
**UNIVERSITI PUTRA MALAYSIA**

# *IN VITRO CULTURE ESTABLISHMENT AND SHOOT REGENERATION IN RUBBER (HEVEA BRASILIENSIS MUELL. ARG.)*

**MAHDI MORADPOUR**

**ITA 2015 18**


# *IN VITRO* CULTURE ESTABLISHMENT AND SHOOT REGENERATION IN RUBBER (*HEVEA BRASILIENSIS* MUELL. ARG.)

**By**

**MAHDI MORADPOUR**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science**

**May 2015**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

**_IN VITRO_ CULTURE ESTABLISHMENT AND SHOOT REGENERATION IN RUBBER (_HEVEA BRASILIENSIS_ MUELL. ARG.)**

By

**MAHDI MORADPOUR**

**May 2015**

**Chairman: Associate Professor Maheran Abdul Aziz, PhD**
**Institute: Tropical Agriculture**

The development of *in vitro* methods to produce high quality clonal planting materials of *Hevea brasiliensis* for replanting and new planting is highly desirable and essential. Some procedures do exist but generally do not address well the initial stage of culture establishment. The establishment of *in vitro* cultures of challenging woody plant species like *H. brasiliensis* have frequently been hampered by persistent microbial contamination, phenolic production, phase change and low response to media. Therefore, the overall goal of this study was to establish an efficient *in vitro* propagation method for *H. brasiliensis* through solving the persistent microbial contamination problems, controlling phenolic production and increasing the response of explants to media. Shoot tips and axillary buds derived from one to two year-old grafted plants of *H. brasiliensis* clone RRIM 2025 were used as explants. In determining the most suitable method of reducing explant contamination, different concentrations of sodium hypochlorite, mercuric chloride and nano silver at different immersion times were assessed on explants derived from three physiological leaf stages (bronze, light green and stable green leaf stages) of preculture and non-preculture treated plants. The highest percentage of survival (94.44%) was from explants derived from the light green leaf stage of preculture treated plants immersed in 92.6 µM NS for 20 min. Different types of adsorbents which were silver nitrate, activated charcoal and nano silver were assessed in controlling browning of *in vitro* cultures of *H. brasiliensis.* Nano silver at 37.04 µM significantly produced the highest percentage of explant survival (87.03%) with a low percentage of browning (10%). In an attempt to determine the most suitable medium for *in vitro* culture of *H. brasiliensis* explants, Murashige and Skoog (MS) medium, Woody Plant medium (WPM) and MB medium in combination with 3%, 6% and 9% sucrose concentrations were assessed. After 16 weeks of culture, the highest percentage of shoot formation (80%) was on MB medium with 6% sucrose. In evaluating the effects of benzyl aminopurine (BAP) alone on shoot induction of *H. brasiliensis,* the highest percentage of

axillary shoot emergence (61.11%) was obtained on MB medium containing 22.2 µM BAP after 16 weeks of culture. In the second experiment on shoot induction, different concentrations of BAP in combination with 1.44 µM Gibberellic acid ($GA_3$) were assessed whereby MB medium containing 22.2 µM BAP with 1.44 µM $GA_3$ produced a maximum mean number of 2 shoots per explant after 16 weeks of culture. Lastly, different concentrations of BAP in combination with 2.7 µM naphthalene acetic acid (NAA) were assessed on shoot induction whereby the highest mean number of 3 shoots per explant were obtained on MB medium supplemented 8.8 µM BAP and 2.7 µM NAA after 16 weeks of culture. In the study on multiplication and elongation of *H. brasiliensis* shoots, various combinations of plant growth regulators were assessed. In the first experiment, different concentrations of $GA_3$ in combination with 2.2 µM BAP and 1.23 µM indole-3-butyric acid (IBA) were tested and a maximum mean number of 10 shoots was produced on MB medium supplemented with 1.45 µM $GA_3$, 2.2 µM BAP and 1.23 µM IBA after 16 weeks of culture. In the second shoot multiplication experiment, *in vitro* shoots were placed in different concentrations of thidiazuron (TDZ) combined with 0.1 µM IBA and the highest mean number of shoots per explant (4.6) was obtained on MB medium containing 0.45 µM TDZ and 0.1 µM IBA after 16 weeks of culture, Finally, different concentrations of kinetin (Kin) in combination with 4.4 µM BAP and 2.7 µM NAA were tested for shoot multiplication and a maximum mean number of 4.33 shoots, which were strong and healthy, was obtained on MB medium supplemented with 9.3 µM Kin, 4.4 µM BAP and 2.7 µM NAA. In conclusion, this study indicates that a successful establishment of *in vitro* culture of *H. brasiliensis* requires the understanding of the species leaf developmental stages, the interaction of the explant source with the environment and the effective application of the non-toxic silver nano particles in reducing microbial contamination and browning of *Hevea* explants. An efficient initial culture establishment developed in this study thus opens the way for future application of the microcutting technique for propagation as well as genetic improvement of *H. brasiliensis*.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PEMBENTUKAN KULTUR *IN VITRO* DAN REGENERASI PUCUK UNTUK TANAMAN GETAH (*HEVEA BRASILIENSIS* MUELL. ARG.)**

Oleh

**MAHDI MORADPOUR**

**Mei 2015**

**Pengerusi: Profesor Madya Maheran Abdul Aziz, PhD**
**Fakulti: Institut Pertanian Tropika**

Pembangunan kaedah *in vitro* untuk menghasilkan bahan tanaman klon *Hevea brasiliensis* berkualiti tinggi untuk penanaman semula dan penanaman baru adalah sangat wajar dan penting. Beberapa prosedur memang wujud tetapi umumnya tidak menangani dengan baik peringkat awal pembentukan kultur. Pembentukan kultur *in vitro* bagi spesies tumbuhan berkayu yang mencabar seperti *H. brasiliensis* telah kerap dihalang oleh pencemaran mikrob yang berterusan, pengeluaran fenolik, perubahan fasa dan tindak balas yang rendah terhadap media. Oleh itu, matlamat keseluruhan kajian ini adalah untuk membentuk kaedah pembiakan *in vitro* yang berkesan untuk *H. brasiliensis* melalui penyelesaian masalah pencemaran mikrob yang berterusan, mengawal pengeluaran fenolik, dan meningkatkan tindak balas eksplan terhadap media. Hujung pucuk dan tunas aksil yang diperoleh dari pokok cantuman *H. brasiliensis* klon RRIM 2025 berumur satu ke dua tahun, digunakan sebagai eksplan. Dalam menentukan kaedah yang paling sesuai bagi mengurangkan pencemaran eksplan, kepekatan natrium hipoklorit, merkurik klorida dan nano silver (NS) serta masa rendaman yang berbeza telah diuji ke atas eksplan yang diperoleh daripada tiga peringkat fisiologi daun (peringkat daun gangsa, hijau muda dan hijau stabil) daripada pokok yang diberi rawatan prakultur dan tanpa rawatan. Peratusan hidup tertinggi (94.44%) adalah bagi eksplan yang diperoleh daripada peringkat daun hijau muda pokok yang diberi rawatan prakultur setelah direndam dalam 92.6 µM NS selama 20 min. Pelbagai jenis adsorben iaitu silver nitrat, arang teraktif dan NS telah di uji untuk mengawal pemerangan kultur *in vitro H. brasiliensis*. Didapati NS pada 37.04 µM secara signifikan menghasilkan peratusan hidup eksplan tertinggi (87.03%) dengan peratusan pemerangan yang rendah (10%). Dalam usaha menentukan medium yang paling sesuai untuk kultur *in vitro H. brasiliensis*, medium Murashige and Skoog (MS), medium Tumbuhan Berkayu (WPM) dan

medium MB yang digabungkan dengan kepekatan 3%, 6% dan 9% sukrosa telah diuji. Selepas 16 minggu dikultur, peratusan tertinggi pembentukan pucuk (80%) adalah pada medium MB dengan 6% sukrosa. Untuk mengkaji kesan kepekatan benzyl aminopurine (BAP) secara bersendirian ke atas induksi pucuk *H. brasiliensis,* peratusan pengeluaran pucuk aksil tertinggi (61.11%) adalah pada medium MB yang mengandungi 22.2 µM BAP selepas 16 minggu dikultur. Bagi eksperimen induksi pucuk yang kedua, kepekatan BAP yang berbeza beserta 1.44 µM asid Gibberellic ($GA_3$) telah diuji dan didapai medium MB mengandungi 22.2 µM BAP dengan 1.44 µM $GA_3$ menghasilkan min tertinggi 2 pucuk per eksplan selepas 16 minggu dikultur. Seterusnya, kepekatan BAP yang berbeza beserta 2.7 µM asid naftalen asetik (NAA) telah diuji untuk induksi pucuk dimana min tertinggi 3 pucuk per eksplan diperoleh di atas medium MB yang mengandungi 8.8 µM BAP dan 2.7 µM NAA selepas 16 minggu dikultur. Dalam kajian penggandaan pucuk *H. brasiliensis,* pelbagai kombinasi pengawalatur tumbesaran tumbuhan telah diuji. Dalam eksperimen pertama, kepekatan $GA_3$ yang berbeza beserta 2.2 µM BAP dan 1.23 µM asid indol-3-butirik (IBA) telah diuji dan min tertinggi 10 pucuk per eksplan diperoleh pada medium MB mengandungi 1.45 µM $GA_3$, 2.2 µM BAP dan 1.23 µM IBA selepas 16 minggu dikultur. Bagi eksperimen penggandaan pucuk yang kedua, pucuk *in vitro* telah dikultur pada kepekatan thidiazuron (TDZ) yang berbeza yang digabungkan dengan 0.1 µM IBA dan min bilangan puck tertinggi per eksplan (4.6) diperoleh pada medium MB yang mengandungi 0.45 µM TDZ dan 0.1 µM IBA selepas 16 minggu kultur. Akhir sekali, kepekatan kinetin (Kin) yang berbeza beserta 4.4 µM BAP dan 2.7 µM NAA telah diuji untuk penggandaan pucuk. Rumusannya, kajian ini menunjukkan bahawa kejayaan membangunkan kultur *in vitro H. brasiliensis* memerlukan kefahaman mengenai peringkat pembentukan daun spesies tersebut, interaksi sumber eksplan dengan persekitaran dan aplikasi berkesan partikel nano silver yang tidak toksik bagi mengurangkan pencemaran microbial dan pemerangan eksplan *Hevea.* Pembentukan pengkulturan yang efisien di dalam kajian ini membuka jalan untuk aplikasi teknik keratan mikro bagi pembiakan dan pembaikan genetik *H. brasiliensis* di masa hadapan.

**ACKNOWLEDGEMENTS**

I would like to thank three important groups of people, without whom this thesis would not have been possible: my supervisory committee, my wonderful lab-mates, and my family.

Most of all, I would like to thank my thesis supervisor, Assoc. Prof. Dr. Maheran Abdul Aziz, a talented teacher and passionate scientist. Dr. Maheran seemed to be wise beyond her experience. I am indebted and thankful for the fresh new opportunities she offered. I also thank Dr. Maheran for appreciating my research strengths and patiently encouraging me to improve in my weaker areas. Her strong support of my own ideas and research directions, and confidence in my abilities were benefits not all graduate students enjoy. I am proud to say my experience in the Agrotechnology lab was intellectually exciting and fun, and has energized me to continue in academic research. I sincerely hope I continue to have opportunities to interact with Dr. Maheran for the rest of my research career. I would also like to thank Prof. Datin Dr. Siti Nor Akmar Abdullah for serving as a member on my thesis committee.

To all my lab-mates, thank you for your understanding and encouragement in my many, moments of crisis. Your friendship makes my life a wonderful experience. I cannot list all the names here, but you are always on my mind. Finally, but not least, I want to thank my parents and my brothers Mohsen and Moein, with whom I shared so much growing up, we have always been encouraged by our parents to ask questions and to be curious about how things work.

I certify that a Thesis Examination Committee has met on 28th of May 2015 to conduct the final examination of Mahdi Moradpour on his thesis entitled ***In Vitro* Culture Establishment and Shoot Regeneration in Rubber (*Hevea Brasiliensis* Muell. Arg.)** in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

**Halimi bin Mohd Saud, PhD**
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

**Saleh bin Kadzimin, PhD**
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

**Nor Hasnida Hassan, PhD**
Forest Biotechnology Division
Forest Research Institute Malaysia (FRIM)
Country
(External Examiner)

**BUJANG KIM HUAT, PhD**
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of requirement for degree of Master of Science. The members of the Supervisory Committee were as follows:

**Maheran Abdul Aziz PhD**
Associate Professor
Institute of Tropical Agriculture
Universiti Putra Malaysia
(Chairman)

**Siti Nor Akmar Abdullah, PhD**
Professor
Institute of Tropical Agriculture
Universiti Putra Malaysia
(Member)

---

**BUJANG BIN KIM HUAT, PhD**
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

**Declaration by graduate student**

I hereby confirm that:

- This thesis is my original work;
- Quotations, illustrations and citations have been duly referenced;
- This thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- Intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- Written permission must be obtained from supervisor and the office of Deputy Vice- Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- There is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012- 2013) and the Universiti Putra Malaysia (Research) Rules 2012.The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: Mahdi Moradpour, (GS31719).

**Declaration by Members of Supervisory Committee**

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature:
**Name of Chairman of Supervisory Committee:**

Signature:
Name of Member of Supervisory Committee:

**TABLE OF CONTENTS**

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| µg | Microgram |
| µM | Micromolar |
| AC | Activated charcoal |
| ANOVA | Analysis of variance |
| BAP | 6-Benzyl aminopurine |
| CaOCl | Sodium hypochlorite |
| cm | Centimeter |
| CRD | Complete Randomized Design |
| DMRT | Duncan multiple range test |
| $GA_3$ | Gibberellic acid |
| $HgCl_2$ | Mercuric chloride |
| IBA | Indole-3-butyric acid |
| Kin | Kinetin |
| MB | Enjarlic and Carron medium |
| mg | Milligram |
| mL | Milliliter |
| mm | Millimeter |
| MS | Murashige and Skoog |
| NAA | α-Naphthalene acetic acid |
| NS | Nano silver |
| PGR | Plant Growth Regulator |
| RCBD | Randomized Complete Block Design |
| SN | Silver nitrate |
| TDZ | Thidiazuron |
| WPM | Woody plant medium |

# CHAPTER 1

# INTRODUCTION

Natural rubber, produced from the latex of the rubber tree *Hevea brasiliensis,* accounts for about 40% of the world's total rubber utilization while 60% is produced by synthetic processes (Lieberei, 2007). The annual world demand for natural rubber is constantly growing because of its distinguishing physicochemical properties, which are still not achievable in synthetic products. World natural rubber production has been steadily increasing since the turn of the century and it currently stands at 11,603,000 tonnes per year (Department of Statistics Malaysia, 2014). Most rubber plantations are located in South East Asia, especially in Thailand, Indonesia and Malaysia, with growing production areas in Vietnam and China (Lieberei, 2007).The increasing demand for natural rubber should persist in the coming decades due to the foreseeable increases in utilization in many countries. The possibilities of extending the planted areas seem limited nowadays and it is primarily through yield increases that growers will be able to satisfy the demand. The quality of the planting material used is an essential component of yield (Jain *et al.,* 2000).

True-to-type multiplication of selected planting materials through cutting has long been troubled with difficulty in developing a cutting technique, due to early loss of rhizogenesis capacity during young tree growth, and the observation of deficient root systems on cuttings (Jain *et al.,* 2000). Rubber trees have been traditionally propagated by grafting buds from selected clones on seedlings or plants from seed orchards. This process is lengthy, since one to two years are required before the plants can be transplanted to the field. Furthermore, no evaluation on the interaction between rootstock and scion has been carried out. For this reason the grafted plants sometimes do not produce the natural rubber at the expected levels (Mendanha *et al*., 1998).

Certain non-uniformity and heterogeneity is seen in bud-grafted clones of rubber, which is particularly ascribable to the non-selected stocks. Such handicaps would be potentially removed by the *in vitro* propagation of plants on their own roots (Carron *et al*., 1989) as well as reduce the cost and time required for plant production. Micropropagation, or propagation *in vitro* of complete plants, is a promising technique for large scale multiplication of selected clones of rubber. The success or failure of micropropagation of tree species including rubber often depends on the condition of the plant material at the time of collection. This is particularly true when explants are obtained from trees grown in the field. The physiological conditions of tissues vary with

season, position within the tree and climatic factors. Each of these conditions can affect the manner in which tissue responds in culture. In addition, environmental conditions have an effect on the degree of microbial infection on plant tissues and hence the degree of contamination would depend on the time explants are collected (Bonga, 1982). Internal and external contamination of plant tissues turns out to be a prevailing problem, because microorganisms, mostly fungi and bacteria, can grow much faster than plant cells and take up all the nutrients, preventing the plants from growing (Cassells, 1990). Although there are techniques to minimize the possibility of bacterial and fungal contaminations during *in vitro* propagation, such as meristem culture and repetitive subcultures, designing a more efficient approach to sterilize plant tissues still seems necessary to eliminate labour intensive trial and errors, and time-consuming decontamination procedures. In order to eliminate the persistent fungal and bacterial contaminations, treatment with antibiotics and antifungal agents may be used. However, it has been reported that antibiotics are normally phytotoxic, and have an inhibitory effect on multiplication, callus induction, regeneration, and explant survival (Teixeira *et al*., 2003).

To date, the *in vitro* culture establishment of *H. brasiliensis* is still problematic. Beside the occurrence of contamination, rapid browning and/or necrosis of the explants is another obstacle. These problems are at least partly caused by oxidation of polyphenols which are abundant in *Hevea* species. Also, inhibition of shoot organogenesis and necrosis of the explants are associated with considerable leakage of exudates into the culture medium (Mederos and Trujillo, 1999). Lack of an optimal protocol for sterilization and debrowning of field-, orchard- or greenhouse-derived (*ex vitro*) explants may result in a paucity of samples for further research (Mahna *et al*., 2013). To optimise an efficient tissue culture method for *Hevea* rubber, explant preparation is one of the important steps that need to be given attention in order to overcome the problem of microbial contamination and browning. A detailed knowledge of mother plant and microbial contamination interaction or host-pathogen combination requires understanding of the dynamics of *Hevea* leaf development and the biochemical potential of cyanide liberation from the species. This study therefore seeks to provide an improved and efficient tissue culture technique for the production of *H. brasiliensis* microcuttings.

The specific objectives of this study were:

1- To determine the effects of sterilant type, preculture treatment and leaf development stage on overcoming contamination of *H. brasiliensis* explants;
2- To determine the effects of different types of adsorbent in controlling browning of *in vitro* culture of *H. brasiliensis;*
3- To optimize the basal medium and plant growth regulator requirements for shoot induction of *H. brasiliensis.*

## REFERENCES


Abdi, G. (2012). Evaluation on the potential of Nano silver for removal of bacterial contaminants in valerian (*Valeriana officinalis* L.) tissue culture. *Journal of Biological and Environmental Science,* 6(17), 199–205.

Abdi, G., Salehi, H. and Khosh-Khui, M. (2008). Nano silver: a novel nanomaterial for removal of bacterial contaminants in valerian (*Valeriana officinalis* L.) tissue culture. *Acta Physiologiae Plantarum,* 30(5), 709–714.

Aremu, A. O., Bairu, M. W., Novák, O., Plačková, L., Zatloukal, M., Doležal, K. and van Staden, J. (2012). Physiological responses and endogenous cytokinin profiles of tissue-cultured 'Williams' bananas in relation to roscovitine and an inhibitor of cytokinin oxidase/dehydrogenase (INCYDE) treatments. *Planta*, 236(6), 1775–90.

Audley, B. G. and Wilson, H. (1978). Metabolism of 2-chloroethylphosphonic acid (ethephon) in suspension cultures of *Hevea brasiliensis*. *Journal of Experimental Botany*, 29(6), 1329–1336.

Bhattacharyya, R. and Bhattacharya, S. (2001). High frequency *in vitro* propagation of *Phyllanthus amarus* Schum. & Thom. by shoot tip culture. *Indian Journal of Experimental Biology*, 39, 1184–1187.

Blazquez, C. H. and Owen, J. (1963). Histological studies of *Dothidella ulei* on susceptible and resistant *Hevea* clones. *Phytopathology*, 53, 58–65.

Bonga, J. (1981). Organogenesis *in vitro* of tissues from mature conifers. *In Vitro*, 17, 511–518.

Bonga, J. M. (1982). Vegetative propagation in relation to juvenility, maturity and rejuvenation. In J. M. Bonga, and D. J. Durzan (Eds.), *Tissue Culture in Forestery,* (pp. 387–412). Springer Nethereland.

Bouychou, J. (1953). Culture des tissus *d'hévéa* in vitro. *Revue Generate du Caoutchouc et des Plastl-que*, *26*(9), 630.

Brand, M. H. and Lineberger, R. (1986). In vitro propagation of *Halesia carolina* L. and the influence of explantation timing on initial shoot proliferation. *Plant Cell, Tissue and Organ Culture*, 7, 103–113.

Cailloux, F., Julien-Guerrier, J., Linossier, L. and Coudret, A. (1996). Long-term somatic embryogenesis and maturation of somatic embryos in *Hevea brasiliensis*. *Plant Science*, 120, 185–196.

Capelle, S. C., Mok, D. W., Kirchner, S. C. and Mok, M. C. (1983). Effects of thidiazuron on cytokinin autonomy and the metabolism of N6-(Δ2-

isopentenyl) [8-14C] adenosine in callus tissues of *Phaseolus lunatus* L. *Plant Physiology*, 73(3), 796–802.

Carron, M. P. and D'Auzac, J. (1992). Biochemical and histological features of somatic embryogenesis in *Hevea brasiliensis*. *Indian Journal of Natural Rubber Research,* 5, 7-17.

Carron, M. P. and Etienne, H. (1995). Somatic embryogenesis in rubber tree (*Hevea brasiliensis* Müll. Arg.). In Y. P. S. Bajaj (Ed.), Somatic Embryogenesis and Synthetic Seed I Biotechnology in Agriculture and Forestry, 30, 353–369.

Carron, M. P., Deschamps, A., Enjalric, F. and Lardet, L. (1985). Vegetative *in vitro* propagation by microcutting of selected rubber trees: A widespread technique before 2000. *In Proceeding of 1st International Rubber Tissue Culture Workshop* (pp. 22–26).

Carron, M. P., Enjalric, F. Lardet, L. and Deschamps, A. (1989). Rubber (*Hevea brasiliensis* Müll. Arg.). In Y. P. S. Bajaj (Ed.), *Biotechnology in Agriculture and Forestery*, Vol. 5 *Trees II*, (pp. 222–245). Springer Berlin Heidelberg.

Carron, M. P., Lardet, L., Julien, J. and Boko, C. (2001). Somatic embryogenesis in *Hevea brasiliensis* (Muell. Arg.); Current advances and limits. *Annual IRRDB Meeting*. CIRAD, Montpellier, France, 110–118.

Cassells, A. C. (1990). Problems in tissue culture: culture contamination. In P. C. Debergh, and R. H. Zimmerman (Eds.), *Micropropagation,* (pp. 31–44). Dordrecht: Springer Netherlands.

Chee, K. (1976). Assessing susceptibility of *Hevea* clones to *Microcyclus ulei*. *Annals of Applied Biology*, 84(2), 135–145.

Chen, X. and Schluesener, H. (2008). Nanosilver: a nanoproduct in medical application. *Toxicology Letters*, 176(1), 1–12.

Chen, Z. (1984). Rubber (*Hevea*). Handbook of Plant Cell Culture- Crop Species, 2, 546–571.

Chong, C., and Taper, C. (1972). Malus tissue cultures. I. Sorbitol (D-glucitol) as a carbon source for callus initiation and growth. *Canadian Journal of Botany*, 50, 1399–1404.

Chu, S. (1966). Studies on tissue culture of *Hevea brasiliensis*. I. Role of osmotic concentration carbohydrate and pH value in induction of callus growth in plumule tissue from *Hevea* seedling. *Journal of the Rubber Research Institute of Malaya*, 19, 272–276.

Combe, J. C. and Gener, P. (1977). Effect of the stock family on the growth and production of grafted *Hevea*. *Journal of Rubber Research Institute of Sri Lanka*, *54*, 83–92.

Compton, M. E. and Preece, J. E. (1986). Exudation and explant establishment. *IAPTC Newsl*, 50, 9–18.

Conceiçao, L. F., Ferreres, F., Tavares, R. M. and Dias, A. C. (2006). Induction of phenolic compounds in *Hypericum perforatum* L. cells by *Colletotrichum gloeosporioides* elicitation. *Phytochemistry*, 67(2), 149–155.

Debergh, P. C. and Maene, L. J. (1981). A scheme for commercial propagation of ornamental plants by tissue culture. *Scientia Horticulturae*, 14(4), 335–345.

Department of Statistics, Malaysia (2014). Monthly rubber statistics Malaysia. March- 2014. ISSN 0127-6778.

Dibax, R., Eisfeld, C. D. L., Cuquel, F. L., Koehler, H. and Quoirin, M. (2005). Plant regeneration from cotyledonary explants of *Eucalyptus camaldulensis*. *Scientia Agricola*, 62(4), 406–412.

Dibrov, P., Dzioba, J., Gosink, K. K. and Häse, C. C. (2002). Chemiosmotic mechanism of antimicrobial activity of $Ag^{+}$ in *Vibrio cholerae*. *Antimicrobial Agents and Chemotherapy*, 46(8), 2668–2670.

Dijkman, M. (1951). *Hevea*, *Thirty years of research in the Far East*. Univ Miami Press, Coral Gables, Florida, USA.

Durand-Cresswell, R., Boulay, M. and Franclet, A. (1982). Vegetative propagation of *Eucalyptus*. In J. M. Bonga and D. J. Durzan (Eds.) *Tissue Culture in Forestry,* Vol. 5, (pp. 150–181). Springer Netherlands.

El Hadrami, I., Carron, M. P. and D'Auzac, J. (1991). Influence of exogenous hormones on somatic embryogenesis in *Hevea brasiliensis*. *Annals of Botany*, 67(6), 511–515.

Elechiguerra, J. L. and Burt, J. (2005). Interaction of silver nanoparticles with HIV-1. *Journal of Nanobiotechnology*, 3(6), 1–10.

Enjalric, F. and Carron, M. P. (1982). *In vitro* microcutting of *Hevea brasiliensis* (Kunth.,Mull. Arg.) young plants. *Comptes Rendus des Seances de l'Academie Des Sciences. Serie III. Sciences de La Vie*, 295, 259-264.

Enjalric, F., Carron, M. P. and Lardet, L. (1987). Contamination of primary cultures in tropical areas: the case of *Hevea brasiliensis*. *Acta Horticulturae,* 225, 57–66.

Etienne, H., Berger, A. and Carron, M. (1991). Water status of callus from *Hevea brasiliensis* during induction of somatic embryogenesis. *Physiologia Plantarum*, 82(2), 213–218.

Etienne, H., Lartaud, M., Michaux-Ferriere, N., Carron, M. P., Berthouly, M. and Teisson, C. (1997). Improvement of somatic embryogenesis in *Hevea brasiliensis* (Müll. Arg.) using the temporary immersion technique. *In Vitro Cellular and Developmental Biology-Plant*, 33(2), 81–87.

Etienne, H., Sotta, B., Montoro, P., Miginiac, E. and Carron, M. P. (1993). Comparison of endogenous ABA and IAA contents in somatic and zygotic embryos of *Hevea brasiliensis* (Müll. Arg.) during ontogenesis. *Plant Science*, 92(1), 111–119.

Fan, L., Linker, R., Gepstein, S., Tanimoto, E., Yamamoto, R. and Neumann, P. M. (2006). Progressive inhibition by water deficit of cell wall extensibility and growth along the elongation zone of maize roots is related to increased lignin metabolism and progressive stelar accumulation of wall phenolics. *Plant Physiology*, 140(2), 603–612.

Fink, C. V. M. and Sticklen, M. (1986). *In vitro* organogenesis from shoot tip, internode, and leaf explants of *Ulmus* x ―Pioneer. *Plant Cell, Tissue and Organ Culture*, 7(3), 237–245.

Garton, S, Hosier, M. A., Read, P. E. and Farnham, R. (1981). In vitro propagation of *Alnus glutinosa* Gaertn. *HortScience;(United States)*, *16*(6)*758*.

Ghanti, K. S. and Govindaraju, B. (2004). High frequency shoot regeneration from *Phyllanthus amarus* Schum. and Thonn. *Indian Journal of Biotechnology*, 3, 103–107.

Gholamhoseinpour, A. Sh., Carapetian, J. and Dejampour, J. (2012). Effects of nanosilver and vancomycin on sterilization of Peach × Almond hybrids in the in vitro cultures. *International Journal of AgriScience*, 2(5), 457–465.

Hallé, F. and Martin, R. (1968). Etude de la croissance rythmique chez l'Hevea (*Hevea brasiliensis* Mull.-Arg. Euphorbiacees-Crotonoidees). *Adansonia*, 8, 475–503.

Housti, F., Coupe, M. and D'Auzac, J. (1992). Browning mechanisms and factors of influence in *in vitro Hevea* calli cultures. *Indian Journal of Natural Rubber Research*, 5, 86–99.

Hua, Y. W., Huang, T. D. and Huang, H. (2010). Micropropagation of self-rooting juvenile clones by secondary somatic embryogenesis in *Hevea brasiliensis*. *Plant Breeding*, 129(2), 202–207.

Huang, L. C., Lee, Y. L., Huang, B. L., Kuo, C. I. and Shaw, J. F. (2002). High polyphenol oxidase activity and low titratable acidity in browning bamboo tissue culture. *In Vitro Cellular Developmental Biology Plant*, 38(4), 358–365.

Huetteman, C. A. and Preece, J. E. (1993). Thidiazuron: a potent cytokinin for woody plant tissue culture. *Plant Cell, Tissue and Organ Culture*, 33(2), 105–119.

Hui, Z., Ming, P., Xu, W., HuiCai, Z. and XiongTing, C. (2009). Micropropagation of rubber tree (*Hevea brasiliensis*) by employing mature stem as explants. *Genomics and Applied Biology*, 28(6), 1169–1176.

Jain, S. M., Gupta, P. K. and Newton, R. J. (2000). *Somatic Embryogenesis in Woody Plants* (Vol. 6). Springer Science and Business Media.

Jayashree, P. K. and Thomas, V. (2001). Optimization of parameters affecting somatic embryogenesis in *Hevea brasiliensis*. *Indian Journal Natural Rubber Research*, 14, 20–29.

Jayashree, R., Rekha, K., Venkatachalam, P., Uratsu, S. L., Dandekar, A. M., Kumari Jayasree, P., Kala, R. G., Priya, S., Sushma Kumari, S., Sobha, S., Ashokan, M. P., Sethuraj, M. R. and Thulaseedharan, A. (2003). Genetic transformation and regeneration of rubber tree (*Hevea brasiliensis* Muell. Arg) transgenic plants with a constitutive version of an anti-oxidative stress superoxide dismutase gene. *Plant Cell Reports*, 22(3), 201–9.

Jayasree, P. K. and Asokan, M. (1999). Somatic embryogenesis and plant regeneration from immature anthers of *Hevea brasiliensis* (Muell.) Arg. *Current Science*, 76(9), 1242–1245.

Jo, Y. K., Kim, B. H. and Jung, G. (2009). Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. *Plant Disease*, 93(10), 1037–1043.

Kavitha, M., Nair, R. R., Thilaga, S. and Ganesh, D. (2012). Standardization of protocol for explant preparation and plant regeneration from apical bud and nodal explants of *Anthocephalus cadamba*. *International Journal of Biological Technology*, 3(2), 8–15.

Kefeli, V. I., Kalevitch, M. V. and Borsari, B. (2003). Phenolic cycle in plants and environment. *Journal of Cellular and Molecular Biology*, 2, 13–18.

Ko, W. H., Su, C. C., Chen, C. L. and Chao, C. P. (2008). Control of lethal browning of tissue culture plantlets of Cavendish banana cv. Formosana with ascorbic acid. *Plant Cell, Tissue and Organ Culture*, 96(2), 137–141.

Kondamudi, R., Murthy, K. S. R. and Pullaiah, T. (2009). *Euphorbiaceae* a critical review on plant tissue culture. *Ropical and Subtropical Agroecosystems*, 10(3), 313–335.

Lansdown, A. (2002). Silver I: its antibacterial properties and mechanism of action. *Journal of Wound Care*, 11(4), 125–133.

Lardet, L., Aguilar, M. E., Michaux-Ferriere, N. and Berthouly, M. (1998). Effect of strictly plant-related factors on the response of *Hevea brasiliensis* and *Theobroma cacao* nodal explants cultured *In vitro*. *In Vitro Cellular and Developmental Biology-Plant*, 34(1), 34–40.

Lardet, L., Dessailly, F., Carron, M. P., Montoro, P. and Monteuuis, O. (2009). Influences of aging and cloning methods on the capacity for somatic embryogenesis of a mature *Hevea brasiliensis* genotype. *Tree Physiology*, 29(2), 291–8.

Laukkanen, H., Häggman, H., Kontunen- Soppela, S. and Hohtola, A. (1999). Tissue browning of *in vitro* cultures of Scots pine: Role of peroxidase and polyphenol oxidase. *Physiologia Plantarum*, 106(3), 337–343.

Lieberei, R. (1986). Cyanogenesis of *Hevea brasiliensis* during infection with *Microcyclus ulei*. *Journal of Phytopathology*, 115(2), 134–146.

Lieberei, R. (2007). South American leaf blight of the rubber tree (*Hevea* spp.): new steps in plant domestication using physiological features and molecular markers. *Annals of Botany*, *100*(6), 1125–42.

Lieberei, R. Selmar, D. and Biehl, B. (1985). Metabolization of cyanogenic glucosides in *Hevea brasiliensis*. *Plant Systematics and Evolution*, 150(1-2), 49–63.

Lieberei, R., Fock, H. P. and Biehl, B. (1996). Cyanogenesis inhibits active pathogen defence in plants: inhibition by gaseous HCN of photosynthetic CO2 fixation and respiration in intact leaves. Angewandte Botanik. (Germany), 70(5-6), 230-238.

Linossier, L. Veisseire, P. Cailloux, F. and Coudret, A. (1997). Effects of abscisic acid and high concentrations of PEG on *Hevea brasiliensis* somatic embryos development. *Plant Science*, 124(2), 183–191.

Lisowska, K. and Wysokinska, H. (2000). *In vitro* propagation of *Catalpa ovata* G. Don. *Plant Cell, Tissue and Organ Culture*, 60(3), 171–176.

Lloyd, G. and McCown, B. (1980). Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. *In Combined Proceedings, International Plant Propagators' Society*, 30, 421–427.

López Arnaldos, T., Muñoz, R., Ferrer, M. A. and Calderón, A. A. (2001). Changes in phenol content during strawberry (*Fragaria* × *ananassa*, cv. Chandler) callus culture. *Physiologia Plantarum*, 113(3), 315–322.

Lubick, N. (2008). Nanosilver toxicity: ions, nanoparticles-or both? *Environmental Science and Technology*, 42(23), 8617–8617.

Mahna, N., Vahed, S. Z. and Khani, S. (2013). Plant *in vitro* culture goes nano: nanosilver-mediated decontamination of *ex vitro* explants. *Journal of Nanomedicine and Nanotechnology*, 4(02), 4–7.

Majumder, S. (1964). Chromosome studies of some species of *Hevea*. *Journal of Rubber Research Institute of Malaysia*, 18, 269–273.

Martin, KP. Zhang, CL. Slater, A. and Madassery, J. (2007). Control of shoot necrosis and plant death during micro-propagation of banana and plantains (*Musa* spp.). *Plant Cell, Tissue and Organ Culture*, 88(1), 51–59.

Mathes, M. C., Morselli, M. and Marvin, J. W. (1973). Use of various carbon sources by isolated maple callus cultures. *Plant and Cell Physiology*, 14(4), 797–801.

McCown, B. H. and Sellmer, J. (1987). General media and vessels suitable for woody plant culture. In J. M. Bonga and D. J. Durzan (Eds.) *Cell and tissue culture in forestry sciences* Vol. 24-26 (pp. 4–16). Springer Netherlands.

Mederos-Molina S. and Trujillo M.I. (1999). Elimination of browning exudate and *in vitro* development of shoots in *Pistacia vera* L. cv. Mateur and *Pistacia atlantica* Desf. Culture. *Acta Societatis Botanicorum Poloniae*, *68*, 21–24.

Meier, K. and Reuther, G. (1994). Factors controlling micropropagation of mature *Fagus sylvatica*. *Plant Cell, Tissue and Organ Culture*, 39(3), 231–238.

Meiners, J., Schwab, M. and Szankowski, I. (2007). Efficient *in vitro* regeneration systems for *Vaccinium* species. *Plant Cell, Tissue and Organ Culture*, 89(2-3), 169–176.

Mendanha, A. B. L., Torres, R. A. D. A. and Freire, A. D. B. (1998). Micropropagation of rubber trees (*Hevea brasiliensis* Muell. Arg). *Genetics and Molecular Biology*, *21*, 3.

Mok, M. C. and Mok, D. W. (1985). The metabolism of [14C]-thidiazuron in callus tissues of *Phaseolus lunatus*. *Physiologia Plantarum*, 65(4), 427–432.

Montoro, P., Etienne, H., Michaux-Ferrière, N. and Carron, M. P. (1993). Callus friability and somatic embryogenesis in *Hevea brasiliensis*. *Plant Cell, Tissue and Organ Culture*, 33(3), 331–338.

Montoro, P., Rattana, W., Pujade-Renaud, V., Michaux-Ferriere, N., Monkolsook, Y., Kanthapura, R. and Adunsadthapong, S. (2003). Production of *Hevea brasiliensis* transgenic embryogenic callus lines by

*Agrobacterium tumefaciens*: Roles of calcium. *Plant Cell Reports*, 21(11), 1095–1102.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, 15(3), 473–479.

Nayanakantha, N. M. C. and Seneviratne, P. (2007). Tissue culture of rubber: past, present and future prospects. *Ceylon Journal of Science (Biological Sciences)*, 36(2), 116–125.

Ng, A. P., Ho, C. Y., Sultan, M. O., Ooi, C. B. and Lew, H. L. (1982). Influence of six rootstocks on growth and yield of six scion clones of *Hevea brasiliensis*. *In Proceedings of the Rubber Research Institute of Malaysia Planters' Conference. Kuala Lumpur.*

Paranjothy, K. and Ghandimathi, H. (1975). Tissue and organ culture of Hevea. In *International Rubber Conference, Kuala Lumpur (Malaysia).*

Pereira, A. M. S., Bertoni, B. W., Appezzato-da-Glória, B., Araujo, A. R., Januário, A. H., Lourenço, M. V. and França, S. C. (2000). Micropropagation of *Pothomorphe umbellata* via direct organogenesis from leaf explants. *Plant Cell, Tissue and Organ Culture*, 60(1), 47–53.

Potrykus, I. (1990). Gene transfer to plants: assessment and perspectives. *Physiologia Plantarum*, 79(1), 125–134.

Pretto, F. R. and Santarém, E. R. (2000). Callus formation and plant regeneration from *Hypericum perforatum* leaves. *Plant Cell, Tissue and Organ Culture*, 62(2), 107–113.

Priyadarshan, P. (2011). *Biology of Hevea rubber*. Wallingford, Oxfordshire, U. K; Cambridge, Mass. CABI.

Pua, E. C. and Chong, C. (1984). Requirement for sorbitol (D-glucitol) as carbon source for *in vitro* propagation of *Malus robusta* No. 5. *Canadian Journal of Botany*, 62(7), 1545–1549.

Quoirin, M., Boxus, P. and Gaspar, T. (1974). Root initiation and isoperoxidases of stem tip cuttings from mature *Prunus* plants. *Physiologie Vegetale,* 12, 165-174.

Razali, M., Nur Aida, M. P., Habsah, M., Hairiyah, M., Rohaya, M. A., Zaipun, M. Z. and Hazwana, H. (2012). Influence of nanosilver packaging on quality retention of peeled garlic. In *VII International Postharvest Symposium* 1012, 1317–1322.

Rhodes, M. J. C., Wooltorton, L. S. C. and Lourenço, E. J. (1979). Purification and properties of hydroxycinnamoyl CoA quinate hydroxycinnamoyl transferase from potatoes. *Phytochemistry*, 18(7), 1125–1129.

Rostami, A. and Shahsavar, A. (2009). Nano-silver particles eliminate the *in vitro* contaminations of olive 'Mission' explants. *Asian Journal of Plant Sciences*, 8(7), 5011–501.

Seigler, D. S. (1998). *Plant Secondary Metabolism.* Kluwer Academic Publishers, Springer US.

Selby, C. and Harvey, B. M. R. (1985). The influence of natural and *in vitro* bud flushing on adventitious bud production in Sitka spruce bud and needle cultures. *New Phytologist*, 100(4), 549–562.

Selmar, D., Lieberei, R., Biehl, B. and Conn, E. E. (1988). Mobilization and utilization of cyanogenic glycosides the linustatin pathway. *Plant Physiology*, 86(3), 711–716.

Selmar, D., Lieberei, R., Biehl, B. and Conn, E. E. (1989). α-Hydroxynitrile lyase in *Hevea brasiliensis* and its significance for rapid cyanogenesis. *Physiologia Plantarum*, 75(1), 97–101.

Seneviratne, P. and Wijesekara, G. (1996). The problem of phenolic exudates in *in vitro* cultures of mature *Hevea brasiliensis*. *Journal of Plantation Crops*, 24, 54–62.

Seneviratne, P. and Wijesekera, G. (1994). The growth phase and its effects on bud proliferetion and growth of in vitro culture of *Hevea brasiliensis*. *Journal of the National Science Council of Sri Lanka*, *22*(4), 313–324.

Seneviratne, P. and Wijesekera, G. (1997). Effect of $GA_3$ on the growth of axillary buds of *Hevea brasiliensis in vitro*. *Journal of Rubber Research Institute of Sri Lanka,* 37–44.

Seneviratne, P., Flegmann, A. W. and Wijesekera, G. (1995). The problem of surface sterilization of shoot materials of *Hevea*. *Journal of Rubber Research Institute of Sri Lanka*, 75(1), 51–60.

Seneviratne, P., Flegmann, A. W. and Wijesekera, G. (1996). The effects of the basic medium and the carbohydrate content on shoot cultures of *Hevea brasiliensis*. *Journal of Rubber Research Institute of Sri Lanka*, 78, 60–68.

Sirisom, Y. and Te-chato, S. (2012). The effect of peptone and silver nitrate on in vitro shoot formation in *Hevea brasiliensis* Muell Arg . *Journal of Agricultural Technology*, 8(4), 1509–1516.

Smart, D. R., Ferro, A., Ritchie, K. and Bugbee, B. G. (1995). On the use of antibiotics to reduce rhizoplane microbial populations in root physiology and ecology investigations. *Physiologia Plantarum*, 95(4), 533–540.

Sondi, I. and Salopek-Sondi, B. (2004). Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. *Journal of Colloid and Interface Science*, 275 (1), 177–82.

Sukamto, L. A. (2011). Effect of physiological age and growth regulators on callus browning of coconut endosperm. *Journal of Biotropia*, 18(1), 31–41.

Sushamakumari, S., Asokan, M. P., Anthony, P., Lowe, K. C., Power, J. B. and Davey, M. R. (2000). Plant regeneration from embryogenic cell suspension-derived protoplasts of rubber. *Plant Cell, Tissue and Organ Culture*, 61(1), 81–85.

Tahardi, J. S. (1998). Plant regeneration in *Hevea brasiliensis* via somatic embryogenesis. *Menara Perkebunan,* 66(1), 1–8.

Tao, F. J., Zhang, Z. Y., Zhou, J., Yao, N. and Wang, D. M. (2007). Contamination and browning in tissue culture of *Platanus occidentalis* L. *Forestry Studies in China*, 9(4), 279–282.

Te-chato, S. and Chartikul, M. (1993). Tissue culture of rubber: Certain factors affecting callus formation from integument seed. *Songklanakarin Journal of Science and Technology*, 15, 227–233.

Teixeira da Silva, J. A., Nhut, D. T., Tanaka, M. and Fukai, S. (2003). The effect of antibiotics on the in vitro growth response of chrysanthemum and tobacco stem transverse thin cell layers (tTCLs). *Scientia Horticulturae*, *97*(3-4), 397–410.

Toth, K., Haapala, T. and Hohtola, A. (1994). Alleviation of browning in oak explants by chemical pretreatments. *Biologia Plantarum*, 36(4), 511–517.

Vaughn, K. C. and Duke, S. O. (1984). Function of polyphenol oxidase in higher plants. *Physiologia Plantarum*, 60(1), 106–112.

Veisseire, P., Cailloux, F. and Coudret, A. (1994a). Effect of conditioned media on the somatic embryogenesis of *Hevea brasiliensis*. *Plant Physiology and Biochemistry*, *32*(4), 571–576.

Veisseire, P., Linossier, L. and Coudret, A. (1994b). Effect of abscisic acid and cytokinins on the development of somatic embryos in *Hevea brasiliensis*. *Plant Cell, Tissue and Organ Culture*, *39*(3), 219–223.

Wang, Z. Y. and Chen, X. N. (1995). Effect of temperature on stamen culture and somatic plant regeneration in rubber. *Acta Agronomic Science*, 21, 723–726.

Wang, Z. Y., Wu, H. D. and Chen, X. T. (1998). Effects of altered temperatures on plant regeneration frequencies in stamen culture of rubber trees. *Journal of Tropical and Subtropical Botany*, 6, 166–168.

Wilson, H. M. and Street, H. E. (1975). The growth, anatomy and morphogenetic potential of callus and cell suspension cultures of *Hevea brasiliensis*. *Annals of Botany*, 39(4), 671–682.

Wilson, P. J. and van Staden, J. (1990). Rhizocaline, rooting co-factors, and the concept of promoters and inhibitors of adventitious rooting. A Review. *Annals of Botany*, 66(4), 479–490.

Zhang, W., Yao, Y., Sullivan, N. and Chen, Y. (2011). Modeling the primary size effects of citrate-coated Silver Nanoparticles On Their Ion Release Kinetics. *Environmental Science and Technology*, 45(10), 4422-4428

## LIST Of PUBLICATIONS

**Moradpour, M.,** Aziz, M. A. and Abdullah, S. A. (2014). The Effects of Basic Medium and Sucrose Concentration on Growth of *Hevea Brasiliensis* Microcuttings. Poster presented at UPM- Shizuoka University International Colloquium, Universiti Putra Malaysia, Selangor, Malaysia.

**Moradpour, M.,** Aziz, M. A., Abdullah, S. A. and Ravanfar, S. A. (2013). Nanotechnology: The Solution to High Contamination and Browning of Field-Derived *Hevea* Explants Cultured *In Vitro*. Poster presented at International Conference on Crop Improvement (ICCI 2013) Issues and Prospects for Biotechnology Intervention, Bangi, Selangor, Malaysia. 25th-26th November, 2013.

**UNIVERSITI PUTRA MALAYSIA**

**STATUS CONFIRMATION FOR THESIS / PROJECT REPORT AND COPYRIGHT**

**ACADEMIC SESSION : 2014- 2015**

**TITLE OF THESIS / PROJECT REPORT:**

***IN VITRO* CULTURE ESTABLISHMENT AND SHOOT REGENERATION IN RUBBER (*HEVEA BRASILIENSIS* MUELL. ARG.)**

**NAME OF STUDEN: MAHDI MORADPOUR**

I acknowledge that the copyright and other intellectual property in the thesis/project report belonged to Universiti Putra Malaysia and I agree to allow this thesis/project report to be placed at the library under the following terms:

1. This thesis/project report is the property of Universiti Putra Malaysia.
2. The library of Universiti Putra Malaysia has the right to make copies for educational purposes only.
3. **The library of Universiti Putra Malaysia is allowed to make copies of this thesis for academic exchange.**

I declare that this thesis is classified as:

***Please tick (√)**

☐ **CONFIDENTIAL (Contain confidential information under Official Secret Act 1972).**

☐ **RESTRICTED (Contains restricted information as specified by the organization/institution where research was done).**

☐ **OPEN ACCESS I agree that my thesis/project report to be published as hard copy or online open access.**

This thesis is submitted for:

☐ Embargo from _____ until _____

**(date)** **(date)**

**Approved by:**

| | |
|---|---|
| **(Signature of Student)** | **(Signature of Chairman of Supervisory Committee)** |
| **New IC No/ Passport No.:** | **Name:** |
| **Date :** | **Date :** |

**[Note : If the thesis is CONFIDENTIAL or RESTRICTED, please attach with the letter from the organization/institution with period and reasons for confidentially or restricted. ]**