A LOW COST TEMPORARY IMMERSION BIOREACTOR FOR MICROPROPAGATION OF LOCAL PINEAPPLE (*Ananas comosus* L.)

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To my beloved mother and father
ACKNOWLEDGEMENT

Praise be to Allah, the Most Gracious and Most Merciful for giving me the chance to complete this dissertation.

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A laboratory scale temporary immersion bioreactor or also known as TIB has been developed in this study by applying the principle of temporary immersion system due to lacking of TIB development for local pineapple Sarawak cultivar production and higher production cost needed by using semi-solid culture system. The effect of immersion time on shoot production frequency using a modified temporary immersion bioreactor was determined and the shoot induction efficiency of TIB and semi-solid system was also evaluated. The modified TIB was operated by adjusting the time for controlled solenoid valve to allow the transfer of MS liquid medium from medium container to the culture container. Different immersion time was tested by setting up the timer to 3 min/2h and 15 min/2h throughout the three weeks culture period. Results showed that 100% shoot regenerated with maximum number of shoots produced per explant (3 ± 0.58 shoots) when cultured in the TIB with 3 min/2h immersion time. Meanwhile, out of the two culture systems, the shoot regeneration efficiency in TIB managed to produce 3 ± 0.58 shoots within three weeks whereas the semi-solid system required six weeks to produce similar amounts of shoots (6 ± 1.16) which reduced shoot production time by half compared to the semi-solid system. From this study, it is suggested that modified TIB has promoted shoot regeneration frequency thus enhancing pineapple plantlets productivity.
ABSTRAK

Dalam kajian ini, sebuah bioreaktor yang mengaplikasikan sistem rendaman sementara atau juga dikenali sebagai TIB telah dicipta untuk pembiakan secara in vitro nenas tempatan kultivar Sarawak pada peringkat makmal. Usaha ini telah diambil bagi mengatasi masalah kekurangan kajian dalam kemajuan TIB bagi pembiakan nenas kultivar Sarawak secara in vitro dan juga kerana tingginya kos penghasilan yang diperlukan dalam sistem terdahulu iaitu sistem media separa pejal. Kesal masa rendaman yang digunakan oleh TIB terhadap bilangan pucuk yang dihasilkan telah dikenal pasti dan keberkesanan penggunaan TIB berbanding sistem media separa pejal dalam induksi pucuk turut dinilai. TIB yang telah diubah suai dalam kajian ini beroperasi dengan menyelenggara masa kawalan bagi injap solenoid untuk membenarkan pengaliran media cecair MS dari bekas media ke bekas tanaman. Masa rendaman yang berbeza telah dikaji dengan menetapkan masa 3 minit/2 jam dan 15 minit/2 jam pada alat pengawal masa sepanjang tempoh tiga minggu dikultur. Hasil kajian menunjukkan 100% pucuk yang dihasilkan secara maksimum (3 ± 0.58) dapat dilihat pada bahagian tanaman yang dikultur menggunakan TIB dengan masa rendaman selama 3 minit/2 jam. Perbandingan bagi keberkesanan penghasilan pucuk antara sistem rendaman sementara (TIB) dan sistem media separa pejal pula menunjukkan TIB mengurangkan masa penghasilan pucuk kepada separuh berbanding sistem separa pejal dengan menghasilkan 3 ± 0.58 pucuk dalam masa tiga minggu manakala sistem media separa pejal memerlukan enam minggu untuk menghasilkan jumlah yang hampir sama (6 ± 1.16). Kesimpulannya, TIB yang telah diubah suai ini berupaya meningkatkan penghasilan pucuk sekaligus berpotensi untuk meningkatkan kadar penghasilan anak pokok nenas.
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>BAP</td>
<td>6-benzylaminopurine</td>
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<td>B.I.B®</td>
<td>Bioreactor Immersion by Bubbles</td>
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<tr>
<td>CO₂</td>
<td>Carbon Dioxide</td>
</tr>
<tr>
<td>ETFE</td>
<td>Ethylene Tetrafluoroethylene</td>
</tr>
<tr>
<td>FEP</td>
<td>Fluorinated Ethylene Propylene</td>
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<tr>
<td>g/L</td>
<td>Gram per liters</td>
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<td>mg/L</td>
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<td>mm</td>
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<td>MS</td>
<td>Murashige and Skoog Medium</td>
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<td>PFA</td>
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<td>Plant Growth Regulator</td>
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<td>sec</td>
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<td>°C</td>
<td>Degree Celcius</td>
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<td>µM</td>
<td>Micromolar</td>
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<td>%</td>
<td>Percentage</td>
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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

*Ananas comosus* L. Merr or commonly known as pineapple is a tropical plant which is indigenous to warm climates and dry region with infrequent rainfall (Coppens d’Eeckenbrugge *et al.*, 2011). This monocotyledonous plant has gained so much interest in the world trade and considered as one of the major tropical crops due to its powerful uses. For instance, the stems and fruits of pineapple have great potential in producing protease and bromelain for commercial purpose whereas the pineapple leaf fibers can be used to make cloth and cordage and also paper in Philippines and Taiwan since decades ago (Liu, 2005). Additionally, this fruit is also rich in many good nutrients such as vitamin A, B, C as well as some other important minerals like manganese that can prevent osteoporosis (Mhatre, 2007).

Increasing demands of this plant from various countries all over the world has led to the revolution of varieties of propagation techniques. It is conventionally propagated by asexual methods from various parts of the plant such as suckers, ratoons,
slips, crown, crown slips, and stumps (Coppens d’Eeckenbrugge et al., 2011). These parts give different measure of time taken from planting to harvesting. For instance, crowns take longer time than slips of suckers (about 24 months) whereas suckers are normally the fastest to mature as the crop can be harvested after 18 months of planting compared to slips which requires almost 20 to 22 months (Evans et al., 2002). Figure 1.1 shows the structure of pineapple and its important parts that can be used for asexual propagation.

![Figure 1.1 Structure of pineapple plant (Coppens d’Eeckenbrugge et al., 2011).](image)

Unfortunately, the propagation method using the crown, slips and suckers is quite time consuming and laborious as the propagation rate is only one to two propagules per plant per year (Evans et al., 2002). Therefore, tissue culture propagation or also known as micropropagation has been selected as an alternative method due to massive production about 2000 plants obtained from one crown within a year as reported
by Fitchet (1985). Micropropagation can be defined as a cell cultivation method that multiplied the genetically identical copies of a cultivar under controlled and sterile conditions by asexual reproduction (Raven et al., 2005). Hence, the establishment of this method has induced many studies on pineapple micropropagation in order to increase its productivity for the sake of the world population.

Previously, conventional micropropagation system has utilized solid medium with added gelling agent like agar in small jars to culture the explants (Welander et al., 2014). As time passes, the system has been upgraded to the usage of liquid medium in the bioreactors which allows fast proliferation of the plants due to maximum uptake of nutrients (Welander et al., 2014). This recent method has led to another system of liquid culture which is known as a temporary immersion time (TIS) that has been considered as a good technique and great alternative in terms of automation to lower the labor cost in comparison with conventional solid media (Ethienne and Berthouly, 2002).

1.2 Problem Statement

Micropropagation has been known as a highly potential technique to produce plants in vast amounts with minimal space and less number of matured mother plant used. However, the use of semi-solid medium in micropropagation is quite restrained for commercial purpose as higher cost is needed especially for production costs (Welander et al., 2014). This is mainly due to the facts that cultured shoots need to be divided and placed in small jars which require larger space, more quantity of jars and more energy to handle the overall process (Welander et al., 2007). In addition, conventional micropropagation also may lead to other problems such as low multiplication rate as well as low survival rate of plantlets after acclimatization (Escalona et al., 1999). Therefore, a doable and tangible approach is needed to reduce the cost and maximize the plant production for A. comosus micropropagation.
Unlike the conventional micropropagation, the use of liquid media in semi-automatic bioreactors which operated based on temporary immersion principle is quite reliable nowadays as regular contact and uniform access of nutrients between plants and the media would ensure better growth of the plants (Watt, 2012). Since less number of effective low cost temporary immersion bioreactors (TIB) is available for Sarawak cultivar micropropagation, hence an approach has been initiated in this study to develop a low cost bioreactor that may enhance the productivity of pineapple micropropagation.

1.3 Objectives of the Study

The objectives of this study are:

1. To construct a low cost temporary immersion bioreactor for pineapple micropropagation.
2. To determine the optimum immersion time for newly modified temporary immersion bioreactor.
3. To compare the efficiency of newly modified temporary immersion bioreactor and MS semi-solid medium in shoots regeneration.
1.4 Scope of the Study

Shoot explants of Sarawak cultivar were used as starting materials for shoot regeneration using a low cost temporary immersion bioreactor. In this study, the ability of newly designed temporary immersion bioreactor to propagate shoots was investigated. The effect of the immersion time on shoot induction was also evaluated.

1.5 Significance of the Study

An efficient micropropagation technique is very crucial in producing high quantity amount of local pineapple plantlets. The temporary immersion system has been established by numerous studies as a good alternative in enhancing shoot multiplication for in vitro plant tissue culture since late 1990s (Watt, 2012) due to some advantages of this system in ensuring adequate nutrient and oxygen supply, minimize the subculturing process, ease of medium changes and allowing for control of contamination (Ziv, 2005; Arencibia et al., 2008). Therefore, the initiative of developing a low cost temporary immersion bioreactor would be a great advantage and effort in reducing the production cost and optimizing the yield of pineapple micropropagation.
REFERENCES


