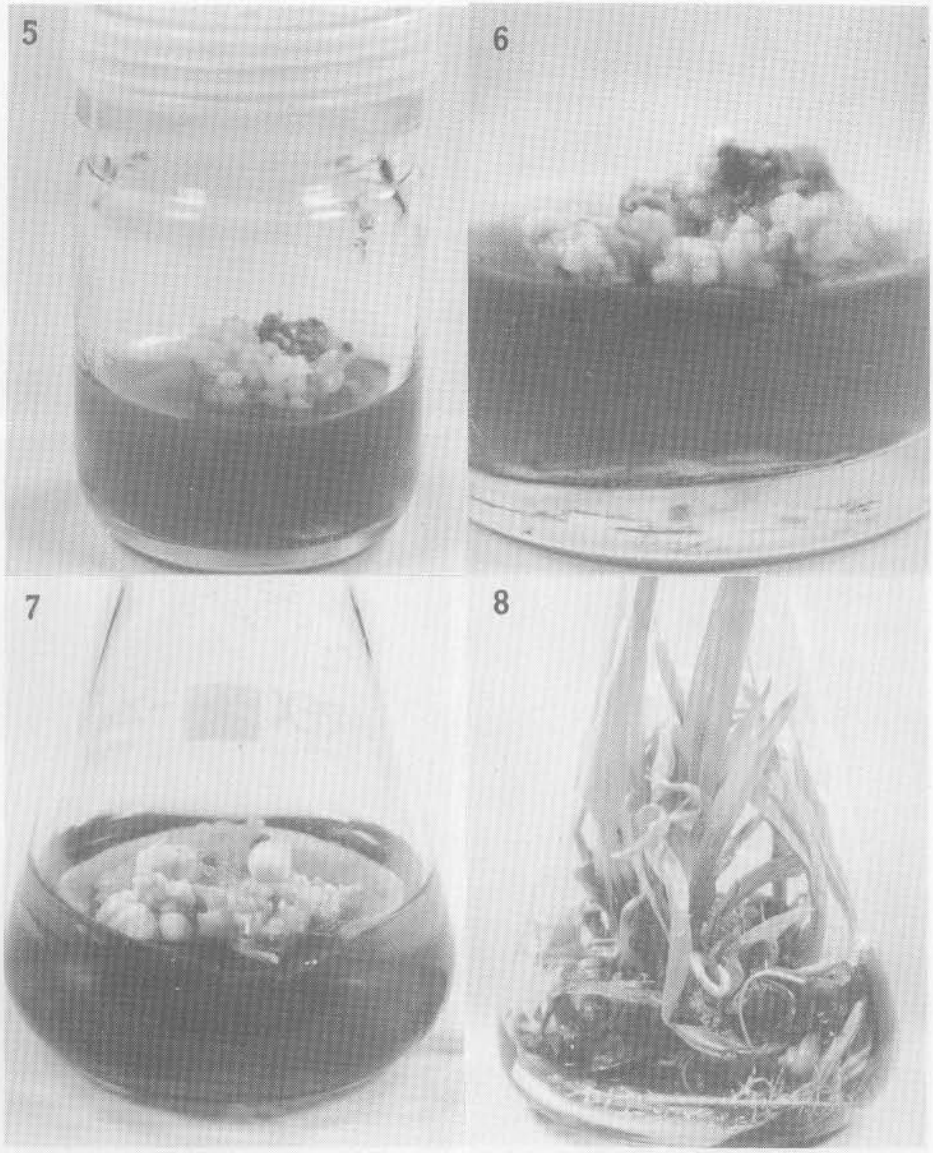

Fig. 5. Fast-growing callus cultured on 1/2 MS medium containing 5 mg/l 2,4-D and 0.05% AC.

Fig. 6. Opaque white formation and chlorophyllian embryoids derived from fast-growing callus.

Fig. 7. Organized structures differentiated from embryoids cultured on 1/2 MS medium lacking 2,4-D with 15% CW.

Fig. 8. Complete plants cultured on 1/2 MS medium lacking 2,4-D with 15% CW.

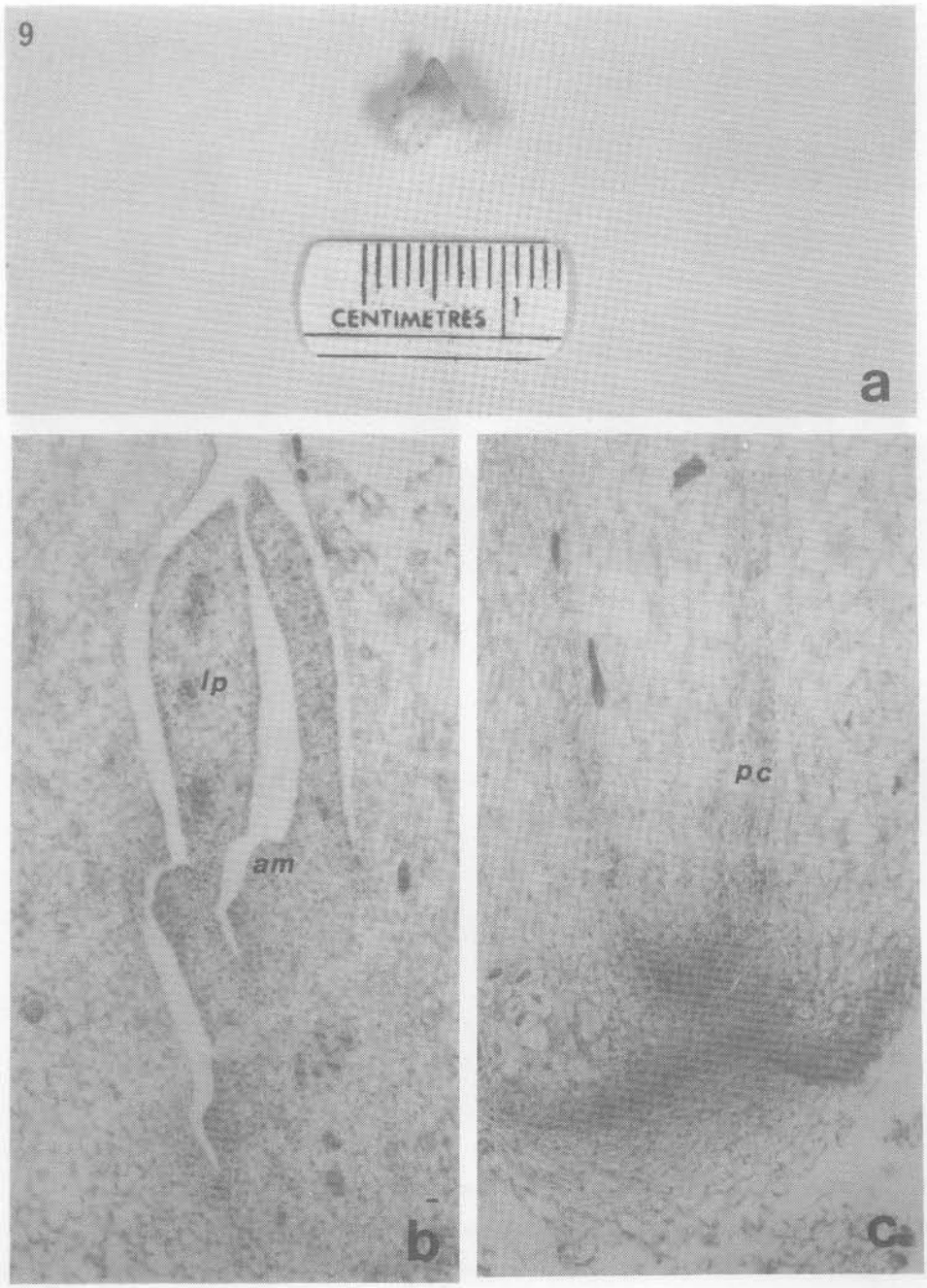

Fig. 9. Morphogenetic sequences in cultured embryoid derived from fast-growing callus. (a) Intense green spots appeared on the embryoid. Longitudinal section of an embryoid showing shoot apex (b) and root apex (c). (lp = leaf primordium; am = apical meristem; pc = procambial cells).

explants[17,21,22] and cytokinins tend to inhibit callus formation[6]. 2,4-D is the most successful auxin, though NAA at a high level will also produced callus. The disadvantage of NAA for callus induction is that it also acts at a lower level to induce roots or pneumathodes[23].

The sequence of appearance of nodules and subsequent plantlet can be correlated to the presence of growth regulators in the medium[15]. The conditions under which the phenomenon presented are the same for oil palm as for other species. In our system, nodules were first induced by providing a nutrient medium rich in auxins. The increase of auxins was essential for fostering embryoid formation. If maintained on this medium, the embryoids failed to develop. Plantlets developed from these embryoids by transferring them to the regeneration medium lacking auxins and supplemented with CW, indicating that a change in growth regulator type (from auxin to cytokinin) was a necessary prelude to somatic embryogenesis development. In general, cytokinins enhance shoot formation and auxins promote rooting. Therefore, exogenous application of CW stimulated the development process of embryoids into plantlets. This is in agreement with Tisserat[18] that excised date embryos on medium containing high auxin concentrations produced callus and organized structures. However, in the view of Jones[7] the formation of embryoids appears to be more a function of the origin of the starting innoculum than that of the subsequent cultural conditions. Our success is probably attributable to the distinct maturity of the embryos employed as explants. This is in contrast with the results of Alang[24], who reported that mature sago embryos were less responsive *in vitro* and that plantlets could not be regenerated from calli originating from embryos.

The results of this study have shown that plantlet production *in vitro* of oil palm can be vegetatively propagated *via* somatic embryogenesis. We used callus that originated from mature embryo explants to study nutritional requirements and concurrently the results obtained from these experiments have been applied to somatic tissues from selected oil palm ortets. Research is continuing with the aim of improving the methodology for the differentiation of somatic embryos in this important palm.

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บทคัดย่อ

เพาะเลี้ยงแคลลัสจากเอ็มบริโอปาล์มน้ำมันบนอาหารสูตร 1/2 MS (Murashige and Skoog, 1962) ที่เติม NAA (α–Naphthaleneacetic acid) 30 มก/ล หรือ 2,4–D (2,4–Dichlorophenoxyacetic acid) 3 มก/ล น้ำตาลซูโครส 3% และผงถ่าน 0.05% เมื่อย้ายแคลลัสที่ได้ไปเลี้ยงบนอาหารสูตรเดิม แต่เพิ่มความเข้มข้นของ NAA และ 2,4–D เป็น 70 มก/ล และ 5 มก/ล ตามลำดับ จะได้เอ็มบริโอเจนิคแคลลัสที่เจริญเร็ว เมื่อทำการย้ายเลี้ยงเอ็มบริโอ เจนิคแคลลัสเหล่านี้บนอาหารสูตรเดิมหลายๆ ครั้ง พบว่า เอ็มบริโอเจนิคแคลลัสสร้างเอ็มบริออยด์ขึ้นมามากมาย เอ็มบริออยด์ที่ได้เจริญกลายเป็นต้นปาล์มน้ำมันที่สมบูรณ์ เมื่อย้ายไปเลี้ยงบนอาหารสูตรเดิมที่ไม่มี NAA และ 2,4–D แต่เพิ่มน้ำมะพร้าว 15% เมื่อศึกษากายวิภาคของเอ็มบริออยด์ในระยะต่างๆ พบว่าจะมีโครงสร้างเหมือนเอ็มบริโอที่ได้จากไซโกต