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CLONAL MULTIPLICATION OF OIL PALM (*ELAEIS GUINEENSIS* JACQ)

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ABSTRACT

Five mm long tender leaf explants and leaf base explants taken from 3-year-old seedlings were cultured on defined media supplemented with various auxins and cytokinins. Cultures were incubated in the light. The expalnts produced callus from the veins at the cut surface in 2-3 weeks on R medium containing 20 mgl^{-1} 2,4-D and 0.1 mgl^{-1} BAP. Somatic embryoids developed on lowering the 2,4–D concentration and replacing it with NAA. Bipolar and tripolar embryoids originated as snow-white protuberences from the sub-surface layers of the callus. The process of germination of the somatic embryoids was similar to that of normal zygotic embryos. However, the formation of adventitious roots was enhanced with the addition of 0.01 mgl^{-1} IBA to the medium.

INTRODUCTION

Crop improvement in oil palm through conventional methods has been slow due to its perennial nature, long juvenile phase and high level of heterozygosity due to cross pollination and lack of conventional methods for vegetative propagation. The crop is essentially seed propagated and the commercial plantations are with hybrid *tenera (dura x pisifera)* palms. The use of hybrids comes in the way of establishing uniform plantations as the inbred parental lines are not available (Nwankwo and Krikorian, 1982). Recently much emphasis has been given to the oil quality aspects as well. It is thus attractive to develop methods for the vegetative propagation in order to establish uniform plantations of elite genotypes. There are many reports on the vegetative propagation of oil palm through tissue culture (Jones, 1984; Rabechault and Martin, 1976, Paranjothy, 1984; 1986). In this communication the development of clonal oil palms from tender leaf explants of three year-old *tenera* seedlings through somatic embryogenesis with a minimum callus phase is presented.

Explant

The central tender leaf column of the seedling along with a covering leaf sheath was dissected with the growing apex and, wiped with 70% ethanol and flamed. After trimming the cut ends which came in contact with ethanol the covering leaf sheath was removed. Tender leaf was then cut into 5 mm long explants with 2 or 3 leaf folds.

Callus Induction

For callus induction the leaf explants

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were placed on a medium containing 2,4-D. Addition of charcoal to the medium seemed necessary to minimise the toxic effects of the oxidative products of polyphenols released by the injured tissues. The cultures were incubated at 29 ± °C under varying intensities of fluorescent light (16h photoperiod). The relative humidity was 60-70 per cent.

The best results were obtained where 2,4-D (20 mgl^{-1}) was used along with BAP (1 mgl^{-1}). After two to twelve weeks of incubation depending on the plant used, callus proliferation appeared at the morphologically lower cut end of the explant (Fig. 1). In some cultures proliferation was seen at the other end also. The callus at the lower end had small compactly arranged cells with dense cytoplasm while at the other end it was filamentous with large round vacuolated cells.

Somatic Embryogenesis

The explants with a few cell layers of thick primary callus were cultured on a series of media in which 2,4-D was gradually replaced by NAA. This led to the development of somatic embryoids in about 8 – 20 weeks. The somatic embryoids appeared as small, white, club shapped structures (Fig. 2) on compact calli. These structures attained a size of 3-5 mm in length within 6-8 weeks of their appearance. They had well defined shoot and root poles (Fig. 3). 10-15% of the embryoids had a tri-polar structure where the third pole represented the haustorium (Fig. 4) which never developed beyond 5 mm in length.

The embryoids were not connected to each other or to the explant by vascular elements. They were independent structures which developed from different regions of the callus. After the embryoids had attained a minimum of 2 mm size, the embryoids could be separated from the explant tissues easily.

Germination of Embryoids

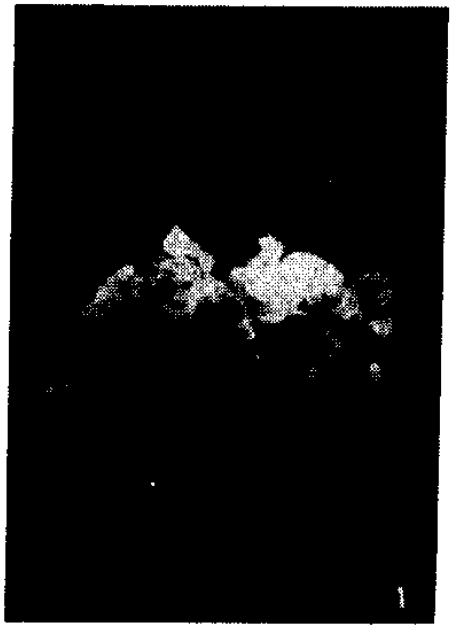
Once the embryoids attained a minimum length of 4 mm, they could be handled individually. On a germination medium over 50 per cent of the embryoids had shoot emergence first (Fig. 5), 30 per cent had simultaneous shoot and root development, 15 per cent with root development followed by shoot and 5 per cent shoots failed to grow after root development.

Hardening

The plants after attaining a size of 5 cm with at least two roots having laterals were transferred to a mixture of sand and vermiculite moistened with Hoagland's solution with 0.01 mgl^{-1} IBA in mineral pots and kept under high humidity conditions (Fig. 6). The growth of the plants was faster in the potting mixture. In three weeks time they were transferred to the garden mixture. Over 80 per cent of the plants survived in the garden mixture.

DISCUSSION

Ever since the success in clonal propagation of oil palm was demonstrated by Jones (1974) much attention has been centred on the commercial exploitation of this method. Tissue culture method currently followed comprises the elaborate callus phase followed by shooting from meristemoids and rooting (Paranjothy, 1986). In



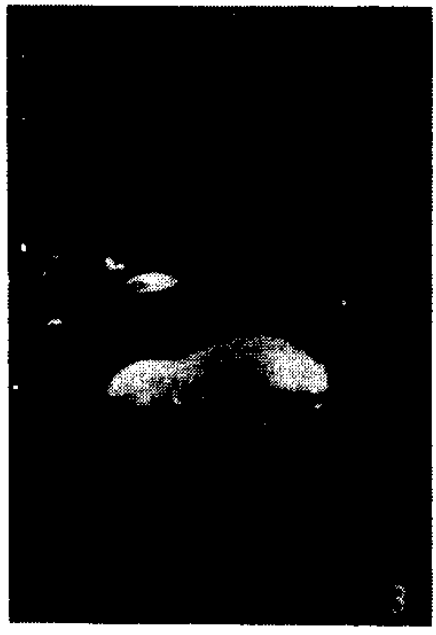


Fig. 1. Leaf explant with callus lining at the cut end.

Fig. 2. Embryoids developing from the callus lining.

Fig. 3. Single bipolar embryoid with shoot pole (s) and root pole (r).

Fig. 4. A tripolar embryo with shoot (s), root (r) and haustorial (h) regions.

Fig. 5. Germination of the embryoid with shoot and root poles developing simultaneously.

Fig. 6. Plantlet transferred to mineral pot.

contrast to the meristemoid formation which essentially needs separate shooting and rooting treatments for developing the plantlets (Wooi, 1984), the method described here produces embryoids from the compact calli at the lower cut end of the explant, very similar to the sexual embryos and germinate with both shoot and root. The germination of the embryoids was found to be 75 to 80%. The time taken for the production of the plantlet of 5 cm height in tube from the leaf explant was only four months. This time was, how- ever, varying with the plant used for the initiation of the culture.

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