ELICITATION OF ANTHRAQUINONES WITH ITS ASSOCIATED ENZYMIC AND NON-ENZYMIC ANTIOXIDANT RESPONSES IN *MORINDA ELLIPTICA* CELL CULTURE

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MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA

2004

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By

CHONG TZER MIIN

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

December 2004

Especially dedicated to ……

father and mother
sisters
nieces and nephew
and last but not least
my dearest friends

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements of the degree of Master of Science

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December 2004

Chairman: **Mohd. Azmuddin Abdullah, Ph.D.**

Faculty: **Biotechnology and Biomolecular Science**

Morinda elliptica (Rubiaceae) cell suspension culture was used as a model system to understand the effects of elicitation and enzymic and non-enzymic antioxidant responses. The kinetic profiles of anthraquinone (AQ) compounds, hydrogen peroxide (H_2O_2) level, lipid peroxidation and antioxidant vitamins were determined for cultures grown in maintenance (M), intermediary (G) and production (P) medium strategies, and compared with the profiles from callus and leaf. Screening experiments were carried out to select the most suitable elicitors and effective elicitation factors that could enhance AQs without affecting cell growth in G medium. The selected elicitor was then used to study the effects on enzymic and non-enzymic antioxidant responses. High performance liquid chromatographic

(HPLC) method was developed to identify and quantify AQ constituents from the two methods of elicitation – medium strategies and jasmonic acid (JA).

P medium strategy produced the highest registered cell growth at 49 g l^{-1}, intracellular AQ content at 42 mg g^{-1} (dry cell weight) DW and H_2O_2 level at 9 μmol g^{-1} (fresh weight) FW, compared to other media. The extent of lipid peroxidation at 40.4 nmol g^{-1} FW and total carotenoids at 13.3 mg g^{-1} FW for cultures in P medium were comparable to the leaf. Vitamin C content in all culture systems was almost half the leaf content. On the other hand, vitamin E was around 400 to 500 μg g^{-1} FW in 7-day-old cultures from all medium strategies which was 2-fold to the leaf. In P(21) cultures, nordamnacanthal at 5.6 mg g^{-1} DW and 1-hydroxy-2-methylanthraquinone at 17.2 mg g^{-1} DW were detected 4-fold and 12-fold, respectively, higher than the roots. The mechanism to counter reactive oxygen species (ROS) in the whole plant and plant cell cultures is dynamic depending on the availability and level of different types of antioxidants.

Different elicitors depending on the concentration and day of treatment exerted different effects on cell growth and AQ production. Fifty μM JA treated on day 12 with cultures harvested on day 15 enhanced AQ content to 39.6 mg g^{-1} DW, which was 2.1-fold to control. JA elicitor treatment during early and exponential growth phase showed significant AQ induction than stationary growth phase. With JA treatment on day 12 and cultures harvested on day 17, nordamnacanthal, 1-hydroxy-2-methylanthraquinone and lucidin-ω-methyl ether were registered at 3.3, 3 and 1.8 mg g^{-1} DW, which were 2, 3 and 4-fold, respectively, to control. Early exponential

growth phase elicitation can induce extracellular AQ such as 1 g l^{-1} *Aspergillus flavus* (AF) elicitation which significantly promoted extracellular AQ at 5 mg l^{-1}.

In the early stage of elicitation, the increase in antioxidant activities as compared to control show that antioxidative mechanisms were functioning to counter the increase in H_2O_2 and lipid peroxidation. JA elicitation could enhance total carotenoids and vitamin E to the level comparable to P medium strategy and the leaf. The reduction of both catalase (CAT) and ascorbate peroxidase (APO) activities in elicited cultures after 6 days could suggest a greater antioxidative role being played by AQ, tocopherols and carotenoids. On the other hand, the higher vitamin C in elicited culture than control corresponds well with low APO, but high glutathione reductase (GR) activities suggest the need to maintain glutathione (GSH) level. The induction or reduction of antioxidant activities provides evidence for occurrence of oxidative burst in elicited cell cultures and the versatility of plant secondary compounds. The better understanding of stress responses in plant cell culture could pave the way for rational design and distribution of plant cell-based products.

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

PENINGKATAN ANTRAKUINON DAN TINDAKBALAS ANTIOKSIDAN ENZIM DAN BUKAN-ENZIM DI DALAM *MORINDA ELLIPTICA* SEL KULTUR

Oleh

CHONG TZER MIIN

Disember 2004

Pengerusi: **Mohd. Azmuddin Abdullah, Ph.D.**

Fakulti: **Bioteknologi dan Sains Biomolekul**

Ampaian sel *Morinda elliptica* (Rubiaceae) telah digunakan sebagai sistem model untuk mengetahui kesan peningkatan dan tindakbalas antioksidan enzim dan bukan-enzim. Profil kinetik komponen anthrakuinon (AQ), paras hidrogen peroksida (H_2O_2), pengoksidaan lipid dan vitamin antioksidan ditentukan daripada sel kultur yang tumbuh di dalam strategi media penjagaan (M), perantaraan (G) dan penghasilan (P) dan dibandingkan dengan profil kultur kalus dan daun. Ujikaji awal dilakukan untuk memilih "elisitor" yang sesuai dan faktor peningkatan yang berkesan dalam meningkatkan AQ dengan tidak merencatkan pertumbuhan sel di dalam media G. "Elisitor" terpilih kemudian digunakan untuk mengkaji kesan terhadap tindakbalas enzim dan bukan-enzim antioksidan. Teknik kromatografi

cecair bertekanan tinggi (HPLC) dibangunkan untuk mengenalpasti komponen-komponen AQ dan kuantitinya daripada dua jenis cara peningkatan – strategi media dan asid jasmonik (JA).

Strategi media P telah menghasilkan tahap tertinggi pertumbuhan sel pada 49 g l^{-1}, 42 mg g^{-1} (berat kering) DW kandungan AQ intrasel dan 9 μmol g^{-1} (berat basah) FW paras H_2O_2, jika dibandingkan dengan media lain. Pengoksidaan lipid sebanyak 40.4 nmol g^{-1} FW dan jumlah karotenoid sebanyak 13.3 mg g^{-1} FW di dalam kultur media P adalah sebanding dengan jumlah yang diperolehi daripada daun. Kandungan vitamin C di dalam semua sistem kultur adalah separuh daripada kandungan daun. Kandungan vitamin E pula adalah 400 hingga 500 μg g^{-1} FW dalam kultur berumur 7 hari menggunakan semua strategi media, iaitu 2 kali ganda daripada daun. Dalam kultur P(21), sebanyak 5.6 mg g^{-1} DW nordamnakantal dan 17.2 mg g^{-1} DW 1-hidrosil-2-metilanthrakuinon telah diperolehi, iaitu 4 dan 12 kali ganda lebih tinggi daripada akar, mengikut untan. Mekanisme untuk menangani spesis oksigen reaktif (ROS) di dalam tumbuhan dan kultur tisu tumbuhan adalah dinamik dan ianya bergantung kepada keupayaan dan paras setiap antioksidan yang berbeza-beza.

Faktor kepekatan dan hari rawatan "elisitor" yang berbeza memberi kesan yang berbeza terhadap pertumbuhan sel dan penghasilan AQ. Rawatan 50 μM JA pada hari ke-12 dengan penuaian pada hari ke-15 telah meningkatkan kandugan AQ 39.6 mg g^{-1} DW, iaitu 2.1 kali ganda lebih banyak daripada kawalan. Rawatan "elisitor" JA pada fasa pertumbuhan awal dan pertengahan eksponen meningkatkan AQ secara ketara berbanding dengan fasa pertumbuhan pegun. Dengan rawatan JA pada hari

ke-12 dan tuaian pada hari ke-17, nordamnakantal, 1-hidrosil-2-metilantrakuinon dan lucidin-ω-metil eter masing-masing menghasilkan 3.3, 3 and 1.8 mg g^{-1} DW, iaitu 2, 3 dan 4 kali ganda secara turutan berbanding dengan kawalan. Rawatan "elisitor" pada pertumbuhan awal eksponen dapat meningkatkan AQ ekstrasel dengan ketara di mana 1 g l^{-1} "elisitor" *Aspergillus flavus* (AF) menghasilkan 5 mg l^{-1} AQ ekstrasel.

Pada tahap awal rawatan "elisitor", peningkatan aktiviti antioksidan berbanding dengan kawalan menunjukkan mekanisma antioksidan yang berfungsi untuk menghadapi peningkatan paras H_2O_2 dan pengoksidaan lipid. Rawatan "elisitor" JA boleh meningkatkan jumlah karotenoid dan vitamin E ke paras setanding dengan strategi media P dan daun. Penurunan pada aktiviti katalase (CAT) dan askorbat peroksidase (APO) pada kultur berelisitor selepas hari ke-6 menunjukkan bahawa tugas utama sebagai antioksidan dimainkan oleh AQ, tokoferol dan karotenoid. Selain daripada itu, vitamin C pada kultur berelisitor adalah lebih tinggi berbanding dengan kawalan dan selaras dengan penurunan aktiviti APO, namun peningkatan aktiviti glutation reduktase (GR), dicadangkan dengan keperluan untuk memastikan paras sekata glutathion (GSH). Peningkatan atau penurunan aktiviti antioksidan menjadi suatu bukti tentang kewujudan kesan oksidatif melampau pada kultur sel berelisitor dan keupayaan komponen sekunder tumbuhan yang pelbagai. Kefahaman tentang isyarat tegasan pada kultur sel tumbuhan dapat menyediakan asas untuk pendekatan yang lebih rasional dalam meningkatkan produktiviti sel tumbuhan.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my greatest appreciation to Dr. Mohd. Azmuddin Abdullah, for his invaluable guidance, constructive comments and ideas. He gave me the chance to improve my knowledge, to be more independent and to take things in stride as an explorer. My deep sense of gratitude is also to Assoc. Prof. Dr. Lai Oi Ming for her kindness, patience and encouragement. My gratitude goes to Dr. Nor'Aini Mohd. Fadzillah and Prof. Dr. Nordin Hj. Lajis for their advice and support throughout my project and thesis writing.

To En. Rosli, Kak Fadhilah, Mr. Ong, Pn. Raonah and others in the Department of Biotechnology, UPM - thanks for all the assistance. Special thanks to Kak Yati and Kak Intan from the Department of Biology, Tissue Culture Lab, UPM for their assistance in antioxidant experiments. My special appreciation to the Malaysian Government for the PASCA scholarship that has enabled me to complete my study without having to worry about whose going to pay for the fee.

To all my friends - Fu Siaw Liung and wife, Rozita Omar, Hii Siew Ling, Yii Choo Ying, Levina, Lim Long Chang, Poh Yang Ming, members of Phytotron and Enzyme lab - Kong Ching, Shamsina, Kavita, Madhiyah, Chiang Lim, Susan, Suzailin, Su, Ziha and others whom I may have not listed here (sorry). Thanks for the camaraderie, without which my life will not be as meaningful and colourful. Last but not least, my utmost gratitude to my parents and sisters for their continuous support, love and encouragement throughout my life. God bless you all!

I certify that an Examination Committee met on 7 Dec 2004 to conduct the final examination of Chong Tzer Miin on his Master of Science thesis entitled "Elicitation of anthraquinones with its associated enzymic and non-enzymic antioxidant responses in *Morinda elliptica* cell culture" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on 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 UPM or other institutions.

_____

CHONG TZER MIIN

Date :

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