UNIVERSITI PUTRA MALAYSIA

IMPACT OF CO2 ENRICHMENT AND ITS INTERACTION WITH NITROGEN ON GROWTH , PHYSIOLOGY AND SECONDARY METABOLITES OF *Labisia pumila* Benth

MOHD HAFIZ BIN IBRAHIM

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By

MOHD HAFIZ BIN IBRAHIM

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

September 2012

DEDICATION

For my beloved mother Sharifah bte Hassan, for all of your sacrifices and hardships in caring and teaching me as your son, you have raised me excellently. And for my future wife Nurul Amalina bte Mohd Zain who believed in me, without you there would be no excuses for me to stand still and work hard to achieve my dreams. My heartfelt gratitude for all love, encouragement and support through the years of my quest for knowledge. May this achievement shall be our stepping stone towards living our dreams and ambitions……...

The vegetation of a good land comes forth (easily) by the Permission of its Lord; and that which is bad, brings forth nothing but (a little) with difficulty. Thus do We explain variously the Ayât (proofs, evidences, verses, lessons, signs, revelations, etc.) for a people who give thanks".

"[Al-A'râf 7 : 58]

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Doctor of Philosophy

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September 2012

Chairman : Associate Professor Hawa ZE Jaafar, PhD

Faculty : Agriculture

The demand for *Labisia pumila* (Kacip Fatimah) keeps increasing annually as this plant is believed to have high phytoestrogen activity that widely used in neutraceutical industries. However the plant is relatively difficult slow to grow, and takes about 16 to 36 months before it is ready for use in medicinal preparation. Carbon dioxide (CO_2) enrichment technique is known to be able to enhance slow growing plants and also to improve and alter plant bioactive compounds. Enriching *L. pumila* with elevated CO_2 may extend these benefit. Therefore, a project was conducted with the objectives ; (i) to determine and establish the most suitable microclimate for CO_2 enrichment of *L. pumila* under growth house based on ventilation per floor rate area and shading levels (ii) to investigate the effects of different CO_2 concentration on the growth, leaf gas exchange and secondary metabolites of three varieties of *L. pumila* (iii) to determine the interaction between CO_2 and nitrogen levels on growth, leaf gas exchange, primary and secondary metabolites of *L. pumila* and (iv) to establish and understand the biochemical regulation of secondary metabolites in *L. pumila* seedlings under interaction effects between CO_2 and nitrogen levels. There were four experiments

conducted to fulfill the objectives. In the first experiment , two levels of shade (0% , and 90%) and five different ventilation to floor area (v/f) ratio (0 = close; 0.375, =open-up; 0.450 = open-doors; 0.750 = open-bottom; 1.35 = open-all) were conducted to determine the best combination of these factors for establishment of the suitable microclimate for *L. pumila* under growth house for CO_2 enrichment. Result showed that shade+open-all give the best microclimate inside the growth chamber. Analysis of variance revealed that means of temperature and vapor pressure deficit (VPD) were significantly ($P \leq 0.01$) influenced by v/f ratio. Mean relative humidity (RH) inside the chamber was significantly ($P \leq 0.01$) enhanced by the shade used. Under shade+open-all, the stomatal conductance (gs) of *L. pumila* was found to be very sensitive to VPD. At VPD, temperature and RH of 1.41 kpa, 33^0C and 68% respectively, gs of *L. pumila* started to decrease from 1100 am onwards suggesting that CO_2 enrichment should be carried out before this time to optimize benefit from CO_2 exposure. In the second experiment, three varieties of *L. pumila* seedlings (*alata*, *pumila* and *lanceolata*) were exposed to three levels of CO_2 (400, 800,1200 µmol mol^{-1}). In this experiment, the manipulation of CO_2 enrichment on *L. pumila* seedling seemed to be able to reduce the nursery period through growth enhancement, that was solely contributed by CO_2 enrichment. Neither varietal differences nor its interaction effects with CO_2 were observed. Increasing CO_2 from 400 to 1200 µmol mol^{-1} had significantly improved growth, net photosynthesis (A), water use efficiency, maximum efficiency of photosystem II, carbohydrate, total phenolics and total flavonoids. However, chlorophyll content, specific leaf area, and net assimilation rate were found to decrease by end of the experiment. From this experiment, it was found that the production of secondary metabolites was negatively correlated with chlorophyll content implying that nitrogen levels might be playing an important role

in the production of secondary metabolites in *L. pumila*. In the third experiment, three varieties of *L. pumila* seedlings were exposed to four levels of nitrogen (0, 90, 180 , 270 kg N ha^{-1}) under 1200 µmol mol^{-1} CO_2 in a split plot design. Absolute control at 400 µmol mol^{-1} CO_2 and 180 kg N ha^{-1} (Standard) were also included. Under CO_2 enrichment, application of low nitrogen (0 and 90 kg N ha^{-1}) managed to enhance the production of secondary metabolites significantly regardless of differences in varieties. As levels of nitrogen increased, plant growth, photosynthesis, photosynthesis nitrogen use efficiency (PNUE) and chlorophyll content were enhanced. However, carbohydrate, total phenolics and flavonoids were reduced with increase in nitrogen fertilization. Increase in the production of secondary metabolites was found to be positively correlated with increase in the carbon to nitrogen ratio (C/N) and PNUE. In the last experiment, two CO_2 levels (400 and 1200 µmol mol^{-1}) and three nitrogen levels (0, 90, 270 kg N ha^{-1}) were used to establish the mechanism of enhancement of secondary metabolites under varied CO_2 levels and nitrogen fertilization. It was found that the highest production of secondary metabolites was obtained at 1200 µmol mol^{-1} + 0 kg N ha^{-1} and lowest at 400 µmol mol^{-1} + 270 kg N ha^{-1}. The interaction effect between CO_2 and nitrogen was found to influence the production of secondary metabolites, fructose, PAL activity and protein in *L. pumila*. Generally, these findings support the protein competition model that suggest the increase in production of secondary metabolites under high CO_2 and low nitrogen levels was due to increased availability of phenylalanine (precursor for phenolics and flavonoids) due low sink ratio which increases the availability for production of secondary metabolites. From the project, it can be concluded that CO_2 enrichment onto *L. pumila* was able to enhance growth and medicinal properties especially at low nitrogen fertilization.

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

PENGARUH PERKAYAAN CO_2 DAN INTERAKSI DENGAN NITROGEN TERHADAP PERTUMBUHAN, FISIOLOGI DAN METABOLIT SEKUNDER *Labisia pumila* Benth

Oleh

MOHD HAFIZ IBRAHIM

September 2012

Pengerusi : Profesor Madya Hawa ZE Jaafar, PhD

Fakulti Pertanian

Permintaan terhadap *Labisia pumila* (Kacip Fatimah) terus meningkat setiap tahun kerana tanaman ini diyakini memiliki kandungan fitoestrogen tinggi yang banyak digunakan dalam industri neutraseutikal. Walaubagaimanapun, pertumbuhan relatif pokok ini lambat, dan memerlukan waktu sekitar 16 - 36 bulan untuk boleh digunakan untuk pernyediaan ubat. Perkayaan karbon dioksida pada *L. pumila* mungkin berpotensi untuk meningkatkan pertumbuhan *L. pumila* serta sebatian bioaktifnya. Perkayaan dengan karbon dioksida mungkin melanjutkan faedah tersebut. Disebabkan itu satu penyelidikan telah dijalankan dengan objektif; (i) untuk menentukan mikroiklim yang sesuai untuk perkayaan CO_2 pada *L.pumila* berdasarkan nisbah ventilasi per luas lantai dan naungan (ii) untuk mengenalpasti kesan pelbagai kepekatan CO_2 pada pertumbuhan, pertukaran gas daun dan metabolit sekunder pada tiga varieti *L.pumila* (iii) untuk mengkaji kesan interaksi perkaryaan CO_2 dan nitrogen pada pertumbuhan, pertukaran gas daun dan metabolit primer dan sekunder dan (iv) untuk memahami regulasi biokimia metabolit sekunder pada interaksi antara

CO_2 dan nitrogen di dalam *L.pumila*. Empat eksperimen telah dijalankan untuk memenuhi objektif tersebut. Pada eksperimen pertama, dua tahap naungan (0% , 90%) dan lima nisbah ventilasi kepada luas lantai (v/f) (0, tertutup sepenuhnya; 0.375, terbuka-atas; 0.450, terbuka-pintu;0.750, terbuka-bawah; 1.35, terbuka-penuh) telah dijalankan untuk menentukan kombinasi terbaik faktor tersebut untuk pewujudan mikroklimat yang sesuai untuk *L. pumila* di bawah rumah perkayaan CO_2. Keputusan menunjukkan yang naungan+ terbuka-penuh memberikan mikroklimat sesuai di dalam rumah perkayaan CO_2. Analisis varians menunjukkan yang purata suhu, defisit tekanan vapor (DTV) secara signifikan ($P \leq 0.01$) mempengaruhi kadar v/f. Purata kelembapan bandingan (KB) secara signifikannya ($P \leq 0.01$) mempengaruhi naungan yang digunakan. Di bawah terbuka-penuh kekonduktasi stomata (gs) *L. pumila* didapati sangat sensitif terhadap defisit tekanan Vapor (DTV). Pada DTV, suhu dan KB 1.41 kPa, 33^0C dan 68% masing-masing, gs *L. pumila* mulai menurun bermula dari pukul 1100. Ini menunjukkan bahawa perkaryaan CO_2 harus dilakukan sebelum waktu ini untuk mendapat manfaat optima dari perkayaan. Dalam eksperimen kedua, tiga varieti *L. pumila* (*alata, pumila* dan *lanceolata*.) didedahkan pada tiga kepekatan CO_2 (400 800 dan 1200 µmol mol^{-1}). Dalam kajian ini, didapati perkaryaan boleh mengurangkan tempoh di dalam tapak semaian melalui peningkatan pertumbuhan yang secara tunggalnya dipengaruhi oleh perkaryaan CO_2. Sepanjang eksperimen tiada kesan variati dan interaksi didapati. Didapati bahawa peningkatan CO_2 dari 400 - 1200 µmol $mo1^{-1}$ secara signifikan meningkatkan pertumbuhan, fotosintesis bersih (A), kecekapan penggunaan air (WUE), kecekapan maksimum fotosistem II (f_v/f_m), karbohidrat, total fenol dan flavonoid. Namun, kandungan klorofil, luas daun spesifik (SLA), dan kadar assimilasi bersih (NAR) telah didapati menurun pada akhir eksperimen. Dari kajian ini, didapati bahawa pengeluaran metabolit sekunder

berkorelasi negatif dengan kandungan klorofil dan boleh disimpulkan bahawa nitrogen mungkin memainkan peranan langsung dalam pengeluaran metabolit sekunder di dalam *L. pumila*. Pada eksperimen ketiga, tiga variati *L. pumila* telah didedahkan pada empat aras nitrogen yang berbeza (0, 90, 180 dan 270 kg N Ha^{-1}) di bawah 1200 µmol mol^{-1} CO_2 di dalam rekabentuk split plot. Kawalan mutlak pada 400 µmol mol^{-1} CO_2 dan 180 kg N ha^{-1} juga disertakan. Dalam perkayaan CO_2, pembajaan nitrogen yang rendah (0 dan 90 kg N ha^{-1}) telah didapati berjaya meningkatkan pengeluaran metabolit sekunder walaupun tiada kesan variati didapati. Pada pembajaan nitrogen yang tinggi didapati pertumbuhan tanaman, fotosintesis, photosynthesis nitrogen use efficiency (PNUE) dan kandungan klorofil telah meningkat. Namun, kandungan karbohidrat, fenol dan flavonoid berkurang dengan peningkatan pembajaan nitrogen. Peningkatan pengeluaran metabolit sekunder didapati berkorelasi positif dengan peningkatan nisbah karbon kepada nitrogen (C/N) dan PNUE. Pada eksperimen terakhir, dua tahap CO_2 (400 dan 1200 µmol mol^{-1}) dan tiga tahap nitrogen (0, 90 dan 270 kg N Ha^{-1}) telah digunakan untuk mengkaji mekanisme peningkatan metabolit sekunder di bawah perkaryaan CO_2 dan pembajaan nitrogen. Didapati pengeluaran tertinggi metabolit sekunder diperolehi pada 1200 µmol mol^{-1} + 0 kg N Ha^{-1} dan terendah di 400 µmol mol^{-1} + 270 kg N Ha^{-1}. Kesan interaksi antara CO_2 dan nitrogen didapati mempengaruhi pengeluaran metabolit sekunder, fruktosa, aktiviti phenyll alanine lyase (PAL) dan kandungan protein di dalam *L.pumila*. Secara umumnya, penemuan ini menyokong model protein competition model (PCM) yang menunjukkan peningkatan pengeluaran metabolit sekunder di bawah perkaryaan CO_2 dan pembajaan nitrogen rendah disebabkan peningkatan phenylalanine (prekursor untuk fenol dan flavonoid) kerana "keupayaan sinki rendah" yang meningkatkan ketersediaan untuk pengeluaran metabolit sekunder.

Dari projek ini, boleh disimpulkan perkayaan CO_2 pada *L. pumila* mampu meningkatkan pertumbuhan dan khasiat perubatan terutamanya pada pembajaan nitrogen yang rendah.

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I certify that an Examination Committee has met on 21/09/2012 to conduct the final examination of Mohd Hafiz Bin Ibrahim on his Doctor of Philosophy thesis entitled "IMPACT OF CO_2 ENRICHMENT AND ITS INTERACTION WITH NITROGEN ON GROWTH, PHYSIOLOGY AND SECONDARY METABOLITES OF *Labisia pumila* Benth", 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. Member of the Examination Committee are as follows:

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

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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 or and is not concurrently submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

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MOHD HAFIZ BIN IBRAHIM

Date :

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