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

AMILADZIFFAR BINTI RAMLI

A dissertation submitted in partial fulfilment of the
requirements for the award of the degree of
Master of Science Specialization Biotechnology

Faculty of Biosciences and Medical Engineering
Universiti Teknologi Malaysia

MARCH 2018

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.

I would like to take this opportunity to thank both of my dedicated supervisors, Dr. Azman bin Abd Samad and Dr. Wan Rosmiza Zana binti Wan Dagang (co-supervisor) for their valuable guidance, knowledge, and advices throughout this study.

Huge thanks conveyed to all the staffs and laboratory assistants in Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia for their support and tolerance to ensure this project could be done in a timely manner and under safe conditions.

Finally, special thanks to my beloved ones; my family and friends, especially my dearest mother for their endless prayers, supports and understandings all this while. Last but not least, to my late father, thank you for lifting up my spirit from afar.

ABSTRACT

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. Kesan 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.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS / SYMBOLS

ANOVA	-	Analysis of Variance
BAP	-	6-benzylaminopurine
B.I.B®	-	Bioreactor Immersion by Bubbles
CO_2	-	Carbon Dioxide
ETFE	-	Ethylene Tetrafluoroethylene
FEP	-	Fluorinated Ethylene Propylene
g/L	-	Gram per liters
h	-	Hour
mg/L	-	Milligram per liters
min	-	minute
mL	-	Milliliter
mm	-	Millimeter
MS	-	Murashige and Skoog Medium
MPIB	-	Malaysian Pineapple Industry Board
PFA	-	Phenoleformaldehyde
PGR	-	Plant Growth Regulator
PP	-	Polypropylene
PTFE	-	Polytetrafluoroethylene
RITA®	-	Recipient for Automated Temporary Immersion System
SE	-	Standard Error

sec	-	Second
TIS	-	Temporary Immersion System
TIB	-	Temporary Immersion Bioreactor
℃	-	Degree Celcius
µM	-	Micromolar
%	-	Percentage

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.

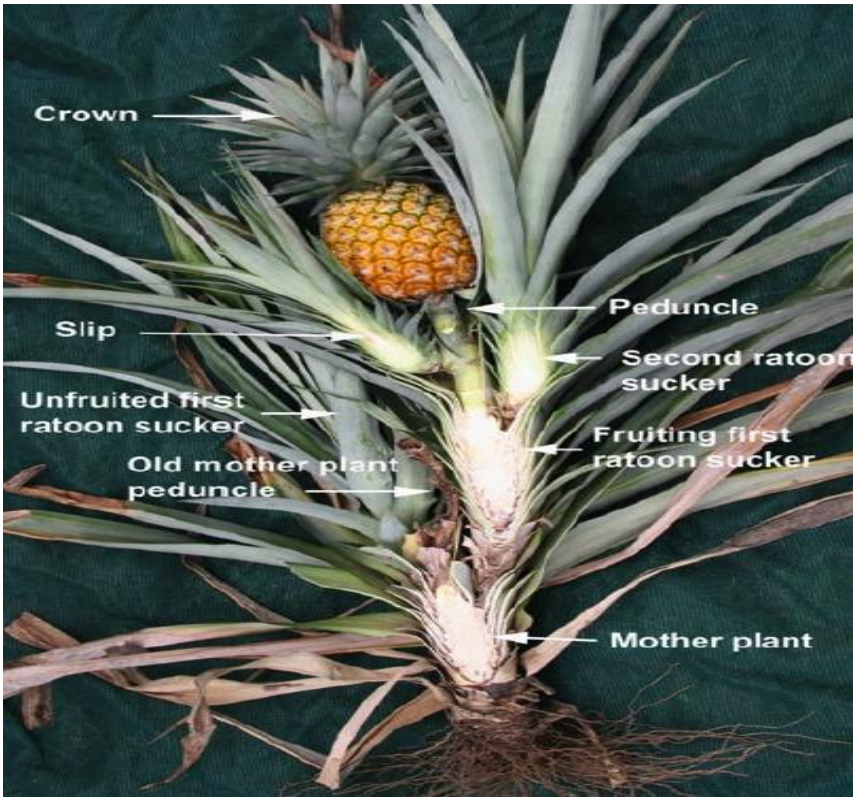

Figure 1.1 Structure of pineapple plant (Coppens d'Eeckenbrugge *et al*., 2011).

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

Aitken-Christie, J., & Jones, C. (1987). Towards automation: radiate pine shoot hedges *in vitro*. *Plant Cell, Tissue and Organ Culture*, 8, 185-196.

Aitken–Christie, J., & Davies, H. E. (1988). Development of a semi-automated micropropagation system. *Acta Horticulturae*, 230, 81–87.

Aitken-Christie, J. (1991). Automation. In: Debergh, P. C., & Zimmerman, R. J. (Eds.) *Micropropagation: Technology and Application* (pp. 363-388). Kluwer Academic Publishers: Dordrecht, The Netherlands.

Akdemir, H., Süzerer, V., Onay, A., Tilkat, E., Ersali, Y., & Ozden, Y. (2014). Micropropagation of the pistachio and its rootstocks by temporary immersion system. *Plant Cell, Tissue and Organ Culture*, 117, 65–76.

Akita, M., & Takayama, S. (1994). Stimulation of potato (*Solanum tuberosum* L.) tuberization by semi-continuous liquid medium surface level control. *Plant Cell Reports*, 13, 184-187.

Alabarran, J., Bertrand, B., Lartraud, M., & Etienne, H. (2005). Cycle characteristics in a temporary immersion bioreactor affect regeneration, morphology, water and mineral status of coffee (*Coffea arabica*) somatic embryos. *Plant Cell, Tissue and Organ Culture*, 81, 27-36.

Almeida, W. B. D., Santana, G. S., Pinheiro, A., Rodriguez, M., Angelica, M., & Carvalho, P. D. E. (2002). Optimization of a protocol for the micropropagation of pineapple. *Revista Brasileira de Fruticultura*, 24(2), 296-300.

Alvard, D., Cote, F., & Teisson, C. (1993). Comparison of methods of liquid medium culture for banana micropropagation. Effects of Temporary Immersion on Explants. *Plant Cell, Tissue and Organ Culture*, 32, 55-60.

Aragon, C. E., Escalona, M., Rodriguez, R., Canal, M. J., Capote, I., Pina, D., & Gonzales-Olmedo, J. (2010). Effect of sucrose, light, and carbon dioxide on plantain micropropagation in temporary immersion bioreactors. *In Vitro Cell Development Biology Plant*, 46, 89-94.

Archambaut, J., Williams, R. D., Lavoie, L., Pepin, M-F., & Chavarie, C. (1994). Production of somatic embryos in a helical ribbon impeller bioreactor. *Biotechnology and Bioengineering*, 44, 930-943.

Arencibia, A. D., Bernal, A., Yang, L., Cortegaza, L., Carmona, E. R., Pérez, A., Hu, C. J., Li, Y. R., Zayas, C. M., & Santana, I., (2008). New role of phenylpropanoid compounds during sugarcane micropropagation in Temporary Immersion Bioreactors (TIBs). *Plant Science*, 175, 487–496.

Arib, R. M. N., Sapuan, S. M., Ahmad, M. M. H. M., Paridah, M. T., & Khairul Zaman, H. M. D. (2004). Mechanical properties of pineapple leaf fibre reinforced polypropylene composites. *Materials & Design*, 43, 169-183.

Awal, A., Nor Fazilah, N., Azvin, M. P., Najwa, M., Shamsiah, A., & Norrizah, J. (2011). Micropropagation of pineapple (*Ananas comosus* L. Merr. 'Josapine'). *Acta Horticulturae*, 923, 163-168.

Bandeira, F. S.; Xavier, A.; Otoni, W. C.; & Lani, E. R. G. (2007). *Ex vitro* acclimatization of plants propagated by the *in vitro* grafting of *Eucalyptus urophylla* x *E.grandis*.clones *Arvore Magazine*, 31, 773-781.

Bertoni, M. S. (1919). Contributions a l'etude botanique des plantes cultivees, I: essai d'une monographie du genre Ananas. *Ann Cient Paraguay* (Ser. II), 4, 250-322.

Coppens d'Eeckenbrugge, G., Sanewski, G. M., Smith, M. K., Duval, M., & Leal, F. (2011). Ananas. In: Kole, C. (Ed.). *Wild Crop Relatives: Genomic and Breeding Resources, Tropical and Subtropical Fruits* (pp. 21-41). Springer-Verlag: Berlin, Heidelberg.

Cabasson, C., Alvard, D., Dambier, D., Ollitrault, P., & Teisson, C. (1997). Improvement of *Citrus* somatic embryo development by temporary immersion. *Plant Cell, Tissue and Organ Culture*, 50, 33–37.

Da Silva, A., Pasqual, M., Teixeira, J. B., & de Araujo, A. G. (2007). Micropropagation methods of pineapple. *Pesquisa Agropecuaria Brasileira, Brasilia*, 42, 1257-1260 (in Portuguese).

Debergh, P. (1988). Improving mass propagation of *in vitro* plantlets. In: Kozai, T. (Ed) *Horticulture in High Technology Era* (pp 45-57). International Symposium on High Technology In Protected Cultivation: Tokyo.

Debergh, P. C., Aitken-Christie, J., Cohen, S., Von Arnold, S., Zimmerman, R., & Ziv, M. (1992). Reconsideration of the term "vitrification" as used in micropropagation. *Plant Cell, Tissue and Organ Culture*, 30, 135-140.

Debnath, S. C. (2010). A scaled-up system for *in vitro* multiplication of thidiazuron-induced red raspberry shoots using a bioreactor. *Journal of Horticultural Science and Biotechnology*, 85(2), 94-100.

Escalant, J-V, Teisson, C., & Côte, F. (1994). Amplified somatic embryogenesis from male flowers of triploid banana and plantain cultivars (*Musa* spp.). *In Vitro Cell Development Biology-Plant*, 30, 181–186.

Escalona, M., Lorenzo, J. C., González, B., Daquinta, M., González, J. L., Desjardins, Y., & Borroto, C. G. (1999). Pineapple (*Ananas comosus* L. Merr) micropropagation in temporary immersion systems. *Plant Cell Reports*, 18, 743-748.

Etienne-Barry, D., Bertrand, B., Vasquez, N., & Etienne, H. (1999). Direct sowing of *Coffea arabica* somatic embryos mass-produced in a bioreactor and regeneration of plants. *Plant Cell* Reports, 19, 111-117.

Etienne, H., Lartaud, M., Michaux-Ferriere, N., Carron, M. P., Berthouly, M., & Teisson,C. (1997). Improvements of somatic embryogenesis in *Hevea brasiliensis* (Mull. Arg.) using the temporary immersion technique. *In Vitro Cell Development Biology-Plant*, 33, 81-87.

Etienne, H., & Berthouly, M. (2002). Temporary immersion systems in plant propagation. *Plant Cell, Tissue and Organ Culture*, 69, 215-231.

Evans, D. O., Sanford, W. G., & Bartholomew, D. P. (2002). Growing pineapple. Cooperative Extension Service, Fruits and Nuts – 7, CTAHR, (pp. 4-8).

Firoozabady, E., & Gutterson, N. (2003). Cost-effective *in vitro* propagation methods for pineapple. *Plant Cell Reports*, 21(9), 844-850.

Fitchet, M. (1985). Tissue culture of pineapples. *Information-Bulletin, Citrus-and-Subtropical-Fruit-Research-Institute*, 149, 1-2. 1

Gaspar, T., Kevers, C., Debergh, P. C., Maene, L., Paque, M., & Boxus, P. (1987). Vitrification: morphological, physiological and ecological aspects. In Bonga, J. M. & Durzan, D. J. (Eds.), *Cell and Tissue Culture In Forestry*, Vol. 1 (pp. 152-166). Martinus Nijhoff Publisher: Dordrecht, The Netherlands.

George, E. F., & Klerk, G. J. (2008). The components of plant tissue culture media I: macro- and micro- nutrients. In: George, E. F. *et al*. (Eds.) *Plant Propagation by Tissue Culture* (pp. 65-113). 3rd ed. Dordrecht, The Netherlands: Springer.

Georgiev, V., Schumann, A., Pavlov, A., & Bley, T. (2014). Temporary immersion systems in plant biotechnology. *Engineering in Life Sciences*, 14(6), 607-621.

Gupta, P., Mascarenhas, A., & Jagannathan, V. (1981). Tissue culture of forest trees clonal propagation of mature trees of *Eucalyptus citriodora* Hook, by tissue culture. *Plant Science Letters*, 20(3), 195-201.

Hale, S. A., Young, R. E., Adelberg, J. W., Keese, R. J., & Camper, N. D. (1992). Bioreactor development for continual-flow liquid plant tissue culture. *Acta Horticulturae*, 319, 107-112.

Harris, R. E., & Mason, E. B. (1983). Two machines for *in vitro* propagation of plants in liquid media. *Canadian Journal of Plant Science*, 63, 311-316.

Hammerschlag, F. (1982). Factors affecting establishment and growth of peach shoot *in vitro*. *HortScience*, 17, 85-86.

Ilczuk, A., Winkelman, T., Richartz, S., Witomska, M., & Serek, A. (2005). *In vitro* propagation of *Hippeastrum* x *chmielli* Chm. - influence of flurprimidol and the culture in solid or liquid medium and in temporary immersion systems. *Plant Cell, Tissue and Organ Culture*, 83, 339-346.

Jimenez, E., Perez, N., de Feria, M., Barbon, R., Capote, A., Chavez, M., Quiala, E., & Perez, J. C. (1999). Improved production of potato microtubers using a temporary immersion system. *Plant Cell, Tissue and Organ Culture*, 59, 19-23.

Jones, A. M., & Petolino, J. F. (1988). Effects of support medium on embryo and plant production from cultured anthers of soft-red winter wheat. *Plant Cell, Tissue and Organ Culture*, 12, 243-261.

Jova, M. C., Kosky, R. G., Cuellar, E. E., & Cuellar, A. E. (2012). Efficiency of semi-automated culture systems on microtubers formation of yam (*Discorea alata* L.). *Biotechnology, Agronomy, Society and Environment*, 16, 45-47.

Krueger, S., Robacker, C., & Simonton, W. (1991). Culture of *Amelanchier* x *grandiflora* in a programmable micropropagation apparatus. *Plant Cell, Tissue and Organ Culture,* 27, 219-226.

Lorenzo, J. C., Gonzalez, B. L., Escalona, M., Teisson, C., Espinosa, P., & Borroto, C. (1998). Sugarcane shoots formation in an improved temporary immersion system. *Plant, Cell, Tissue and Organ Culture*, 54, 197–200.

Lemos, E. E. P.; Ferreira, M. S.; Alencar, L. M.C., Oliveira, J. G. L.; & Magalhães, V. S. (2001). Micropropagação de clones de banana cv. *Terra* embiorreator de imersão temporária. *Revista Brasileirade Fruticultura*, 23, 482-487.

Leal, F. (1989). On the history, origin and taxonomy of the pineapple. *Interciencia*, 14, 235-241.

Leal, F., & Coopens d' Eeckenbrugge, G. (1996). Pineapple. In: Janick, J. & Moore, J. N. (Eds.). *Fruit breeding, Vol 1: Ttree and Tropical Fruit* (pp. 515-556). John Wiley and Sons: New York.

Linnaeus, C. (1753). *Species Plantarum*, Vol 2. Stockolm.

Lieu, P. N., Tinh, N. N., Vui, P. V., Khai, T. P., & Teisson, C. (2004). Study of multiplication rate of conventional propagation *in vivo* of Cayenne (*A. comosus* L.). *Impact de 10 annees de cooperation francaise sur l'amelioration des productions fruitieres au Vietnam* – SOFRI. Conference, (pp 1-11).

Liu, W., Misra, M., Askeland, P., Drzal, L. T., & Mohanty, A. K. (2005). Green composites from soy based plastic and pineapple leaf fiber: fabrication and properties evaluation. *Polymer,* 46, 2710-2721.

Maier, C., & Calafut, T. (1998). Polypropylene: The definitive user's guide and databook. In William Andrew (Ed.). *Technology and Engineering* (pp. 268). Plastics Design Library, William Andrew Inc.: United States of America, Norwich, NY.

Malaysian Pineapple Industry Board, 2017. Kultivar. Retrieved from the Official Portal of Malaysian Pineapple Industry Board website http://www.mpib.gov.my/en/web/guest/kultivar1

McAlister, B., Finnie, J., Watt, M. P., & Blake way, F. C. (2005). Use of temporary immersion bioreactor system (RITA) for the production of commercial *Eucalyptus* clones at Mondi Forests (SA). *Plant Cell, Tissue and Organ Culture*, 81, 347-358.

Merril, E. D. (1917). *An interpretation of Rumphius's Herbarium Amboinense*. Bureau of Science, Manila.

Merillon, J. M. (Ed.). (2013). *Bulbous Plants: Biotechnology*. CRC Press.

Mhatre, M. (2007). Micropropagation of pineapple, *Ananas comosus* (L.) Merr. In: Jain, S. M., & Haggman, H. (Eds.). *Protocols for Micropropagation of Woody Trees and Fruits* (pp. 499-508). Springer.

Mohan, R.; Chui, E. A.; Biasi, L. A.; & Soccol, C. R. (2005), Alternative *in vitro* propagation: use of sugarcane bagasse as a low cost support material during rooting stage of strawberry cv. *Dover*. *Brazilian Archives of Biology and Technogy*, 48, 37-42.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia plantarum*, 15(3), 473-497.

Nadel, B. L., Altman, A., & Ziv, M. (1990). Regulation of large scale embryogenesis in celery. *Acta Horticulturae*, 280, 75-82.

Niemenak, N., Saare-Surminski, K., Rohsius, C., Ndoumou, D. O., & Lieberei, R. (2008). Regeneration of somatic embryos in *Theobroma cacao* L. in temporary immersion bioreactors and analyses of free amino acids in different tissue. *Plant Cell Reports*, 27, 667-676.

Paek, K. Y., Chakrabarty, D., & Hahn, E. J. (2005). Application of bioreactor systems for large production of horticultural and medicinal plants. *Plant Cell, Tissue and Organ Culture*, 81, 28-300.

Paque, M., & Boxus, P. (1987). Vitrification: a phenomenon related to tissue water content. *Acta Horticulturae,* 212, 245-252.

Patino, V. M. (1963). Plantas cultivadas y animals domesticos en America equinoccial. Tomo 1. Frutales. Imprenta Departamental: Cali, Colombia.

Pérez, M., Bueno, M. A., Escalona, M., Toorop, P., Rodríguez, R., & Cañal, M. J. (2013). Temporary immersion systems (RITA) for the improvement of cork oak somatic embryogenic culture proliferation and somatic embryo production. *Trees,* 27, 1277–1284.

Preece, J. E., & Sutter, E. (1991). Acclimatization of micropropagated plants to the green-house. In Debergh, P. C., & Zimmerman, R. H. (Eds.). *Micropropagation: Technology and Application* (pp. 71-91). Kluwer Academic Publishers: Dordrecht, The Netherlands.

Preece, J. E., & Read, P. E. (2005). The biology of horticulture. *An introductory textbook*. 2nd ed. John Wiley & Sons, Inc.: United States of America.

Preil, W., Florek, P., Wix, U., & Beck, A. (1988). Towards mass propagation by use of bioreactors. *Acta Horticulturae,* 226, 99-106.

Ramírez-Mosqueda, M. A., & Iglesias-Andreu, L. G., (2016). Evaluation of different temporary immersion systems (BIT®, BIG and RITA®) in the micropropagation of *Vanilla planifolia* Jacks. *In Vitro Cellular & Developmental Biology-Plant*, 52, 154–160.

Ramos-Castellá, A., Iglesias-Andreu, L. G., Bello-Bello, J. L., & Espinosa, H. (2014). Improved propagation of vanilla (*Vanilla planifolia* Jacks. Ex Andrews) using a temporary immersion system. *In Vitro Cell Development Biology-Plant,* 50, 576–581.

Ramos-Castellá, A., & Iglesias-Andreu, L. G. (2016). Evaluation of different temporary immersion systems (BIT®, BIG, and RITA®) in the micropropagation of *Vanilla planifolia* Jacks. *In Vitro Cell Development Biology Plant*, 52, 154-160.

Rangan, T. S. (1984). Pineapple: In Ammirato, P. V., Evans, D. A., Sharp, W. R., & Yamada, Y. (Eds.). *Handbook of Plant Cell Culture*. McMillan, New York.

Raven, P. H., Evert, R. F., & Eichhorn, S. E. (2005). *Biology of Plants*. 7th ed. W. H. Freeman and Company: United States of America.

Reinhardt, A., & Rodriguez, L. V. (2009). Industrial processing of pineapple-trends and perspectives. *Acta Horticulturae*, 822, 323-328.

Robert, M. L., Herrera-Herrera, J. L., Herrera-Herrera, G., Herrera-Alamillo, M. G., & Fuentes-Carillo, P. (2006). A new temporary immersion bioreactor system for micropropagation. From: *Methods in Molecular Biology, Plant Cell Culture Protocols*, 18, 121-129.

Roels, S., Escalona, M., Cejas, I., Noceda, S., Rodriguez, R., Canal, M. J., Sandoval, J., & Debergh, P. (2005). Optimization of plantain (*Musa* AAB) micropropagation by temporary immersion system. *Plant Cell, Tissue and Organ Culture*, 82, 57-66.

Roostika, T. I., & Mariska, I. (2003). *In vitro* culture of pineapple by organogenesis and somatic embryogenesis: Its utilization and prospect. *Buletin AgroBio*, 6(1), 34-40.

Ruslina binti Jimin (2016). Propagation of Local Pineapple (*Ananas comosus* L.) using RITA Temporary Immersion System. Bachelor Thesis, Universiti Teknologi Malaysia, Skudai.

Scheidt, G. N., Arakaki, A. H., Chimilovski, J. S., Portella, A. C. F., Spier, M. R., Woiciechowski, A. L., & Soccol, C. R. (2009). Utilization of the bioreactor of immersion by bubbles at the micropropagation of *Ananas comosus* L. Merril. *Brazilian Archives of Biology and Technology*, 52 (special), 37-43.

Scherer, R. F., Garcia, A. C., Fraga, H. P. F., Dal Vesco, L. L., Steinmacher, D. A., & Guerra, M. P. (2013). Nodule cluster cultures and temporary immersion bioreactors as a high performance micropropagation strategy in pineapple (*Ananas comosus* var. *comosus*). *Scientia Horticulture*, 151, 38-45.

Scragg, A. H. (1990). Fermentation systems for plant cells. In: Charlwood, B. V., & Rhodes, M. J. C. (Eds.). *Secondary products from plant tissue culture* (pp. 243-263). Clarendon Press, London.

Scragg, A. H. (1992). Large-scale plant cell culture: methods, applications and products. *Current Opinion Biotechnology*, 3, 105-109.

Snyman, S. J., Meyer, G. M., Koch, A. C., Banasiak, M., & Watt, M. P. (2011). Applications of *in vitro* culture system for commercial sugarcane production and improvement. *In Vitro Cell Development Biology-Plant*, 47, 234-249.

Soccol, C. R.; Scheidt, G. N.; & Mohan, R. (2008). Biorreator do tipo imersão por bolhas para as técnicas de micropropagação vegetal. Universidade Federal do Paraná. Patente, (DEPR. 01508000078), 3 Março.

Steward, F. C, Caplin, S., & Millar, F. K. (1952). Investigations on growth and metabolism of plant cells. I. New techniques for the investigation of metabolism, nutrition and growth in undifferentiated cells. *Annal Botany*, 16, 57–77.

Steward, F. C., & Shantz, E. M. (1956). The chemical induction of growth in plant tissue cultures. In: Wain, R. L., & Wingman, F. (Eds) *The Chemistry and Mode of Action of Plant Growth Substances*, 165-186.

Styer, D. J. (1985). Bioreactor technology for plant propagation. In: Henke, R. R., Hughes, K. W., Constantin, M. J., & Hollaender, A. (Eds.). *Tissue Culture Of Forestry And Agriculture* (pp. 117-130). Plenum Press: New York.

Sutter, E. G. (1985). Morphological physical and chemical characteristics of epicuticular wax on ornamental plants regenerated *in vitro*. *Annals of Botany*, 55, 321-329.

Takayama, S., & Akita, M. (1998). Bioreactor techniques for large-scale culture of plant propagules. *Advances in Horticultural Science*, 12, 93-100.

Tautorus, T. E., Lulsdorf, M. M., Kikcio, S. I., & Dunstan, D. I. (1994). Nutrient utilization during bioreactor culture and maturation of somatic embryo culture of *Picea Marianna* and *Picea glauca-engelmannii*. *In vitro Cellular and Developmental Biology Plant,* 30: 58-63.

Teisson, C., & Alvard, D. (1995). A new concept of plant *in vitro* cultivation liquid medium: temporary immersion. In: Terzi, M. *et al*. (Eds.) *Current Issues In Plant Molecular And Cellular Biology* (pp. 105-110). Kluwer Academic Publishers: Dordrecht.

Teisson, C., Alvard, D., Berthouly, B., Cote, F., Escalant, V., Etienne, H., & Lartaud, M. (1996). Simple apparatus to perform plant tissue culture by temporary immersion. *Acta Horticulturae*, 440, 521– 526.

Teixeira da Silva, J. A., Giang, D. T. T., & Tanaka, M. (2005). Micropropagation of sweet potato (*Ipomea batatas*) in a novel CO_2-enriched vessel. *Journal of Plant Biotechnology*, 7, 1-8.

Tisserat, B., & Vandercook, C. E. (1985). Development of an automated plant culture system. *Plant, Cell, Tissue and Organ Culture*, 5, 107–117.

Usman, I. S., Abdulmalik, M. M., Sani, L. A., Muhammad, A. N., Science, P., & University, A. B. (2013). Development of an efficient protocol for micropropagation of pineapple (*Ananas comosus* L. var. Smooth cayenne), 8(18), 2053-2056.

Watt, M. P. (2012). The status of temporary immersion system (TIS) technology for plant micropropagation. *African Journal of Biotechnology*, 11(76), 14025-14035.

Wee, Y. C. (1974). The Masmerah: a new cultivar for the Malaysian pineapple industry. *World Crops*, 26, 64-67.

Welander, M., Zhu, L. H., & Li, X. Y. (2007). Factors influencing conventional and semi-automated micropropagation. *Propagation of Ornamental Plants*, 7(3), 103-111.

Welander, M., Persson, J. Asp, H., & Zhu, L. H. (2014). Evaluation of a new vessel system based on temporary immersion system for micropropagation. *Scientia Horticulturae*, 179, 227-232.

Werker, E., & Leshem, B. (1987). Structural changes during vitrification of carnation plantlets. *Annals in Botany*, 59, 377-385.

Ziv, M., Schwartz, A., & Fleminger, D. (1987). Malfunctioning stomata in vitreous leaves of carnation (*Dianthus coryophyllus*) plants propagated *in vitro*. *Plant Science*, 52, 127-134.

Ziv, M. (1991). Vitrification: morphological and physiological disorders of *in vitro* plants. In: Debergh, P. C., & Zimmerman, R. H. (Eds.). *Micropropagation – technology and application* (pp. 45-79). Dordrecht: Kluwer Academic Publishers.

Ziv, M., & Ariel, T. (1994). Vitrification in relation to stomatal deformation and malfunction in carnation leaves *in vitro*. In: Lumsden, P. J., Nicholas, J., & Davies, W. J. (Eds.). Physiology growth and development of micropropagated plants (pp. 143-154). Kluwer Academic Publishers: Dordrecht, The Netherlands.

Ziv, M. (2000). Bioreactor technology for plant micropropagation. *Horticulture Reviews*, 24, 1-30.

Ziv, M. (2005). Simple bioreactors for the mass propagation of plants. *Plant, Cell, Tissue and Organ Culture*, 81, 277-285.

Zobayed, S. M. A. (2005). Ventilation and micropropagation. In: Kozai, T., Afreen, F., Zobayed, S. M. A. (Eds.). *Photoautotrophic (Sugar-Free) Medium Micropropagation As A New Micropropagation And Transplant Production System* (pp. 147-186). Springer: The Netherlands.

Zuraida, A. R., Nurul Shahnadz, A. H., Harteeni, A., Roowi, S., Che Radziah, C. M. Z., & Sreeramanan, S. (2011). A novel approach for rapid micropropagation of maspine pineapple (*Ananas comosus* L.) shoots using liquid shake culture system. *African Journal of Biotechnology*, 10(19), 3859-3866.