UNIVERSITI PUTRA MALAYSIA

COMBINED EFFECTS OF GAMMA IRRADIATION AND CARBON DIOXIDE ENRICHMENT ON PHYSIOLOGICAL TRAITS AND FLAVONOID CONTENTS OF *CENTELLA ASIATICA* L.

SINA SIAVASH MOGHADDAM

FP 2011 33

COMBINED EFFECTS OF GAMMA IRRADIATION AND CARBON DIOXIDE ENRICHMENT ON PHYSIOLOGICAL TRAITS AND FLAVONOID CONTENTS OF *CENTELLA ASIATICA* L.

By

SINA SIAVASH MOGHADDAM

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

April 2011

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

COMBINED EFFECTS OF GAMMA IRRADIATION AND CARBON DIOXIDE ENRICHMENT ON PHYSIOLOGICAL TRAITS AND FLAVONOID CONTENTS OF *CENTELLA ASIATICA* L.

By

SINA SIAVASH MOGHADDAM

April 2011

Chairman: Associate Professor Hawa binti Jaafar, PhD

Faculty: Agriculture

The study was counducted to investigate the effects of gamma irradiation on secondary metabolite biosynthesis based on flavonoid, and to provide valuable information on the utilization of beneficial effects of gamma radiation in plant cell culture system. The work also determined the combined effects between gamma irradiation and CO2 enrichment on physiological characteristics and flavonoid compounds of *Centella asiatica* as medicinal herb. Turning to the experimental evidence, significant differences were observed in morphological characters, physiological and total flavonoid contents of eight accessions. Total flavonoid content in the leaf was observed to be highest compared with petiole and root in CA23 (9.33± 0.04mg/g DW) and lowest in CA03 (3.17± 0.01mg/g DW). Meanwhile, CA23 was found to have highest net photosynthesis (PN) and leaf area. Time to harvest was observed to be an important factor for accumulation of total flavonoid content in the plants and 6 weeks after planting was recorded to be the best for all accessions. In tissue culture experiments, analysis of

variance for shoot regeneration from *C. asiatica* nodal explants which cultured on Murashige and Skoog (MS) and shoot media (Duchefa) supplemented with various concentrations of BAP in combination with NAA revealed that frequency of responding explants increased markedly with an enhancing in the concentration of BAP up to 2mg/l in combination of 0.1mg/l NAA in Duchefa medium as well as soaking in TDZ. The highest shoot multiplication, node/explant and leaf/explants of *C.asiatica* were achieved with soaking in (TDZ) at a concentration of 5mg/l for 2 hours. The results of this investigation show that, full-strength MS medium was better than half-strength MS medium for root initiation. A comparison by Duncan's method revealed optimum frequency 95% in shoots cultured on full-strength MS containing 0.5mg/l IBA. The acclimatized plantlets were successfully established with 85% and 77% survival for CA23 and CA03 accessions respectively. The results for radiation sensitivity test based on survival percentage of irradiated and non-irradiated plantlets demonstrated that significant reduction in survival percentage was observed with increasing gamma dosage. For the survival percentage of irradiated nodals to reach 50%, the gamma dosage administered was 60Gy for CA03 and 40Gy for CA23. The most obvious findings to emerge from this study are that, gamma irradiation induced various physiological and biochemical alterations in plants. Biochemical tests unfolded that the irradiated accessions displayed higher total flavonoid content than the non-irradiated ones. The chromatograms obtained from High Performance Liquid Chromatography (HPLC) study revealed that methanolic extracts of *Centella asiatica* under the irradiation treatments displayed significantly variable compositions of flavonoid compounds. Interestingly, among the flavonoids detected, the concentration of rutin in the leaf

extracts was found to be the highest (1.51±0.00014mg/g DW) from those of the other flavonoids. In addition, after rutin, in irradiated plants, fisetin (in CA03) and naringin (in CA23) were detected in the highest amount. Photosynthetic pigments were declined through gamma irradiation with concomitant loss of the photosynthetic ability. The results of this study indicated that under gamma conditions the Malondialdehyde (MDA) (as an indicator of free radicals) and flavonoid contents increased and the Fv/Fm declined concomitantly. This study also investigated that elevated CO2 as excess carbon promoted photosynthesis rate and increased the growth of *C.asiatica*, as well as stimulated the defense mechanism through boosting flavonoid content. The evidence from this study elucidated that the 4- hours CO_2 enrichment at 800µmol/mol signified that there were no significant differences in the measured traits with the exception of carbohydrates, where high level of carbohydrate accumulation caused restriction in potential of net photosynthesis in irradiated and non-irradiated plants of *Centella asiatica*. These findings enhance our understanding about metabolic engineering of flavonoid pathway which gamma and CO_2 concomitantly and synergistically resulted in boosting flavonoid concentration. Although CO_2 enrichment ameliorated the adverse effects of gamma on photosynthesis, the plant also benefits from gamma irradiation to significantly boost flavonoids components.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

GABUNGAN KESAN IRADIASI GAMMA DAN PENGAYAAN KARBON DIOSIDA PADA SIFAT-SIFAT FISIOLOGI DAN KANDUNGAN FLAVONOID DARI *CENTELLA ASIATIC* L.

Oleh

SINA SIAVASH MOGHADDAM

April 2011

Pengerusi: Profesor Madya Hawa binti Jaafar, PhD

Fakulti: Pertanian

Penyelidikan ini telah dijalankan untuk mengkaji kesan penyinaran gamma terhadap biosintesis metabolit sekunder berdasarkan flavonoid dan memberikan maklumat berharga tentang penggunaan kesan yang bermanfaat dari radiasi gamma dalam sistem kultur sel tumbuhan serta untuk menentukan kesan gabungan penyinaran gamma dan pengayaan CO_2 terhadap ciri- ciri fisiologi dan sebatian flavonoid *Centella asiatica* sebagai ramuan ubat. Ciri- ciri morfologi, fisiologi dan kandungan flavonoid telah menunjukkan perbezaan yang signifikan dalam kalangan lapan aksesi yang dikaji. Didapati jumlah kandungan flavonoid pada daun adalah tertinggi berbanding dengan tangkai daun dan akar pada CA23 (9,33 ± 0.04mg/ g BK) dan terendah pada CA03 (3.17 ± 0.01mg/ g BK). Sementara itu, CA23 didapati mempunyai kadar bersih fotosintesis (PN) dan luas daun yang tertinggi. Waktu menuai pula didapati faktor penting untuk akumulasi TFC di tanaman dan 6 minggu selepas tanam didapati yang terbaik untuk

semua aksesi. Analisis varians bagi regenerasi tunas dari eksplan nodal *C. asiatica* yang dikultur pada media MS dan media tunas (Duchefa) yang dilengkapi dengan pelbagai kepekatan BAP dengan kombinasi NAA menunjukkan bahawa frekuensi eksplan yang bergerak balas meningkat secara nyata dengan peningkatan kepekatan BAP hingga 2mg/l dengan kombinasi 0.1mg/l NAA dalam medium Duchefa serta perendaman dalam thidiazuron (TDZ). Pergandaan tunas, nod/eksplan dan daun/eksplan *C.asiatica* yang tertinggi diperolehi dengan perendaman dalam TDZ pada kepekatan 5mg/l selama 2 jam. Keputusan kajian ini menunjukkan bahawa media MS kekuatan- penuh lebih baik daripada media MS kekuatan- separuh untuk inisiasi akar. Hasil perbandingan DMRT pula menunjukkan frekuensi optimum sebanyak 95% pada tunas yang ditanam di media MS kekuatan- penuh yang mengandungi 0.5mg/l IBA. Planlet- plantlet berjaya diaklimatisasi pada kelangsungan hidup masing- masing 85% bagi aksesi CA23 dan 77% bagi CA03. Keputusan ujian sensitiviti radiasi berdasarkan peratusan kelangsungan hidup plantlet yang disinari dan tidak disinari pula menunjukkan pengurangan yang signifikan dalam peratusan kelangsungan hidup dengan peningkatan dos gamma. Dos gamma yang diberikan untuk peratusan kelangsungan hidup nodal yang disinari mencapai 50% adalah 60Gy bagi CA03 dan 40Gy bagi CA23. Penemuan yang paling jelas yang diperoleh dari kajian ini adalah penyinaran gamma menyebabkan pelbagai perubahan fisiologi dan biokimia pada tanaman. Ujian biokimia menunjukkan bahawa aksesi- aksesi yang disinari mengandungi jumlah kandungan flavonoid yang lebih tinggi daripada aksesi- aksesi yang tidak disinari. Kromatogram- kromatogram yang diperoleh dari kajian HPLC mendedahkan ekstrak metanol dari *C. asiatica* di bawah penyinaran menunjukkan komposisi sebatian flavonoid yang berbeza secara signifikan. Menariknya, kepekatan

rutin dalam ekstrak daun adalah yang paling tinggi (1,51 ± 0.00014 mg/g BK) berbanding dengan flavonoid- flavonoid lain yang dikesan. Ia diikuti oleh fisetin (dalam CA03) dan naringin (dalam CA23) yang telah dikesan dalam jumlah yang tertinggi pada tanaman yang disinari. Pigmen- pigmen fotosintesis pula berkurang di bawah penyinaran gamma, beriringan dengan kehilangan kemampuan fotosintesis. Keputusan kajian ini menunjukkan mekanisme di mana MDA (sebagai penunjuk radikal bebas) dan kandungan flavonoid meningkat dan Fv/Fm menurun secara seiringan di bawah keadaan gamma. Penyelidikan ini juga meneliti bahawa kepekatan CO_2 yang tinggi, sebagai sumber karbon yang berlebihan mempercepatkan kadar fotosintesis dan meningkatkan pertumbuhan *C. asiatica*, serta merangsang mekanisme pertahanan melalui peningkatan kandungan flavonoid. Bukti yang diperoleh dari kajian ini menjelaskan bahawa pengayaan CO_2 selama 4 jam pada 800µmol/m^2/s menunjukkan bahawa tidak ada perbezaan yang signifikan pada ciri- ciri yang diukur kecuali pada karbohidrat di mana akumulasi karbohidrat yang tinggi mengehadkan potensi fotosintesis bersih pada tanaman *C. asiatica* yang disinari dan tidak disinari. Penemuan ini meningkatkan pemahaman kita tentang kejuruteraan metabolik flavonoid yang ditingkatkan oleh penyinaran gamma dan CO_2 secara seiringan dan secara sinergetiknya meningkatkan kepekatan flavonoid, melindungi serta memperbaiki sistem fotosintesis. Walaupun, pengayaan CO_2 mengimbangi kesan buruk gamma ke atas fotosintesis, tanaman juga memperoleh manfaat dari penyinaran gamma untuk meningkatkan komponen flavonoid secara signifikan.

I certify that a Thesis Examination Committee has met on 12 April 2011 to conduct the final examination of Sina Siavash Moghaddam on his thesis entitled "Combined Effects of Gamma Irradiation and Carbon Dioxide Enrichment on Physiological Traits and Flavonoid Contents of *Centella Asiatica* L." 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 Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Mohd Razi Ismail, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Mohd Ridzwan A. Halim, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Puteri Edaroyati Megat Wahab, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Chieri Kubota, PhD
Professor
University of Arizona, USA
(External Examiner)

NORITAH OMAR, PhD
Associate Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 12 July 2011

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. Members of the Supervisory Committee were as follows:

Hawa binti Jaafar, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Asmah bt Rahmat, PhD
Professor
Faculty of Medicine
Universiti Putra Malaysia
(Member)

Maheran bt Abdul Aziz, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Rusli Ibrahim, PhD
Principal Researcher
Agrotechnology & Bioscience Division
Agensi Nuklear Malaysia
(Member)

Elizabeth Philip, PhD
Researcher of Climate Change & Forestry Programme
Forest Research Institutes Malaysia (FRIM)
(Member)

HASANAH MOHD GHAZALI, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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, and is not concurrently, submitted for other degree at University Putra Malaysia or other institution.

_____

SINA SIAVASH MOGHADDAM

Date: 12 April 2011

ACKNOWLEDGEMENTS

First of all, praise is to “Allah” the cherisher, and the sustainers of the world for giving me strengths, health and determination to complete this thesis. I was privileged to get the support of several institutes and individuals to make the project concept a reality on its own merits. I would like to thank my thesis supervisor, Associate Professor Dr Hawa binti Jaafar for her guidance and introducing me to Malaysian Nuclear Agency.

I am grateful to the Malaysian Nuclear Agency for approving and enabling me to conduct my research work. The professional assistance of Dr Rusli Ibrahim, Principal Researcher of Nuclear Agency was an inspiration to develop and implement the project. Thank you for your knowledge, expertise and critical comments. I take great pleasure here and wish to express my sincere appreciation to contribution of Prof. Dr. Asmah bt Rahmat for lab work carried out at Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences. My gratitude goes to Dr. Elizabeth Philip Head of Climate Change & Forestry Programme and Assoc. Prof. Dr. Maheran bt Abdul Aziz for their valuable guidance and advice in throughout my study period at UPM. The advisory services of the Malaysian Agricultural Research and Development Institute (MARDI) are commendable. Appreciate Prof Desa Ahmad for assigning growth chamber. The support of my friends during the research activities especially Dr Majid Masoumian deserves great appreciation. I acknowledge my dedicated parents, brother and sisters in law for their devotion and affectionate motivation during a specially demanding period of our lives.

Finally, I wish to thank the one dearest to me, my beloved wife, who continues to astonish me with his patience, resilience and love.

DEDICATION

This thesis is dedicated to my:

Wife

Narges Delafrooz
and our newborn daughter Elena

With innermost and everlasting affection and love

TABLE OF CONTENTS