



**UNIVERSITI PUTRA MALAYSIA**

**IN VITRO PROPAGATION AND ORGAN CULTURE OF  
KACIP FATIMAH (*Labisia pumila* var. *alata*)**

**NITA AZLIN JAAFAR**

**FBSB 2013 31**



***IN VITRO PROPAGATION AND ORGAN CULTURE OF KACIP FATIMAH***  
***(Labisia pumila var. alata)***

By

**NITA AZLIN JAAFAR**

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

**October 2013**

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Abstract of thesis Presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Masters of Science

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(*Labisia pumila* var. *alata*)**

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**October 2013**

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**Faculty: Biotechnology and Biomolecular Sciences**

An *in vitro* propagation of *Labisia pumila* (*L. pumila*) var. *alata* was established using stem explants cultured on MS medium with the presence of different types of cytokinin and auxin. Three healthy shoots with fully expanded leaves per stem were obtained from stem explants culture on MS medium supplemented with 2.2 – 4.4  $\mu\text{M}$  BAP. Addition of higher concentrations of BAP increased the multiple shoots formation however the leaves formed were very small. The shoots were elongated (up to 5 to 7 cm) in medium containing GA3 with a concentration ranging from 2.9 – 4.9  $\mu\text{M}$  GA3. The elongated shoots were rooted using MS supplemented with different level of auxin. The highest number of roots were observed in medium containing 2  $\mu\text{M}$  IBA (i.e average of 20 root segment per plantlet).

Leaf explants cultured onto MS supplemented with NAA promoted the formation of short and thick root, while addition of IBA promoted the formation of long and thin roots. The highest number of root formation was seen in MS medium supplemented with 5 $\mu\text{M}$  NAA (i.e 13 root per explants). Following adventitious root induction, root suspension culture was successfully initiated using MS medium supplemented with 5 $\mu\text{M}$  NAA and 5  $\mu\text{M}$  IBA. The highest fresh weight increment of the root culture was observed in MS medium supplemented with 5 $\mu\text{M}$  NAA (1g of root per month).

In this study, attempts were made to induce hairy root from *L. pumila* var. *alata* using different strains of *A. rhizogenes* and different methods of infection. However, formation of hairy root was not observed even after 14 months of culture although the same set of *A. rhizogenes* were able to induce hairy root from leaf explants of tobacco.

Thus, to understand the mechanism underlying the host-microbe interaction during infection process, a set of virulence (*vir*) genes in *A. rhizogenes* which are responsible for the interaction, transfer and integration of the T-DNA were analyzed. The *vir* genes expression in *A. rhizogenes* was compared in order to study the activity in the susceptible and recalcitrant plant. The expression of *virA* gene was observed to increase over time indicating successful interaction between plant host and *A. rhizogenes*. *VirD2* gene was also observed to be expressed following infection thus it was hypothesized that the T-DNA was processed for further transportation into the plant host genome. The expression of genes responsible for transport channel, *virB5* and *virD4* was also observed thus indicating that the transfer of the T-DNA into the host plant may already take place. However, further analysis on the plant host genome shows that the T-DNA was not present after two days of infection indicating the possible loss of T-DNA.



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

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**Pengerusi: Norihan Mohd Saleh, PhD**

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Protokol bagi pembiakbakaan *Labisia pumila* var. *alata* secara *in vitro* telah dibangunkan dengan menggunakan segmen batang sebagai eksplan. Eksplan ini telah diinkubasi dalam media MS yang ditambah dengan auxin serta cytokinin pada kepekatan yang berbeza-beza. Tiga pucuk dengan daun yang lebar telah diperolehi bagi setiap batang apabila 2.2 – 4.4  $\mu\text{M}$  BAP ditambah ke dalam media MS. Penambahan BAP pada kuantiti yang lebih tinggi telah meningkatkan pembentukan pucuk berbilang, namun pucuk yang terbentuk mempunyai daun yang sangat kecil. Pucuk-pucuk ini telah diinkubasi dalam media yang mengandungi GA3 dengan kepekatan antara 2.9 – 4.5  $\mu\text{M}$  bagi meninggikan pucuk-pucuk tersebut. Pucuk-pucuk yang telah mencapai ketinggian yang sesuai telah diletakkan dalam media yang mengandungi kepekatan auxin yang berbeza bagi tujuan pengakaran. Bilangan akar yang paling tinggi telah didapati dalam media yang mengandungi 2 $\mu\text{M}$  IBA iaitu sebanyak 20 akar secara purata.

Pengkulturan eksplan daun di dalam media MS yang mengandungi NAA telah mempengaruhi pembentukan akar yang tebal dan pendek manakala penambahan IBA telah menyebabkan akar menjadi lebih halus dan panjang. Bilangan akar yang paling tinggi terbentuk dalam media yang mengandungi 5  $\mu\text{M}$  NAA iaitu sebanyak 13 segmen akar. Setelah akar adventitious terbentuk, kultur ampai akar telah berjaya dibangunkan dengan menggunakan media MS yang ditambah dengan sama ada 5  $\mu\text{M}$  IBA atau NAA. Penambahan berat akar yang paling tinggi didapati apabila media MS ditambah dengan 5  $\mu\text{M}$  NAA iaitu sebanyak 1 gram dalam masa sebulan.

Pelbagai usaha turut dilakukan untuk mengaruh pembentukan akar rerambut, iaitu dengan menggunakan pelbagai jenis strain *A. rhizogenes* serta cara melukakan eksplan yang berbeza-beza. Namun begitu, walaupun setelah 14 bulan dalam inkubasi, masih tiada

pembentukan akar rerambut dapat dilihat dari eksplan *L. pumila* sedangkan akar rerambut dari eksplan *Nicotina tabacum* telah terbentuk, di mana set *A. rhizogenes* yang sama telah digunakan bagi kedua-dua jenis tumbuhan.

Maka, bagi memahami mekanisme di sebalik interaksi antara mikrob dan hos semasa proses jangkitan, analisa terhadap sekumpulan gen *vir* yang bertanggungjawab terhadap proses interaksi, perpindahan dan integrasi segmen T-DNA telah dijalankan. Pengekspresan gen *vir* di dalam *A. rhizogenes* telah dikaji bagi membandingkan tumbuhan yang boleh dijangkiti dengan tumbuhan yang sukar dijangkiti. Pengekspresan gen *virA* dilihat bertambah apabila masa bertambah, membuktikan terdapat interaksi antara tumbuhan hos dan bakteria. Kehadiran *virD2* juga bertambah dengan masa, menunjukkan besar kemungkinan bahawa T-DNA telah berjaya diproses untuk dibawa masuk ke dalam hos tumbuhan. Seterusnya, gen *virD2* dan *virB5* juga turut bertambah, berkadar langsung dengan masa, maka adalah dianggarkan bahawa laluan pengangkutan yang membolehkan T-DNA dipindah masuk ke dalam hos tumbuhan telah tersedia seterusnya meninggikan lagi kebarangkalian proses transformasi berlaku. Namun begitu, analisa terhadap hos tumbuhan setelah dua hari proses transformasi dilakukan, menunjukkan bahawa T-DNA tidak dapat dikesan, maka dianggarkan bahawa T-DNA mungkin telah hilang.

## ACKNOWLEDGEMENTS

Alhamdulillah, all praises to Allah for the strengths and his blessing in guiding me to complete this thesis. Special thanks goes to my supervisor, Assoc. Prof. Dr. Norihan Mohd Saleh for her continuous support and for giving me the opportunity to pursue my studies. Her suggestions and advices had helped me through this journey.

I am also grateful beyond words to Dr. Mohd Puad Abdullah who has given me guidance and most valuable knowledge. His encouragement stimulated me to work in this project and his continued help during the research has been invaluable. My highest appreciation also goes to Dr. Kodi Isparan Kandasamy who despite his tight schedule, has managed to be a great help during the writing of this thesis and made many valuable suggestions.

Special thanks are extended to members of the plant tissue culture lab and genetic lab (Department of Cell and Biomolecular, UPM) for their opinions, advices and support. I would also like to thank the staff of plant tissue culture lab in FRIM for being kind and welcoming when I worked in the lab.

Last but not least, I wish to thank my family for loving me, all the constant support, words of encouragements and advices for the past few years.



## APPROVAL

### Approval Sheet No. 1

I certify that a Thesis Examination Committee has met on 10<sup>th</sup> October 2013 to conduct the final examination of Nita Azlin Jaafar on her thesis entitled " *In vitro* propagation and organ culture of Kacip Fatimah (*Labisia pumila* var. *alata*)" 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.

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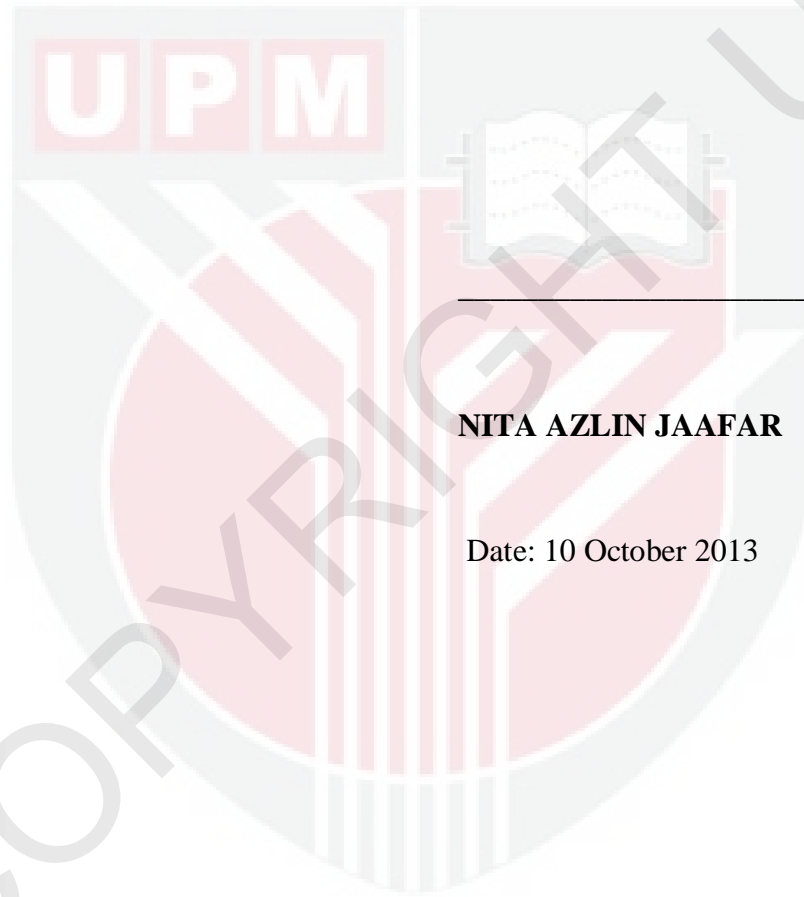
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## DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.



**NITA AZLIN JAAFAR**

Date: 10 October 2013

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