ORIGINAL ARTICLE

Histology of embryoid development in oil palm (*Elaeis guineensis* Jacq.) cell suspension culture

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Abstract

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Embryos of oil palm (*Elaeis guineensis* Jacq.) variety *tenera* were cultured on Eeuwens or Y3 (1976; 1978) medium supplemented with 2 mg/l 2,4-D. Calluses were initiated from these embryos. The eight-week-old calluses derived from embryos were transferred to modified Y3 liquid medium devoid of 2,4-D and supplemented with NAA, BA and coconut water to establish cell suspension culture. After a period of culture, these cells were then subcultured to the same medium without plant growth regulators to induce embryoid formation. The calluses and embryoids were harvested at various times, fixed, sectioned, stained and examined microscopically. Histological study revealed that embryoid occurred from meristematic cells with dense cytoplasm along the callus clumps.

Key words : callus, *Elaeis guineensis* Jacq., embryo culture, histology, oil palm

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บทคัดย่อ

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Histology of embryoid development in oil palm (*Elaeis guineensis* Jacq.) cell suspension culture

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เพาะเลี้ยงเอ็มบริโอปาล์มน้ำมันลูกผสมพันธุ์เทเนอรา บนอาหารสูตร Eeuwens หรือ Y3 (1976; 1978) ที่เติม 2,4-D 2 มิลลิกรัมต่อลิตร สามารถชักนำให้เกิดแคลลัสจากเอ็มบริโอ เมื่อย้ายเลี้ยงแคลลัสอายุ 8 สัปดาห์ที่เกิดจากเอ็มบริโอไปยังอาหารเหลวดัดแปลงสูตร Y3 ที่ปราศจาก 2,4-D แต่เติม NAA, BA และน้ำมะพร้าว เพื่อชักนำเซลล์แขวนลอย หลังจากเพาะเลี้ยงไประยะเวลาหนึ่ง ย้ายเลี้ยงเซลล์แขวนลอยไปยังอาหารเหลวสูตรเดิม แต่ปราศจากสารควบคุมการเจริญเติบโต เพื่อชักนำให้เกิดเอ็มบริออยด์ นำแคลลัสและเอ็มบริออยด์ที่เพาะเลี้ยงในระยะเวลาต่าง ๆ มาฟิกซ์ ตัดชิ้นส่วน ย้อมสีและศึกษาภายใต้กล้องจุลทรรศน์ จากการศึกษาทางเนื้อเยื่อวิทยาพบว่า เอ็มบริออยด์เกิดจากเซลล์เมอริสเต็มที่มีไซโทพลาสซึมเข้มข้นและเกิดเป็นกลุ่มในแคลลัส

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Plant regeneration of oil palm through *in vitro* culture has been reported by several research groups (for review see Kanchanapoom and Domyoas,1999). The establishment of plant regeneration by the utilization of somatic cell genetic technology seems to be satisfactory for oil palm propagation. However, the determination of histology of embryoids is considered inadequate. Few histological studies have dealt with palms (Reynolds and Murashige, 1979; Tisserat and DeMason, 1980; Pannetier and Buffard-Morel, 1982; Nwanko and Krikorian, 1986; DeMason, 1988; Schwendiman *et al*., 1988). In this communication, we describe a study from a histological point of view to observe the ontogenic stage in which somatic embryoids occur in oil palm embryo culture.

Materials and methods

Plant material and initiation of callus

Mature *Tenera* seeds were collected and surface sterilized as described by Kanchanapoom and Chourykaew (1998). The callus induction medium contained Eeuwens or Y3 (1976, 1978) inorganic salts, with (mg/l) Na_2EDTA 37.22; $FeSO_4.7H_2O$ 13.9; myo-inositol 101.97; thiamine-HCl 0.97; pyredoxine-HCl 1.02; nicotinic acid 0.98; glutamine 100; arginine 100; asparagine 100; 2,4-D 2; sucrose 45,000 served as basal medium (designated CY3 medium). Callus was initiated from mature zygotic embryos cultured on CY3. The pH of all media was adjusted to 5.6 prior to the addition of 0.15% Gelrite (Merck & Co., Kelco Division, NJ, USA) and autoclaved at 121ºC for 20 min.

Initiation of callus and cell suspension culture

For induction of suspension culture, approximately 5 gram fresh weight of 2 month-old callus was transferred to 125 ml Erlenmeyer flask containing 25 ml of liquid medium. The medium was modified to include half strength of Y3 inorganic salts, with (mg/l) glycine 4; ascorbic acid 100; NAA 0.5; BA 5 and 20% (v/v) coconut water (designated MY3 medium).

Induction of embryoids

Cells cultured in MY3 liquid medium for 3 weeks were then transferred to the same medium devoid of plant growth regulators (designated FY3

medium). Two experimental procedures were used. Firstly, the cells were incubated in this medium for a period of 3, 6, and 9 days by successive subcultures every 3 days. Secondly, cells were cultured without any subculture for a period of 3, 5 and 7 days. At the end of each period all samples were transferred to FY3 solid media supplemented with 0.05% activated charcoal and subcultured every 2 weeks on these media for 3 months.

Culture conditions

Callus and suspension cultures were incubated at 25±2ºC with a 16-h photoperiod under an illumination of 20 $\mu molm^{-2}s^{-1}$ photosynthetic photon flux density provided by Gro-lux lamps. The inoculum was incubated on a rotary shaker with continuous shaking at 100 rpm.

Histological studies of calluses and embryoids

Calluses and embryoids at various stages of development were selected as representative samples for histological analysis. They were fixed using FAA solution of 90 ml 50% ethyl alcohol, 5 ml glacial acetic acid and 5 ml formalin solution. Tissues were dehydrated through an ethyl-butyl alcohol series for 48 h, embedded in Paraplast-plus, sectioned at 10 µm and then stained with fast green and safranin (Sass, 1958) prior to examination by light microscope.

Results

Initiation of callus and cell suspension culture

Callogenesis was initiated from embryos eight weeks after culture on CY3 medium containing 2-mg/l 2,4-D. Embryo-derived callus was friable, white creamy colored with amorphous texture. To initiate cell suspension, eight-week-old calluses were transferred to MY3 medium. The callus readily disaggregated into fine suspension upon agitation in the culture flask. No morphogenetic response occurred in this medium.

Induction of embryoids

When suspension cells were transferred to FY3 medium, embryoids and rhizogenesis were observed (Figure 1). Embryoid formation was evidenced in the experiment that cells were incubated for a period of 9 days with 3-days interval of subculture. These embryoids underwent complete embryogenesis when they were transferred to FY3 solid media supplemented with 0.05% activated charcoal for 3 months. The embryoids could be distinguished by the presence of white, opaque,

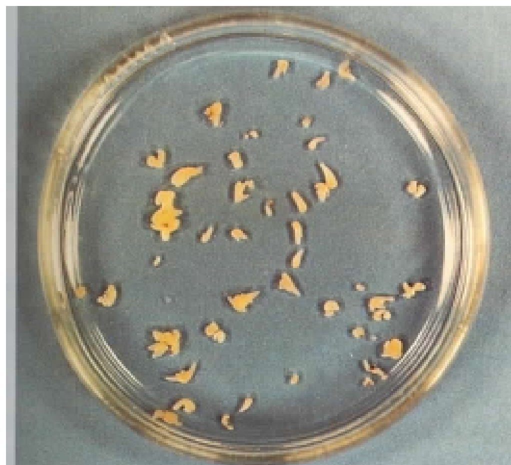

Figure 1 Different morphological conformity of embryoids and rhizogenesis derived from cell suspension cultured on FY3 medium (x 165).

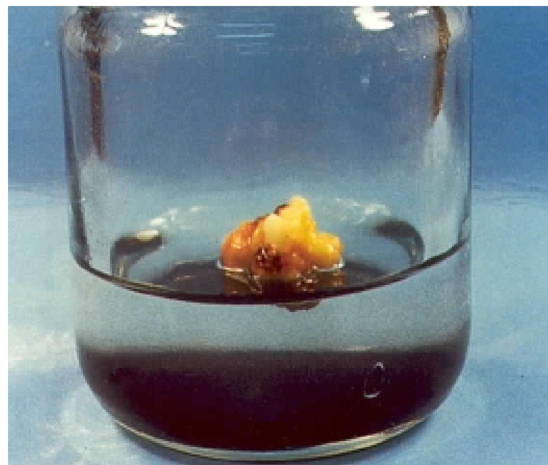

Figure 2 Opaque white and nodular structure formed on FY3 medium supplemented with 0.05% activated charcoal (x 165).

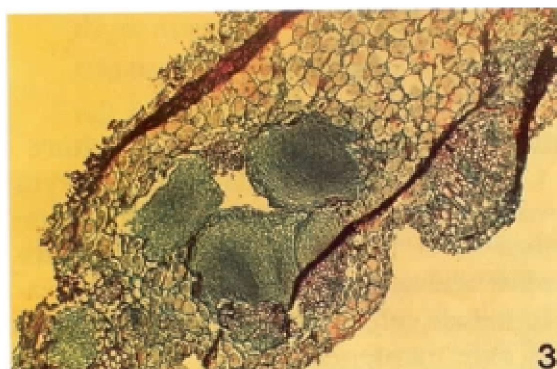

Figure 3 A group of meristematic centers composed of dense cytoplasm emerged on the surface of callus (x 165).

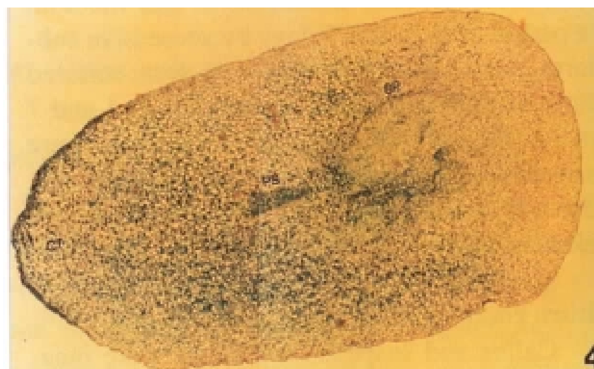

Figure 4 Longitudinal sections through the embryoid showing shoot and root pole. (x 165). (CT = cotyledon; PS = procambial strand; SP = shoot pole).

Figure 5 Transverse sections of procambial cells as individual bundles (arrow) in the embryoid arising at the peripheral of callus (x 165).

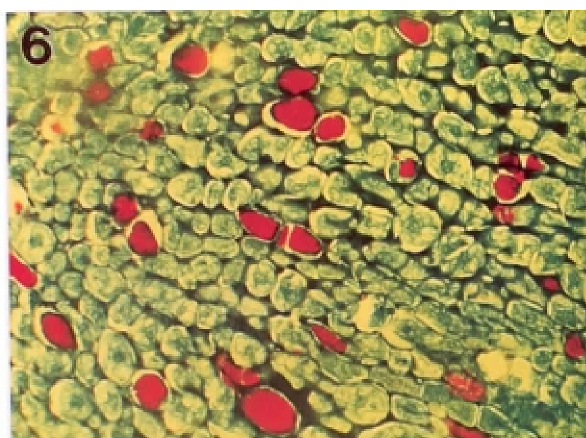

Figure 6 Cells containing storage lipid in droplets. (x 165).

and compact nodules (Figure 2). However, no further differentiation took place when prolonged culture on this medium.

Histological studies of calluses and embryoids

Section of the friable culture revealed well-organized small cells loosely attached to each other. Histological examination showed adjacent numerous centers of meristematic activity. The cells in such centers were smaller with dense cytoplasm

and stained more intensely than those in the outer part of the calluses (Figure 3). The meristematic centers appeared to differentiate into embryoids. The embryoids at this stage consisted of shoot and root pole indicating a bipolar structure characteristic of embryos. The root pole is blunt and flattened. The shoot apex is surrounded by the cotyledon. A small slit, which separates the shoot tip zone from the cotyledon, is evident at the base of cotyledon (Figure 4). Procambial strands are visible as individual bundles in transverse section (Figure 5). At the bipolar stage, differentiated cells containing high storage lipid content were clearly visible as droplets within the cytoplasm (Figure 6).

Discussion

In the case studied here, changes of different media were needed for induction and development of callus and embryoids. In oil palm, a callus was first obtained in the medium rich in 2,4-D (2 mg/l) subsequent transfer to the medium devoid of 2,4-D and supplemented with NAA, BA and coconut water was a prelude to embryogenesis development. Gradient of 2,4-D and addition of NAA, BA, and coconut water may have influenced somatic embryo development. Differentiation takes place when these embryoids were transformed to the medium lacking plant growth regulators. The pattern of culture on several successive media to produce somatic embryos in oil palm is comparable to other plant systems in which the phenomenon has been obtained (Ammirato, 1983).

It is well documented that plant growth regulators are responsible for modification of callus texture. This was held true in oil palm. The absence of 2,4-D in FY3 medium efficiently resulted in callus friability of oil palm cell suspension cultures. Apart from growth regulators, the embryogenic event of the suspension culture appeared to be closely related to the timing of subcultures. This finding is in agreement with Michaux-Ferriere and Carron (1989) who reported that unrenewed medium neither enables *Hevea brasiliensis* cell suspension to conserve its meristematic features nor enables pre-embryogenic cells to become true embryogenic cells.

Histological studies revealed that embryoids were derived from meristematic cells. The oil palm embryoids appeared to be similar to those described by DeMason (1988) in the palm *Washingtonia filifera* and Tisserat and DeMason (1980) in the date palm *Phoenix dactylifera*. The somatic origin of the embryoids was obtained from meristematic cells which were highly differentiated with numerous storage lipids. Such cells contain storage lipids, a source of energy, in the cytoplasm indicating that physiological processes associated with embryogenesis occur (Quinn *et al.*, 1989).

The present study demonstrates that somatic embryogenesis arises from meristematic cells and corresponds to the development morphology of zygotic embryos of oil palm. An improvement of culture conditions and duration of subculture time are still required in order to obtain oil palm capable of germinating.

Acknowledgment

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