Plant regeneration from embryo-derived callus of oil palm – the effect of exogenous polyamines

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Abstract
Regeneration in oil palm was achieved through somatic embryogenesis/organogenesis from embryo-derived callus. Callus was induced from mature embryos of the cross 281 (D)×18 (P) on modified MS medium supplemented with 2,4-D (113.12 μM) and 2-iP (14.76 μM). The embryogenic calluses obtained were transferred to Blaydes medium supplemented with 2,4-D (0.045 μM) and one of the following growth regulators: TDZ (4.54 μM), zeatin riboside (2.85 μM), putrescine (1 mM) and spermine (100 μM). Secondary somatic embryogenesis was found to occur in media supplemented with polyamines. The efficiency of formation of somatic embryos, secondary somatic embryos and shoot meristemoids were significantly higher in putrescine containing medium. Histological studies were also undertaken.

Abbreviations: BA – 6-benzyladenine; 2,4-D – 2,4-dichlorophenoxyacetic acid; 2-iP – 6-(γ,γdimethylallylamino)-purine; IBA – indole-3-butyric acid; MS – Murashige and Skoog medium; TDZ – 1-phenyl-3-(1,2,3-thiadiazol-5-yl) urea (Thidiazuron); t – tonne

Introduction
Oil palm (Elaeis guineensis Jacq.) is a crop species producing high quality oil, which can be obtained from the mesocarp of the fruit (palm oil) and the kernel of the nut (palm kernel oil). Palm oil is used mainly for cooking, preparation of margarine, shortening and also for non-food applications (soap, detergent, cosmetics, etc.). Oil palm is the most productive oil crop with yields of up to 5–7 t of palm oil/ha/year under optimum conditions. Also, the production costs for palm oil in its ecosystem are the lowest among all the crops (Graille and Pina, 1999). All commercial palms are F1 hybrids between selections with small kernels (dura) and large kernels (pisifera), but the hybrids (tenera) show very high variation in oil yield with the best plants yielding 40% more than average. As a monocotyledonous species with a single growing apex, the plant cannot be multiplied vegetatively.

A reliable and efficient procedure for in vitro propagation of elite highest yielding palms will increase yields in a significant way. Extensive research from the 1980s has been successful in regenerating plants from culture (Duval et al., 1995; Parthasarathy et al., 2001). Many of the regenerants show flowering abnormalities (‘mantling’) that lead to fruit abortion and no yield due to a form of somaclonal variation. The fruiting abnormalities (mantleness) in field planted ramets, together with mediocre yield advantage of less than 20% over control dura×pisifera (D×P) seedling materials, created a loss of confidence in clonal oil palm. Unfortunately, many details regarding tissue culture on oil palm are not available because the research was generally carried out by commercial firms. Several papers have reported that long-term culture in the presence of cytokinins could be linked to the flower malformation induced during oil palm regeneration process (Jones, 1990; Besse et al., 1992; Jones et al., 1995).
Earlier studies from our laboratory were based on regeneration from leaf explants using BA and zeatin riboside (Raju et al., 1989; Anitha and Sajini, 1996). Polyamines function in a similar manner to some plant growth regulators with high levels of free polyamines promoting cell growth and DNA stability (Galston and Kaur-Sawhney, 1990). Exogenously supplied polyamines have been known to induce somatic embryogenesis and plant regeneration in many plant species (Galston and Flores, 1991; Martin-Tanguy and Carre, 1993; Adkins et al., 1998; Sargent et al., 1998). However, no work on the use of polyamines has been reported in oil palm. The objective of the present study was to substitute cytokinins with polyamines for improving the formation of somatic embryos and subsequent germination rates from embryo-derived callus of the cross 281 (D)×18 (P).

Materials and methods

**Plant material**

Mature oil palm nuts of the cross 281 (D)×18 (P) were obtained from Regional Station, National Research Centre for Oil Palm, Palode, Kerala, India. Embryos were extracted from the nuts and sterilized in 0.1% (w/v) mercuric chloride with two to three drops of Tween for 15 min. The embryos were then thoroughly washed in sterile water four to five times and inoculated onto the media.

**Culture media**

Modified Murashige and Skoog (1962) medium (Anitha and Sajini, 1996) and Blaydes (1966) medium were used for the experiments. The media were solidified with 0.55% (w/v) agar. The pH was adjusted to 5.7 prior to autoclaving at 1.06 kg cm$^{-2}$ for 20 min.

**Callus induction and maintenance**

Sterilized embryos were inoculated into culture tubes containing 10–15 ml of modified MS medium (Anitha and Sajini, 1996) supplemented with 2,4-D (113.12 μM) and 2-iP (14.76 μM) for callus induction. The medium was solidified with 0.55% (w/v) agar. The cultures were incubated in the dark at 27±2 °C for 12 weeks until sufficient callus was obtained. They were subcultured at monthly intervals in the same medium during this period.

**Plant regeneration**

The calluses obtained were transferred to Blaydes medium (Blaydes, 1966) supplemented with 2,4-D (0.045 μM) and one of the following growth regulators: TDZ (4.54 μM), zeatin riboside (2.85 μM), putrescine (1 mM) and spermine (100 μM). The concentrations of these growth regulators were decided after initial optimization trials. Fifteen explants were used for each experiment and the experiments were repeated twice. Cultures were maintained under a 16-h light intensity. The somatic embryo production and plant regeneration rates were recorded at fortnightly intervals. The germinated plantlets were transferred into plain Blaydes medium solidified with 0.55% (w/v) agar in culture bottles for further growth. Plantlets with 4–5 leaves and with sufficient roots were transferred to a liquid medium (plain Blaydes medium) in big culture tubes (38×200 mm). When the plantlets were obtained from shoot meristemoids, they were transferred to Blaydes liquid medium supplemented with IBA (4.9 mM) for rooting when they reached 4–5 leaf stage.

**Plant acclimatization**

Healthy plants were removed individually from the culture tubes and washed with sterile water. Before transferring to pots, the plantlets were treated with Bavistin (1%) and thereafter with IBA solution (4.9 mM) for 1 h. The potting mixture used was sterilized soil, sand and coir dust in equal portions. Initially, the plantlets were covered with polythene bag. Gradually the bags were perforated and later the bags were removed during the night. After 4 weeks, the bags were removed completely.

**Histological studies**

Specimens for histological studies 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.