

**EFFECTS OF NITROGEN SOURCES ON SELECTED BIOCHEMICAL  
PROPERTIES OF *ORYZA SATIVA* L. CULTIVARS**

**By**

**YAP WAI SUM**

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

**April 2006**

*DEDICATED TO:*

*Souls that entrapped in the mastermind of the body,  
May you find happiness...*

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

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**Chairman : Professor Maziah Mahmood, PhD**

**Faculty : Biotechnology and Biomolecular Sciences**

Fifteen recommended rice cultivars were used in this experiment. They were Setanjung, Muda, MR 84, RU 2242-1-1, IR 64, MR 185, MR 151, MR 159, MR 167, MRQ 34, MR 207, MR 209, MR 211, MR 219 and MR 220. Leaves of three-week old seedlings were analyzed for nitrogen assimilating enzymes, namely nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS), glutamate synthase (GOGAT) and glutamate dehydrogenase (GDH) activities to evaluate the assimilation of N supply whereas ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity was use as an indicator for photosynthesis. The end products of N and C metabolism such as chlorophyll, soluble protein, fresh weight, soluble carbohydrates and starch contents were also analyzed. These biochemical evaluation were carried out in three different growth medium known as ‘control ~ without nitrogen sources’, ‘NO<sub>3</sub><sup>-</sup> containing medium’ and ‘NH<sub>4</sub><sup>+</sup> containing medium’ to determine the effect of different N sources. Treatments were laid out in split-plots in a randomized complete block design with N treatment as the main plot and cultivars as the subplot. In the study obtained, the addition of external

N sources did not help in accelerating the activities of N assimilating enzymes (NR, NiR, GS, GOGAT and GDH) as well as Rubisco when compared to control medium. In the leaves of three-week old seedlings, NR, NiR, GS and Rubisco activities were negatively related with the availability of N sources (-88%, -28%, -22% and -13%, respectively). GOGAT activity was demonstrated not to be influenced by the addition of N sources (no significant different) whereas GDH activity was higher in control (+51%) and  $\text{NH}_4^+$  (+20%) containing medium as compared to  $\text{NO}_3^-$ .

In investigation of the effects of different nitrogen sources on selected N and C metabolites, positive correlation was observed. The increment of nearly 40% under nitrate supply and 62% in ammonium of the soluble protein content was seen with the external addition of N. Similarly, there was also an increased in the fresh weight (FW) content of nearly 0.8-fold in  $\text{NO}_3^-$  grown plants to 0.2-fold in  $\text{NH}_4^+$  fed plants when different N sources were applied (the increased in ammonium supply was not statistically significant in comparison with control). This lower FW content under ammonium supply could be the consequences of the slight chlorosis observed in the leaves tissue. In contrast, chlorophyll content happened to be lower in both  $\text{NO}_3^-$  and  $\text{NH}_4^+$  containing medium, with a reduction of 30% and 14%, respectively. Soluble carbohydrates content was slightly higher under nitrate supply than in ammonium whereas the control medium was found to possess higher starch content. Lower starch content in both medium ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) could possibly relate to higher nitrogen use efficiency which resulted in an increase of soluble protein and FW contents. Apparently, the antioxidative property of ascorbic acid content was significantly higher in both control and  $\text{NH}_4^+$  containing

medium, whereas glutathione reductase (GR) activity was highest under ammonium supply.

Cultivars as the subplot were found to demonstrate high significant evidence ( $P < 0.001$ ) and were in consistency among the entire biochemical analyses, indicating genetic materials (15 rice cultivars) being one of the influential factor in regulating the outcome of the biochemical results obtained.

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

**KESAN SUMBER NITROGEN TERHADAP PARAMETER BOKIMIA  
TERTENTU PADA KULTIVAR *ORYZA SATIVA* L.**

Oleh

**YAP WAI SUM**

**April 2006**

**Pengerusi : Profesor Maziah Mahmood, PhD**

**Fakulti : Bioteknologi dan Sains Biomolekul**

Lima belas kultivar padi pilihan yang disaran digunakan dalam eksperimen ini. Padi tersebut adalah Setanjung, Muda, MR 84, RU 2242-1-1, IR 64, MR 185, MR 151, MR 159, MR 167, MRQ 34, MR 207, MR 209, MR 211, MR 219 dan MR 220. Daun anak pokok padi pada minggu ke-tiga digunakan untuk analisis enzim-enzim asimilasi nitrogen, iaitu aktiviti nitrat reduktase (NR), nitrit reduktase (NiR), glutamin sintetase (GS), glutamat sintase (GOGAT) dan glutamat dehidrogenase (GDH) untuk menilai tahap asimilasi nitrogen manakala aktiviti ribulose 1,5-bisphosphate karbosisilase/oksigenase (Rubisco) digunakan sebagai penunjuk untuk fotosintesis. Analisis hasil akhir untuk metabolit N dan C seperti klorofil, protein pelarut, berat basah, karbohidrat pelarut dan kanji juga dikaji. Penilaian kesemua biokimia ini dilakukan pada tiga jenis media pertumbuhan iaitu 'kawalan ~ tidak mengandungi nitrogen', 'NO<sub>3</sub><sup>-</sup> kandungan media' dan 'NH<sub>4</sub><sup>+</sup> kandungan media' untuk mengkaji kesan sumber N yang berlainan. Kajian ini dilakukan berdasarkan plot-pecah dengan rekaan blok rawak keseluruhan yang mengaplikasikan N sebagai plot utama dan kultivar sebagai subplot. Berdasarkan keputusan yang diperoleh, penambahan sumber N luaran tidak membantu dalam mempertingkatkan aktiviti enzim-enzim asimilasi N (NR, NiR, GS, GOGAT dan GDH) dan juga Rubisco apabila dibandingkan dengan kawalan. Pada daun anak pokok padi minggu ke-tiga, aktiviti NR, NiR, GS dan Rubisco adalah berkadar

songsang dengan kehadiran sumber N (-88%, -28%, -22% dan -13%, masing-masing). Aktiviti GOGAT pula tidak dipengaruhi dengan penambahan sumber N (tiada perbezaan yang signifikan) manakala aktiviti GDH adalah lebih tinggi pada kawalan (+51%) dan pada media  $\text{NH}_4^+$  (+20%) apabila dibandingkan dengan  $\text{NO}_3^-$ .

Berdasarkan kajian ke atas kesan sumber N yang berbeza terhadap metabolit N dan C yang terpilih, perkadaran yang positif dapat dilihat. Peningkatan protein larut sebanyak 40% di bawah sumber nitrat dan 62% di bawah sumber ammonium dapat dikesan dengan penambahan N. Begitu juga dengan kandungan berat basah, di mana penambahan sebanyak 0.8-kali dalam  $\text{NO}_3^-$  dan 0.2-kali dalam  $\text{NH}_4^+$  dapat dilihat apabila sumber N yang berbeza digunakan (peningkatan pada sumber ammonium tidak menunjukkan perbezaan yang signifikan jika dibandingkan dengan kawalan). Kandungan berat basah yang berkurangan ini mungkin disebabkan oleh klorosis yang dapat diperhatikan pada tisu daun anak pokok padi yang dibekalkan dengan sumber ammonia. Sebaliknya, kandungan klorofil yang diperolehi adalah lebih rendah pada kedua-dua kandungan media  $\text{NO}_3^-$  dan  $\text{NH}_4^+$ , dengan penurunan masing-masing sebanyak 30% dan 14%. Kandungan karbohidrat larut pula menunjukkan sedikit peningkatan apabila dibekalkan dengan nitrat barbanding dengan ammonium manakala media kawalan mempunyai kandungan kanji yang lebih tinggi. Kandungan kanji yang lebih rendah di kedua-dua  $\text{NO}_3^-$  dan  $\text{NH}_4^+$  mungkin disebabkan oleh keberkesanan penggunaan nitrogen yang lebih tinggi dengan menyebabkan peningkatan dalam kandungan protein larut dan berat basah. Sebaliknya, kandungan antioksidan asid askorbik pula menunjukkan secara signifikan

lebih tinggi di media kawalan dan  $\text{NH}_4^+$ , manakala glutathion reduktase (GR) menunjukkan aktiviti yang tertinggi pada media ammonium.

Kultivar sebagai subplot pula menunjukkan bukti signifikansi yang tinggi dengan  $P < 0.001$  dan keadaan ini adalah konsisten pada semua analisis biokimia yang dikaji. Ini menunjukkan bahawa bahan genetik merupakan satu faktor penting yang mengawalatur keputusan yang bakal diperolehi.

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*“IN REACHING FOR GOLDS,  
MOST PEOPLE FORGOT ABOUT THE **SILVER LINING**”*

I certify that an Examination Committee has met on 5<sup>th</sup> April 2006 to conduct the final examination of Yap Wai Sum on his Master of Science thesis entitled “Effects of Nitrogen Sources on Selected Biochemical Properties of *Oryza sativa* L. Cultivars” 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.

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**YAP WAI SUM**

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