STAGES IN THE VEGETATIVE 'PROPAGATION OF OIL PALM, Elaeis *guineensis* Jacq. THROUGH TISSUE CULTURE

Keywords: *Elaeis guineensis* Jacq., stages. vegetative propagation, tissue culture. NAA medium, normal inflorescences

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Oil palm (Elaeis guineensis *Jacq.*) *was vegetatively propagated by tissue culture in NIFOR on a NAA medium which provided a very short callus stage. The following stages were identified: callus (initiation and maintenance), embryoid, plantlet (ramet), prenursery (sand culture), nursery, and field planting. Normal male and female inflorescences which led to normal fresh* **fruit** *bunches (FFB), were produced by the tissue culture palms two and a half years after planting the plantlets in the field.*

INTRODUCTION

Vegetative propagation of the oil palm (*Elaeis quineensis* Jacq.) by tissue culture has four significant advantages over conventional breeding. Firstly, it enables rapid multiplication of uniform planting materials with the desired attributes, thereby providing the maximum return for investment and the requisite materials for meaningful agronomic experiments. Secondly, it offers a new opportunity in oil palm breeding. By obtaining haploid palms from pollen/anther culture, the process of hybridization to produce homozygous diploids is considerably reduced. Thirdly, it opens new avenues of research for oil palm biotechnology.

However, the expectations of clonal palms have been dampened by the occurence of fruit and flower abnormalities (Corley et al., 1986; Hartley, 1988; Paranjothy, 1989). The initiation of callus from leaf or root tissue is generally on a medium containing a high concentration of 2,4–Dichlorophenoxy acetic acid (2,4–D) (Duval et *al.,* 1988; Blake, 1983; Rabechault and Martin, 1976; Smith and Thomas, 1973). However, at high concentrations, 2,4–D and 2,4,5–Trichlorophenoxy acetic acid (2,4,5-T) are phytotoxic to broad leaf plants and used as herbicides (George and Sherrington, 1984; Vasil and Vasil, 1972).

Furthermore, cytological examination of callus from roots of oil palm grown on 2,4-D medium contained a high proportion of polyploid and aneuploid cells (Smith and Thomas, 1973). All researchers who have successfully carried out in vitro vegetative propagation of oil palm have used somatic embryogenesis on calluses obtained on 2,4-D media (Jones, 1974; Rabechault and Martin, 1976; Paranjothy and Othman, 1982; Nwankwo and Krikorian, 1983; Blake, 1983; Duval et al., 1988; and Paranjothy, 1989). Hence the abnormalities in clonal propagated oil palm might be due to the high concentration of 2,4, -D routinely used for its culture as 2,4-D is reported to cause mitotic spindle abnormality (Bayliss, 1973).

Corley et al. (1986) however reported that two of their clones which flowered abnormally had not been exposed to 2,4-D at all. This may be due to the prolonged callus stage which is not advantageous to cloning oil palm as explained by Sogeke (1996a). Duval et *al.* (1988) also associated flowering abnormality with fast growth of the callus. However, Butcher et al. (1975) showed that there was a greater tendency for fast growing cultures of *Helianthus annuus* a non-polysomatic species, to become polyploid. Hence, fast growth of callus per se may not necessarily be advantageous to cloning oil palm. Above all, Shamina (1966) and Sunderland (1973) working with *Haplopappus gracilis* observed that cultures maintained on media containing 2,4-D had cells with a greater range of ploidy levels than cultures on media with NAA as the auxin. They both independently concluded that as 2, 4-D cultures had increased growth rates, 2,4-D may favour the growth of tetraploid cell lines.

In several laboratories, therefore, investigation into the hormonal status in abnormal palms are now pursued (Paranjothy, 1989; Besse et al., 1992; 1994; Jones et al., 1995). Furthermore, Corley et al., 1986 and Hartley, 1988 suggested the time of culture and the media be adjusted to overcome the abnormality. 1-Naphthyl-Acetic acid (NAA), which is much milder than 2,4-D, was instead used in this study. The aim was to obtain callus on a non–2,4–d medium with a short callus stage, to determine the other stages in the in vitro vegetative propagation of oil palm, and to observe any abnormality associated with the palms.

MATERIALS AND METHODS

Callus initiation from immature leaf explants of young oil palms (*Elaeis guineensis* Jacq.) was on modified Eeuwens (1976) inorganic medium supplemented with sucrose 30g/l, inositol 100mg/l, aneurine–HCl 0.5mg/l, niacin 0.5mg/l, pyridoxine–HCl 0.5mg/1, casamino acid 500mg/l, and 1-naphthyl acetic acid (NAA) 15mg/l. Agar at 7g/l was added and the pH kept at 5.7. This was the initiation medium Y3(3). All culture up to the plantlet stage were incubated under continuous flourescent light (200 lux) at 28°C±1°C and all the operations done under aseptic conditions. Full details of the methods involved have been published by Sogeke (1996a). Plantlets at the pre-nursery stage were grown at room temperature (28°C–30°C) under fluorescent light (200 lux) with the flasks uncovered while the nursery and field planting were under natural conditions.

RESULTS AND DISCUSSION

Callus Stage (initiation and maintenance)

Callus formation (CF) with pneumathodes (Sogeke 1996a; Hartley, 1988; Blake, 1983) was obtained on Y3(3) 42258 days after inoculation of the medium with surface sterilized leaf explants (*Figures 1* and *2).* The pneumathodes were similar to those observed in this study on roots of sprouted seeds grown inside white polybags under wet conditions. They were therefore exgrowths of roots caused by the moist condition of the media as was also reported by Hartley (1988) for sprouted seeds growth under wet conditions. Maintenance of the calli *was* on Y3(3) containing less NAA (5mg/l)– Maintenance Medium Y3 (3A)-to obtain fast growing calli one month after CF.

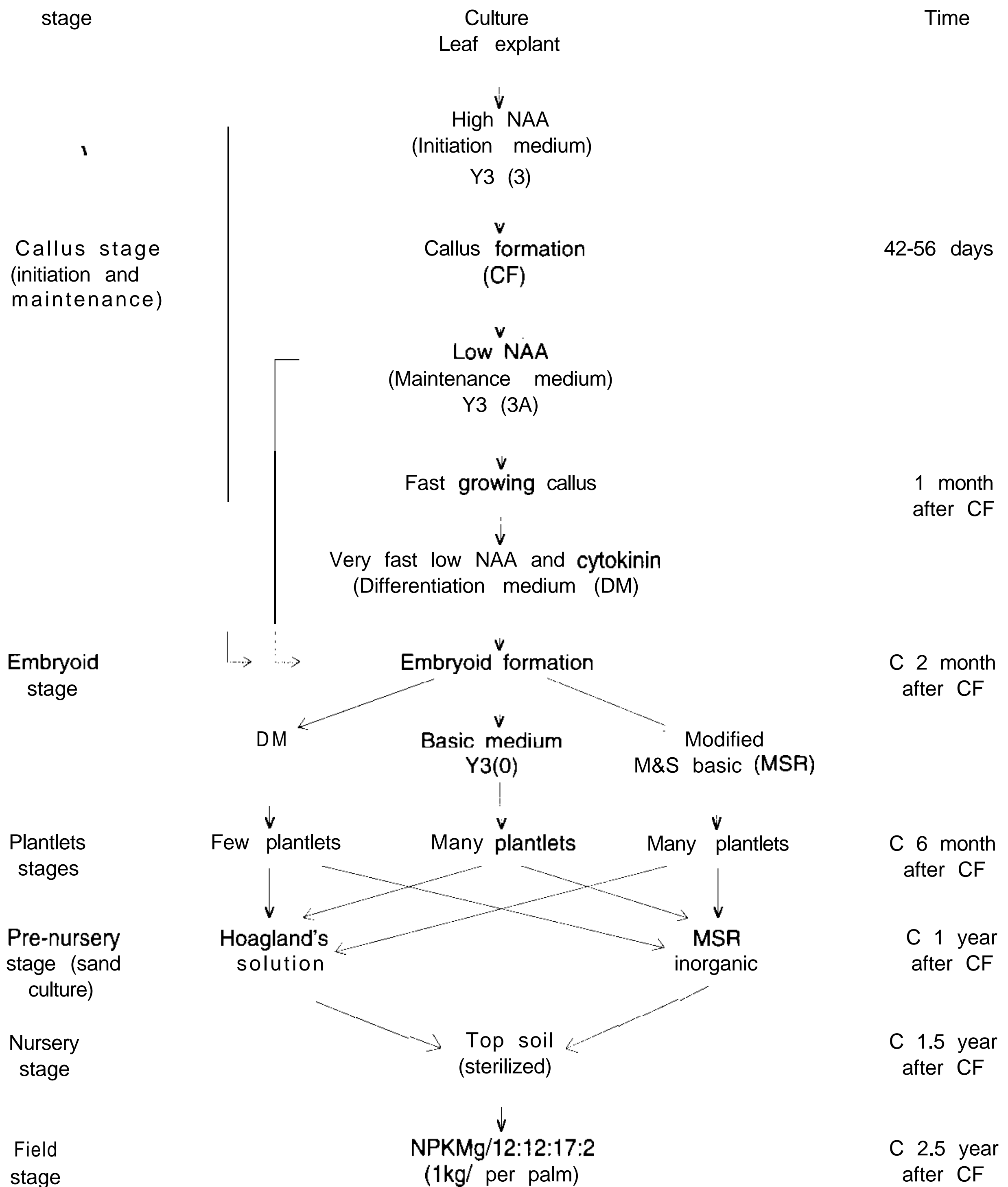

Figure 1. Schematic representation of the stages in vegetative propagation of oil palm by tissue culture

Figure 2. Callus formation with pneumathodes on leaf explant of Elaeis guineensis *Jacq.*

Embryoid Stage

Spontaneous embryogenesis was stimulated *ca.* two month after CF on the Differentiation Medium (DM) which was Y3(3A) containing 0.5mg/l NAA instead of 5mg/l, plus 2mg/l, Kinetin *(Figures 1* and *3)*. Embryoids were also obtained directly on Y3(3) and Y3(3A) without any exogenous cytokinin *(Figure 1)*.

Figure 3. Embryogenesis in callus culture of Elaeis guineensis *Jacq.*

Plantlet (ramet) Stage

Plantlets were obtained on DM less then six months after CF but much enhanced on the basic medium of modified Eeuwens (1976) (Y3(0) and Murashige and Skoog (1962) as modified by Hyndman *et al.* (1982) (MSR) *(Figures 1* and *4)*. Plantlets were also subsequently obtained on Y3(0) and MSR from embryogenic callus without exposure to any kinetin media.

Figure 4. Plantlet (ramet) of Elaeis guineensis *Jacq.*

Pre-nursery Stage (sand culture)

Plantlets were transferred singly into sterilized 250ml Erlenmeyer flasks containing 100g of white sand moistened with 15ml of either sterilized Hoagland's (1950) solution or MSR inorganic solution *(Figure 5)*. The pre-nursery stage lasted six months after the plantlet stage *(Figure 1)*.

Figure 5. Pre-nursery stage (sand culture) of plantlet (ramet) of Elaeis guineensis *Jacq.*

Nursery Stage

Immediately after the pre–nursery stage, the plantlets were transferred to black polythene bags size 28 x 26 x 500 G, containing sterilized top soil shaded from direct sunlight *(Figure 6)*. The nursery stage lasted one year.

Figure 6. Plantlet (ramet) of Elaeis guineensis *Jacq. in polybag at the nursery stage*

Field Stage

The plantlets were finally transplanted into the field two and a half years after CF where they were given rustica fertilizer (NPKMg/ 12:12:17:2) at the rate of 1kg per palm towards the end of the rainy season *(Figures 1* and *7)*.

Figure 7. Two year old oil palm propagated by tissue culture growing in the field

These identifiable stages are comparable to those described by Hartley (1988). The palms which have been grown satisfactorily *(Figure 7)* produced normal female and male inflorescences $2\frac{1}{2}$ years after field planting and eventually normal fresh fruit bunches (FFB) *(Figure 8)*. Full details of the characteristics of these palms will be published later.

Jones *et al.* (1995) reported that in isogenic lines of clonal oil palm, abnormal lines had significantly lower concentrations of cytokinins than normal lines. The only case in which a significantly higher cytokinin content was found in normal versus abnormal tissues was in a comparison within a genotype. While Besse *et al.* (1991) found large well replicated differ

Figure 8. Tissue cultured oil palm bearing normal ripe and unripe bunches three years after planting in the field

ences in the cytokinin contents of mature female flowers from mantled (abnormal) and normal inflourescences, Jones *et al.* (1995) were unable to find any such difference at earlier stages of development when the differences should also have been expressed. The latter authors also found that tissue culture materials contained high concentrations of cytokinin glucosides compared with non-cultured tissues even in cultures not treated with exogenous cytokinins.

Sogeke (1996b) separated plant cell, tissue and organ cultures into four groups on the basis of their exogenous hormonal requirements for growth and development, namely, those which did not require any exogenous phytohormone or natural complexes; those which required only an auxin; those which required an auxin and a cytokinin; and those which required natural complexes which invariably contained at least one or more phytohormones particularly auxins and cytokinins (Haagen-Smit *et al.*, 1946; Stowe and Thimann, 1953; Radley and Dear, 1958; Letham, 1973, 1974; Van Staden and Drewes, 1975 and Van Staden, 1976). It is generally believed that for plant growth and development, auxin and cytokinin are the keys, and they are therefore the prerequisites for totipotency (Skoog and Miller, 1957; Linsmaier and Skoog, 1962, Steward, 1970; Vasil and Vasil, 1971 and Murashige, 1973). Sogeke (1996b) argued that any cell, tissue or organ which does not require an exogenous supply of either or both of these phytohormones must be able to synthesize them *in situ*. Several works have confirmed this (Kulescha and Gautheret, 1948; Einset and Skoog, 1973; Miller, 1974, 1975; Peterson and Miller, 1976; Pengelly and Meins Jr., 1977; Scott *et al.*, 1980; Sogeke and Butcher, 1991).

Jones (1990) and Besse *et al.* (1994) reported a very low cytokinin content in undifferentiated oil palm callus which normally consists of many cells which are non-dividing, while embryoids and nodular calluses, which have many actively dividing cells, contained high levels of cytokinins. Hence, once the emgryogenesis phase has started, exogenous cytokinin should no longer be required as the cells would be able to synthesize cytokinin in abundance. Therefore, the continued use of exogenous cytokinin in the cloning medium, as is the common practice (Blake, 1983; Corley *et al.*, 1986; Duval *et al.*, 1988; Paranjothy, 1989; Sogeke, 1996a), would not be ideal for the growth and development of clonal oil palm.

Jones *et al.* (1995) found a very complex spectrum of cytokinins in tissues of intact oil palm which could explain the non-requirement of a cytokinin in callus initiation (Blake, 1983; Corley *et al.*, 1986; Duval *et al.*, 1988; Sogeke, 1996a). They therefore concluded that concentration *per se* did not cause the flowering abnormality but that a high concentration of cytokinin applied during tissue culture may have disrupted later gene expression. Sogeke (1996a), however, used kinetin to obtain oil palm embryogenesis on NAA medium and observed no abnormality in his tissue cultured palms.

Obasola *et al.* (1978) reported that parthernocarpic fruits, similar to those of clonal oil palms, were induced in oil palms by – (2,4,5-

trichlorophenoxy)-propionic acid. They therefore suggested that flower and bunch abnormalities in oil palms might be due to hormonal factors whereby increase in the concentration of hormones in the palms could cause biochemical and physiological disorders within the palm leading to the expression of abnormality. They concluded that since this abnormality could be induced by both natural and artificial means there was a possibility of these palms having similar hormonal systems which triggers off the formation of these abnormalities.

While tissues of several plants species can be cultured in the absence of an exogenous cytokinin, very few can be grown without an auxin in the medium (Sogeke, 1996b). The notable exceptions are the habituated cultures and plant crown gall tumours (Butcher et al., 1975; Sogeke and Butcher, 1976) which can synthesize both hormones in culture (Einset and Skoog, 1973; Miller, 1974, 1975; Peterson and Miller, 1976; Scott et al., 1980; Sogeke and Butcher, 1991). Sogeke (1996a), however, reported cases of embryogenesis of oil palm callus on a medium with only NAA as the exogenous hormone. Furthermore, he also observed direct root organogenesis from oil palm leaf explant on a NAA medium with no cytokinin. Above all, he was also able to obtain plantlets from callus which had never been cultured on a medium with an exogenous cytokinin.

This report, which is the first detailed publication of vegetatively propagated oil palm by tissue culture on a non-2,4-D medium clearly demonstrates the possibility of cloning oil palm on a medium with NAA as the only exogenous phytohormone. It creates the need to critically asses the role of 2,4-D in clonal oil palm abnormality and necessitates further investigations of the use of NAA and other milder auxins in modified basic media for plant tissue culture.

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