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In Vitro Flowering and Fruit Setting of Oil Palm *Elaeis guineensis* Jacq.

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In vitro flowering and fruit setting was firstly reported in oil palm micropropagation. Vitro-flowering at 10.2% was obtained in MS medium supplemented with 12 mg/l paclobutrazol (PBZ), 8 mg/l α-naphthaleneacetic acid (NAA) in the presence of high concentration of sucrose (7%). However, long term culture of seedlings or plantlets *in vitro* is needed. Practically, 6 months of culture with routine subculture at monthly intervals was observed. Upon two consecutive subcultures (two months) of vitro-flowering plantlets to ARDA medium efficient fruit setting was obtained. The fruits were small in size with three lobe of stigma. Long section of the fruits revealed only mesocarp without seed. This suggest that fruits might develop parthenocapically.

Key words: *In vitro* flowering, oil palm, paclobutrazol, plant growth regulators, fruit sets

Introduction

Oil palm is pioneer tropical edible plant that had been introduced in the last two decades in the developing country in Southeast Asia. This vegetative palm oil had been taking primary categorized as strategic industrial project, due to rise up as much as principal sector income in country producing of oil palm such as Indonesia, Malaysia and Thailand. A huge of scientific reports have been conducted on oil palm micropropagation (Te-chato *et al*. 2003; Te-chato and Hilae, 2007) in order to mass propagation of oil palm to keep pace with the increment world oil palm demand.

Nowadays, there are many reports have been released on *in vitro* flowering study (Nizam and Te-Chato.,2010; Zang e*t al.* 2002; Franklin *et al.* 2000, Jumin and Ahmad. 1999, Jumin and Nito1996). The main objective of this study was to gain a better understanding of the underlying principles and

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control of the flowering and reproductive processes. In fact, those factors were not well defined on *in vitro* flowering and growth of embryo-derived shoots (EDSs) of oil palm. Flowering is the crucial transition point from vegetative to generative growth named "switch-on" mechanism was intensively controlled by various complex factors such as flowering time and meristem identity genes (Kostenyuk *et al*. 1999; Simon *et al*. 1996; Nilsson and Weigel, 1997). There is no clear confirmed the influence on floral mechanism. It may be influenced by various factors affecting plant development (Sachs and Hackett, 1983), including plant hormones (Bonnet Masimbert and Zaerr, 1987; Dickens and Staden, 1988 and 1990) and nutrient in culture medium (Dickens and Staden, 1988; Bernier *et al*. 1993). *In vitro* flowering in oil palm is the understanding way that the factor might be caused by either physiological phenomenon or hormone substance.

In terms of lacking information referred to *in vitro* flowering in oil palm methods, this is a new finding of the development of efficient plant tissue culture procedures for *in vitro* flowering in this plant. This mechanism is important for the application of these technologies for improvement. The main purpose of this study was to create a rapid method that might influence on *in vitro* flowering and subsequence fruit setting in order to optimize the cultural conditions for embryo derived shoot development of oil palm. Especially, several effects of various plant nutrient whether carbon sources and hormone supplied known to affect plant growth development.

Materials and methods

Plant material and culture conditions

EDSs of Thepa clone were selected as plant material. Those shoots were established by the protocol described by Te-Chato *et al.* (2004) which normally lack of roots. They were maintained under two different methods. First method was maintenance on solidified MS medium without plant growth regulators (PGRs). The second method was maintenance in liquidified MS medium which the ingredients were decreased to half strength of original concentration, so called ½ MS. The latter medium was supplemented with 0.06 mg/l ∞-naphthaleneacetic acid (NAA) and 0.03 mg/l 6-benzyladenine (BA). All cultures were maintained under light conditions (25µmol/m^2/sec, 14 hour photoperiod) at 28±2^{o}C and routinely subcultured to fresh medium of the same components at monthly intervals for their proliferation. These shoots were individually excised and used for further experiment.

Root and flower induction from EDSs

Single micro-shoots at approximately 4 cm in length from EDSs were excised and transferred to rooting medium. Rooting medium was solidified ARDA medium modified by supplementation with several concentrations of PGRs and sucrose. Two factors, PGRs and concentrations of sucrose were examined for *in vitro* root and flower induction. For sucrose, concentration at 0, 30, 50, 60, 70, 80 and 90 g l^{-1} was designed. Each concentration of sucrose was combined with NAA at concentration 0, 2, 4, 6 and 8 mg l^{-1} together with paclobutrazol (PBZ) at concentration 0, 3, 6, 9 and 12 mg l^{-1}. All media were adjusted to pH 5.7 before adding agar and autoclaving. The cultures were maintained under 14 h photoperiod of 25µmol/m^2/s at 28±2^{o}C. After 4-6 weeks of culture percentage of root and *in vitro* floral induction were recorded and statically compared in each factor using completely randomized design (CRD).

Fruit setting and morphological observation

In order to stabilize *in vitro* growth and development of flower prolonged subculture period is needed. Vitro-flowering plants were transferred to basal MS with 3% sucrose but without PGRs and subcultured at monthly intervals for 6 months. Fruit setting and morphological of those fruits were examined.

Results and discussions

Root and flower induction from EDSs

Combination of NAA ranging from 2 to 8 mg/l and PBZ at 3 to 9 mg/l influenced on flower formation (Table 1). So far, the two PGRs promoted elongation of leaf sheath surrounded shoot apex. There is abscission layer at basal part of the sheath caused the senescence of those leaves after two weeks of culture. At week three a new leaf with shoot emerged from the apex leading to the collapse of the old one (Fig. 1A). After four weeks of culture healthy plant with shoot was obtained and the first root could be seen (Fig. 1B). Elongation of vegetative organs was confirmed the beginning of the further plantlet growth. The basal part of shoot could response to form adventitious root which was originate from periderm, cell near the vascular cambium and phloem. Growth of those plantlets gradually increased and floral development was observed after 5 months of culture (Table 2). Numerous factors contribute to this process, especially, PGRs are one of the most main factors (Amarjit, 2000). In the absence of PBZ, this phenomenon was not observed. It seems that this mechanism was accelerated by PBZ. The mode of action of PBZ has been

associated with reduction of water potential (Edwin *et al*., 2008) which decreased transpiration, thus, improved root formation and malfunction. Although in tissue culture there seems to be sufficient water flow (Beruto *et al.,* 1999) which may be favored by the stomata being continuously open (De Klerk and Wijnhoven, 2005).

Sugars are another factor affecting physiology processes in plant growth and development. Among those sucrose is commonly used as carbon and energy sources in plant tissue culture (White, 1943; Murashige and Skoog, 1962; Gamborg, 1970) which influences upon plant regeneration (Chen and Chang, 2002). In addition, sucrose is primary source known for reliable in induction and development of flowers *in vitro*. The presence of carbon sources in culture medium is necessary for floral stimulation (Singh *et al*. 2006). In this study, the highest sucrose concentration tested (9%) promoted growth of shoots in association with root formation. Furthermore, sucrose is the key important role in floral induction. Similar results have been reported in many plant species such as rose (Vu *et al.*2006), *Passiflora suberosa* (Scorza and Janick. 1980), *Vigna mungo* (Ignacimuthu *et al*. 1997). Jumin and Nito (1996) reported that addition of 3-7% sucrose to culture medium could induce floral induction in *Fortunella hindsii*. The better response was achieved when cultured on medium containing 7% sucrose in the presence of 8 mg l^{-1}NAA and 12 mg l^{-1}Paclobutrazol (Table 1, Fig. 2). Both PGR and sucrose might have a synergistic effect in plant growth development.

Attempts in root induction of shoot are very important in the final step of any *in vitro* propagation scheme and necessary to adopt a separate rooting procedure using special culture medium. The results from previous study showed that NAA and PBZ containing medium were more efficacies on root induction from EDSs without root. Both NAA and PBZ were recommended to be advantage for root induction in tissue culture of woody plants (Nizam and Te-chato, 2010). Sometime these PGRs containing medium act as promotion effect on shoot elongation before rooting.

Prolonged culture of rooted shoot in ARDA medium in the presence of NAA and PBZ together with higher concentration of sucrose at 7%, were found to induce floral development. However growth of rooted shoot in terms of its elongation was decreased. In addition, reduction of PBZ concentration to 3 mg l^{-1} promoted inhibitory effect on root induction whereas high concentration at 9 mg l^{-1} promoted a healthy growth of all plantlets significantly (Fig. 2). Sucrose is known to be the main carbon source for *in vitro* flowering induction (Rastogi, 1987). Availability of sucrose in aerial parts of the plant promotes flowering in *Arabidopsis thaliana* (Roldan, 1999). Sucrose and cytokinins interact with each other for floral induction in *Sinapis alba* by moving between shoot and root

(Havelange, 2000). In the present study, addition of sugar at high concentration of 7% (70 g/l) to the medium could induce floral induction *in vitro,* both male and female flower.

Occasionally, PBZ could induce flowering and increase the number of flower in several woody species such as *Rhondodendron* (Gent, 1995; Ranney *et al*. 1994). Paclobutrazol act as potent growth regulator activity and high efficacy of the response in ornamental plants. Paclobutrazol is able to exist in the different enantiomers or stereoisomers which plays inhibitory role in sterol biosynthesis (Sugavanam, 1984). One example of those activities is blocking multiple steps in the biosynthesis of gibberellins and sterols.

Fruit setting and morphological observation

The key success on plant tissue and organ culture is depending on artificial culture medium, which supplied the nutrients necessary for growth and development. The primary media constituents are greatly influenced by the nature of the culture medium used. To date, long term maintenance at more than 5 months was considered to induce flowering process. Tissue culture propagated plants of oil palm have been extensively planted in tropical climate, especially, Southeast Asia since past two decades, and healthy, early and synchronously of maturing crops were obtained. However, instances of occurrence of abnormal plants with changed morphology and reduced vigor have been observed in some populations. This could be due to the repeated subculture of the *in vitro* cultures.

The effect of prolonged subculture which initiated floral development process is not well understood. Besides being effected by external factors, the processes might be controlled by internal factors as well. However, the progressive of *in vitro* oil palm growth rate including flowering could be impacted by several subcultures. Due to the complexity factors affecting floral initiation several studies related to subculture time and photoperiod was reported in ornamental plant, C. *niveo-marginatum* (Kostenyuk *et al.* 1999). This phenomenon was inhibited by Gibberellic acid which markedly delayed flowering in C. *niveo-marginatum* even when the flower promoting treatment was applied. However, paclobutrazol, an anti-gibberellin agent, totally blocked the inductive effects of either cytokinin or pruning determined the transition period from the vegetative to reproductive stage of *Cymbidium niveo-marginatum* (Kostenyuk *et al.* 1999). In this investigation, prolonged subcultured to 6 months was long enough to initiate floral formation (10.2%) from plantlets raised *in vitro* under those conditions (Table 2, Fig. 3).

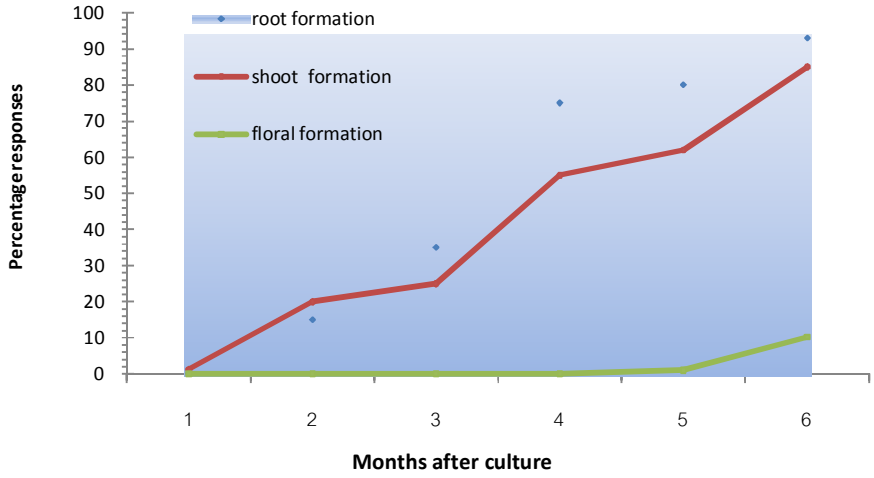

Fig. 1. Prolonged subculture maintenance period of EDS for growth and floral induction after 6 months of culture in medium supplemented with 8 mg l^{-1} of NAA in combination with 12 mg l^{-1} PBZ with 8% sucrose.

Table 1. Effect of PBZ and NAA containing MS medium supplemented with 8% sucrose, 200 mg/l ascorbic acid on flower induction and fruit setting in oil palm after 7 months of culture

PBZ (mg/l)	NAA (mg/l)	Number of flowers	Number of fruit sets
2.0	3.0	1.8 ± 0.31 d	0 b
4.0	6.0	2.0 ± 0.47 cd	0 b
6.0	9.0	4.2 ± 0.37 b	0 b
9.0	12.0	10.2 ± 0.57 a	3.3 ± 0.79 a

Mean sharing common letters within column is not significantly different (p <0.05) by DMRT

Table 2. Growth and development responses of vitro-flowering plantlets at different numbers of subcultures on ARDA medium supplemented with 7% sucrose and 200 mg/l ascorbic

Parameters observed	Growth/development response of 6-month-old vitro flowering plantlets in improved media			
	3th subculture	4th subculture	5th subculture	6th subculture
Stem height (cm)	5.5	6.8	8.5	8.4
Leaf width (cm)	1.7	3.1	3.5	3.2
Shoot number	1.0	1.0	1.0	1.0
Leaf length (cm)	8.3	9.4	11.0	11.5
Floral color	-	-	Dark green	Light green
Fruit color	-	-	Light green	red
Root characters	Long and thin	Long and thin	Short and thick	Short and thick
Root number	3.4	6.2	8.1	8.6

Table 3. Effect of sucrose concentrations on *in vitro* flowering and root formation of EDSs oil palm after 3 months of culture on ARDA medium supplemented with 200 mg/l ascorbic acid, 8 mg/l NAA and 12 mg/l PBZ

Sucrose concentration (g/l)	Root Formation (%)	Formation of Flowers (%)	Shoot elongation (cm)
0	0	0	0
30	7	0	5.5 ± 0.80.c
50	10	0	6.3 ± 0.67 bc
60	12	4.2 ± 0.37 b	7.1 ± 0.69 b
70	83	10.2 ± 0.57 a	14.4 ± 0.31 a
80	10	0	3.2 ± 0.02 d
90	0	0	0

Mean in a column with same alphabets is not significantly different (p <0.05, DMRT)

Fig. 2. Root induction from culturing EDSs on PGR-free MS medium (A) and ARDA medium with 0.5 mg/l NAA after 2 months of culture (B). Both culture media were supplemented with 7% sucrose and 200 mg/l ascorbic.

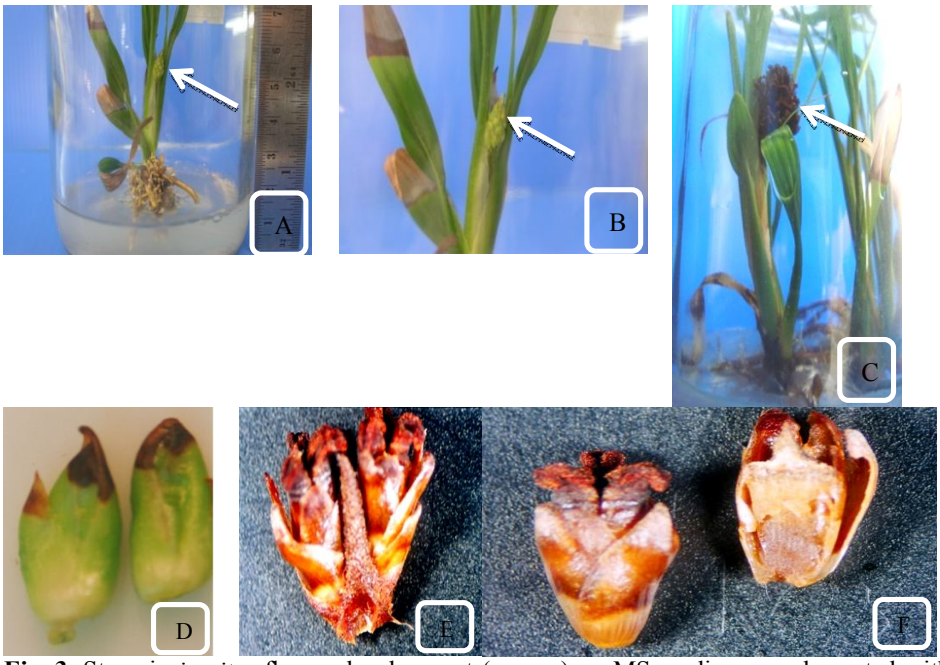

Fig. 3. Stage in *in vitro* flower development (arrows) on MS medium supplemented with 7% sucrose, 200 mg/l ascorbic acid , 8 mg/l NAA and 12 mg/l PBZ for 5 months (A) and 6 months (B) subsequent to transferring to ARDA medium with 0.5 mg/l NAA for 2 months (C) and development of young fruits after 6 months of culture (D) and mature fruit after 8-10 months of culture (E, F). (bar = 1mm.)

Paclobutrazol in combination with NAA was considered as key factor in induction of *in vitro* flowering of oil palm. The increment in plant growth and floral induction was influenced by both PGRs added to the media which were periodically changed with refreshing medium. Although paclobutrazol commonly recognized as growth inhibitor on some plants, but it was the beginning significant initiation of *in vitro* flowering in oil palm. On the other hands, the greatest flowering response occurred when sucrose concentration was raised to 7% or 70 g/l (Table 3). Young or baby green fruits (Fig. 3B, 3D) in inflorescence were observed after 6 month of culture on MS medium supplemented with 7% sucrose, 200 mg/l, 6-8 mg/l NAA and 9-12 mg/l PBZ. Development of mature fruits (Fig.3C, 3E, 3F) was obtained on ARDA medium with 0.5 mg/l NAA for further 2 months. Without subculturing to the new culture medium the green fruits failed to develop into mature fruit. The inflorescence together with the fruits withered and died soon after subculturing. This evident of further development was supported by nutrition factor contained in new culture medium. Unfortunately, the fruits were small in size with three lobe of stigma. Long section of the fruits revealed only mesocarp

without seed suggesting that they might develop parthenocapically. With high concentration of sucrose applied to the culture medium adverse effect of plant growth mechanism was observed. The present study gave clear evidence that sucrose at 9% was harmful on growth and elongation of both shoots and roots.

The advantage of *in vitro* flowering of oil palm obtained in this experiment will help us to understand factors affecting flower induction and their mechanisms. As it has been reported earlier that flowering of oil palm seedling was obtained from pre-nursery stage grown tissue culture plants. All of those plants produce flower or inflorescence at terminal bud leading to stop growing of the plant and finally died. This phenomenon causes a severe damage to oil palm plantation. If these mechanisms are well understood early screening of abnormal plants would be culling prior to hardening and growing to nursery. On the other hands, vitro flowering plants will be used as DNA template for molecular marker assist selection of those abnormal plants. Thus, results obtained from the present study will persue development a new protocol on precocious of nature flowering of oil palm.

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