The Effects of Potent Plant Growth Regulators (PGRs) on in vitro Flowering of Oil Palm

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Abstract

A new protocol on in vitro flowering has been improvements in oil palm using embryo derived shoot (EDS). The woody plant medium (WPM) supplemented with naphthalene acetic acid (NAA) (0, 2, 4, 6 and 8 mg/l) and paclobutrazol (PBZ) (0, 3, 6, 9 and 12 mg/l) were examined. After 5 months of culture the results proven that WPM supplemented with high concentration of sucrose (72 g/l), 8 mg/l NAA and 12 mg/l PBZ gave the best results in inducing in vitro flowering of oil palm at 10%. Flowering occurred in EDS was setting fruits in the same culture medium in the concentration of PGRs as mentioned earlier. This study provides a simple system for rapid in vitro flowering and fruit setting which will be very useful tools for further physiological and molecular biology studies.

Keywords: embryo derived shoots, oil palm (Elaeis guineensis Jacq.), paclobutrazol, in vitro flowering

Introduction

Principally, the oil palm is native to West and Central Africa. It is botanical classification, Elaeis guineensis, Jacq., is derived from the Greek elaiion (oil) and the specific name of guineensis is indicative of its origin from the equatorial Guinea coast (Henderson and Osborne, 2000). Nowadays, oil palm is the multipurpose in every upstream industry sector. Oil palm is used mainly for the purpose of both our daily needs and raw material in industrial sector. Interestingly, oil palm has got greater acceptance than the other oleaginous crops of the tropical belt due to its high yielding potential, contrary with traditional oil seeds, the fruit mesocarp of oil palm has a good reserve of storage lipids given the crop a distinct nutritional status commercially (Sathish and Mohankumar, 2007).

In vitro flowering of oil palm is the new beginning evolution in oil palm, it may possibly significant contribute to genetic development especially, of precious of in vitro oil palm to be reliable and accessible method in future research. Flowering is considered to be a complex process regulated by a combination of environmental and genetic factors (Tisserat and Galletta, 1993). The key important factors for this process are carbohydrates, growth regulators, light and pH of the culture medium (Heylen and Vendrig, 1998). The former researcher in oil palm is mainly conducted due to in vitro propagation by culturing young leaf or zygotic embryos (Te-chato et al., 2008, Thawaro and Te-chato, 2010). To date, several in vitro flowering studies has been revealed in many perennial plant species e.g. Kniphofia leucoccephala (Taylor et al., 1990), Bamboo (Joshi and Nadgouda, 1997) and Date Palm (Allouche et al., 2009) etc. However, in vitro flowering in oil palm is not well established and not yet been reported by any author. To know the mechanism of in vitro flowering of oil palm it will be of great important in studying molecular biology in order to use as marker for early identification of abnormality somaclones derived from tissue culture technique.

In this present study, some plant growth regulators affecting in vitro flowering of oil palm will be elucidated.
**Materials and methods**

In this study embryo derived shoots (EDS) of oil palm induced according to Nizam and Te-chato (2009) were used. Those shoots were derived from culturing young leaves of mature trees taken from Thepa Research Station, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Thailand. Primary callus and embryogenic callus were induced by the protocol of Te-chato et al. (2004) and the callus was maintained on embryogenic callus proliferation medium for at least two years (subculture monthly interval). Vitro-shoot developed on regeneration medium was also used for root induction.

To induce *in vitro* flowering, 3-month-old plantlets (7-8 cm height with 2 to 3 tiny leaves) were transfer to woody plant medium (WPM). All culture media were supplemented with different concentrations of naphthalene acetic acid (NAA) (0, 2, 4, 6 and 8 mg/l) and add paclobutrazol (PBZ) (0, 3, 6, 9 and 12 mg/l). The shoots were subcultured to fresh medium with the same component at every 4 weeks. The pH of culture media was adjusted to 5.6 prior to autoclaving (121 °C for 20 min). The culture was maintained in a culture room at 25 °C under a 12 h photoperiod of white fluorescent light at 60µmol m⁻²·s⁻¹. All above treatments were performed with three replications. Each replication consisted of two shoot. The data on growth and development of shoots were recorded and analysed statistically using SAS 6.0 (Statistically Analysis System).

**Results and discussion**

Embryo derived shoots of oil palm were able to induce root when placed on improved rooting WPM medium supplemented with various concentrations of NAA and PBZ. Whereas the greatest rooting percentage at 80% was obtained using WPM supplemented with 8 mg/l NAA and 12 mg/l PBZ (Figure 1b, c Table 1). Contrary result was found in controlled treatment which poorly result was obtained in WPM medium without those PGRs (Figure 1a).

They are too many factors affecting growth development in *in vitro* of oil palm. PGR is one of those factors promoting flowering in vitro in many plant species (Sim et al., 2007). PBZ, growth retardant, was recognized as inhibitor but acts as stimulator of flower bud induction. PBZ is azole derivative which had been found to promote the shoot inducing capability. Previously result of PBZ at low concentration of 3 mg/l gave the highest shoot number of 3.5 in korarima [*Aframomum corrorima* (Braun) Jansen] (Tefera and Wannakairoj, 2006) while a very low concentration at 0.05-0.075 mg/l gave the best result in floral induction in Friederick's Dendrobium (Te-chato et al., unpublished data). In this present study, it was found that slightly high concentration of PBZ was successfully induced flowering *in vitro*. The different in concentration used for this purpose might be species specific. Nizam and Te-chato (2009) reported that highest concentration of PBZ (6 mg/l) and NAA (9 mg/l) favor root initiation in oil palm and increase chlorophyll content two times higher than that of control.
Figure 1 *In vitro* root induction obtained from several culture media after 4 week of culture. 
A. WPM medium without any PGRs (control) (bar 1.0 cm = 0.92).
B. WPM medium with 6 mg/l NAA and 9 mg/l PBZ (bar 1.0 cm = 0.95).
C. Healthy roots occurred in an optimum culture medium (bar 1.0 cm = 0.92).

Figure 2 *In vitro* flowering from shoots of oil palm cultured in WPM supplemented with 8 mg/l NAA in combination with 12 mg/l PBZ and 7.2% of sucrose.
(A) Shoot was placed on medium after 2 month of culture (bar 1.0).
(B) Shoot stem become bigger after 4 months of culture (bar 1.0 cm).
(C) Shoot with fully developed flowers after 5 months of culture (bar 1.0 cm).
(D) Development of female inflorescence after 8 months of culture (bar 1.0 cm).
(E) Ripened of the fruits (4x).
(F) Longitudinal section of oil palm fruit (4x).
(G) Number of petals per seed of oil palm (4x).

Table 1 Effect of NAA and PBZ at different concentrations containing WPM medium on root formation percentage and *in vitro* flowering of oil palm after 7 months of culture.

<table>
<thead>
<tr>
<th>PGRs</th>
<th>Root Formation %</th>
<th><em>in vitro</em> flowering %</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA (mg/l)</td>
<td>PBZ (mg/l)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>53.3 bcde</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>55.6 bcd</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>60.0 bc</td>
</tr>
<tr>
<td>9</td>
<td>12</td>
<td>75.3 ab</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>80.3 a</td>
</tr>
</tbody>
</table>

F-test ** ** C.V. (%) 25.3 20.9

** Significant difference at p< 0.01

**Conclusion**

WPM supplemented with 8 mg/l NAA and 12 mg/l PBZ yielded rooting percentage at 80% after 4-5 months of culture. At this period of culture in the same PGR containing medium flower bud was initiated to form from axillary or terminal buds. At longer period of culture (7-8 months) fruit setting was found at approx. 10%.

**References**


