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NITROGEN UPTAKE AND EXPRESSION OF NITROGEN TRANSPORTERS OF SELECTED UPLAND RICE

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By

ADIBAH MOHD AMIN

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

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DEDICATION

This thesis is dedicated to

my parents, beloved family and dearest friends.

Thank you for your continuous support.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Doctor of Philosophy

NITROGEN UPTAKE AND EXPRESSION OF NITROGEN TRANSPORTERS OF SELECTED UPLAND RICE

By

ADIBAH MOHD AMIN

September 2016

Chairman : Professor Zaharah Abdul Rahman, PhD
Faculty : Agriculture

Nitrogen (N) is one of the most yield limiting nutrients for upland rice production. The differences in N accumulation in plants have been attributed to the N uptake ability of the roots. The most important aspect of this research is to determine potential factors that can contribute to nitrogen use efficiency (NUE). The objectives of this study are: 1) to determine the NUE of selected upland rice landraces. 2) to determine the root characteristics of upland rice as influenced by nitrogen fertilization, and 3) to identify the expression of high-affinity ammonium transporter that is expressed under different nitrogen level. The first study was focused on NUE of selected upland rice landraces as affected by P fertilization. Five landraces of upland rice seedlings were transplanted in plots treated with two P levels (0kg P/ha and 100kg P/ha). The ^{15}N source is from ^{15}N labelled ammonium sulphate fertilizer [$^{15}(NH_4)_2SO_4$] with 1% ^{15}N atom excess. The %N derived from fertilizer (%Ndff) was calculated using the equation based on isotope dilution technique. The performance of the landraces in taking up the N fertilizer was evaluated by comparing the NUE. At 4 and 8 weeks after transplanting, the NUE had no significant differences between the landraces ($p \geq 0.05$). However, Landrace I had the highest NUE during 8 weeks after transplanting which was 33.59% higher as compared to other landraces. At week 16 after transplanting, the landraces that showed significant effects on NUE ($p \leq 0.05$) was Landrace III with the highest NUE which was 52.59% more than landrace I which had 27.50%. The P fertilization had no significant effects on NUE, dry matter yield and grain yield at week 4, 8 and 16 of all the selected upland rice landraces. The second experiment on root characterization of five upland landraces was planted at Field 10, UPM. Six treatments were applied: (1) 150kg/ha N as Ammonium sulphate (2) 75 kg/ha N as Ammonium sulphate (3) 150kg/ha N as Potassium nitrate (4) 75 kg/ha N as Potassium nitrate (5) Control (0kg/ha N) of Ammonium sulphate (6) Control (0kg/ha N) of Potassium nitrate. The root parameters were recorded since root surface area is important for nutrient uptake. Landrace III had the highest total surface area at both low and high N rates at week 12 and had resulted in high NUE of the landrace. There were significantly positive correlations between bleeding rate and root surface area. The third experiment on the expression of high-affinity ammonium transporter was carried on two upland rice landraces that showed high NUE and low NUE. They were chosen from a previous field experiment. Plants were treated with modified Yoshida nutrient

solution with 0.05mM NH_4NO_3, 0.1mM NH_4NO_3, 1mM NH_4NO_3 and 2mM NH_4NO_3. The expression of ammonium transporter (*OsAMT1;1*) was determined. Landrace III had the highest expression of the transporter compare to Landrace I, thus supporting the results that landrace III had significantly higher NUE compared to Landrace I.

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

PENGAMBILAN NITROGEN DAN EKSPRESI PENGANGKUT NITROGEN OLEH 'LANDRACE' PADI HUMA TERPILIH

Oleh

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Nitrogen adalah salah satu nutrien faktor pengehadkan hasil pengeluaran padi huma. Perbezaan pengumpulan N dalam tumbuh-tumbuhan telah dikaitkan dengan pengambilan keupayaan N akar. Aspek yang paling penting dalam kajian ini adalah untuk menentukan faktor-faktor yang berpotensi untuk menyumbang kepada kecekapan penggunaan nitrogen (KPN). Objektif kajian ini adalah: 1) untuk menentukan KPN 'landrace' padi huma dipilih. 2) untuk menentukan ciri-ciri akar padi huma kesan dipengaruhi oleh pembajaan nitrogen, dan 3) untuk mengenal pasti ekspresi 'ammonium transporter' afiniti tinggi kesan dipengaruhi kepekatan nitrogen yang berbeza. Kajian pertama telah memberi tumpuan kepada KPN 'landrace' padi huma terpilih kesan dipengaruhi oleh pembajaan P. Lima 'landrace' padi huma telah ditanam di dalam plot dan dirawat dengan dua tahap P (0kg/ha P dan 100kg/ha P). Sumber ^{15}N adalah dari baja ammonium sulfat yang dilabel [$^{15}(NH_4)_2SO_4$] dengan 1% ^{15}N atom yang berlebihan. Peratus N berasal dari baja (% Nbdb) dikira menggunakan persamaan berdasarkan teknik isotop pencairan. Prestasi 'landrace' dalam mengambil baja N yang telah dinilai dengan membandingkan KPN. Pada 4 dan 8 minggu selepas dialihkan, KPN tidak mempunyai perbezaan yang signifikan antara varaiti ($p \geq 0.05$). Walau bagaimanapun, 'Landrace' I mempunyai KPN tertinggi semasa 8 minggu selepas diubah iaitu 33.59% lebih tinggi berbanding 'landrace' lain. Pada minggu ke-16 selepas dipindahkan, 'landrace' yang menunjukkan kesan yang besar ke atas KPN ($p \leq 0.05$) adalah 'Landrace' III dengan KPN tertinggi iaitu 52.59% lebih daripada 'Landrace' I yang mempunyai 27.50%. Pembajaan P tidak mempunyai kesan yang besar ke atas KPN, hasil bahan kering dan hasil bijirin pada minggu 4, 8 dan 16 untuk semua 'landrace' padi huma. Kajian kedua pada pencirian akar lima 'landrace' padi huma ditanam di Ladang 10, UPM. Enam rawatan telah digunakan: (1) 150kg/ha N sebagai ammonium sulfat (2) 75kg/ha N sebagai ammonium sulfat (3) 150kg/ha N sebagai Kalium nitrat (4) 75kg/ha N sebagai kalium nitrat (5) kawalan (0kg/ha N) ammonium sulfat (6) kawalan (0kg/ha N) kalium nitrat. Parameter akar telah direkodkan, kerana kawasan permukaan akar adalah penting untuk pengambilan nutrien. 'Landrace' III mempunyai jumlah luas permukaan yang paling tinggi di kedua-dua kadar N rendah dan tinggi pada minggu ke-12, dan telah menyebabkan KPN 'landrace' itu tinggi. Terdapat hubungan yang signifikan positif antara kadar 'root bleeding' dan jumlah luas permukaan akar. Kajian ketiga mengenai ekspresi

pengangkut ammonium afiniti tinggi telah dijalankan pada dua 'landrace' padi huma yang menunjukkan KPN tinggi dan KPN rendah. Mereka dipilih daripada kajian sebelumnya. Pokok telah dirawat dengan larutan nutrien Yoshida diubahsuai dengan 0.05mm NH_4NO_3, 0.1mm NH_4NO_3, 1mm NH_4NO_3 dan 2mm NH_4NO_3. Ekspresi pengangkut ammonium (*OsAMT1;1*) dinilai. 'Landrace' III mempunyai ekspresi tertinggi pengangkut ammonium berbanding 'Landrace' I, oleh itu menyokong keputusan yang 'Landrace' III mempunyai KPN lebih tinggi berbanding dengan 'Landrace' I.

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I certify that a Thesis Examination Committee has met on 30 September 2016 to conduct the final examination of Adibah bt Mohd Amin on her thesis entitled "Nitrogen Uptake and Expression of Nitrogen Transporters of Selected Upland Rice" 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.

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LIST OF ABBREVIATIONS

%	Percentage
µ	Micro
µg	Microgram
µm	Micrometer
µmol	Micromole
^{0}C	Degree Celsius
AA	Auto analyzer
AAS	Atomic absorption spectroscopy
ANOVA	Analysis of variance
AS	Asparagine synthetase
AspAT	Aspartate aminotransferase
C	Carbon
Ca	Calcium
CEC	Cation exchangeable capacity
cm	Centimeter
CO_2	Carbon dioxide
G	Gram
GDH	Glutamate dehydrogenase
GOGAT	Glutamate synthase
GS	Glutamine synthetase
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
ha	Hectare
HCl	Hydrochloric acid
K	Potassium
kg	Kilogram
L	Liter
LSD	Least significant difference
m	Meter
Mg	Magnesium
mg	Milligram

mL	Milliliter
mM	Mili molar
Mn	Manganese
N	Nitrogen
NaOH	Sodium hydroxide
OsAMT1;1	*Oryza sativa* ammonium transporter 1;1
P	Phosphorus
ppm	Part per million
s	Second
SAS	Statistical analysis software
UPM	Universiti Putra Malaysia

CHAPTER 1

INTRODUCTION

Around 3 billion people of the world use rice as a basic food that provides 50 to 80% of their daily calories (Sohrabi et al., 2012). Malaysian per capita consumption on rice is 78.6 kg/year on 2014 (Department of Statistics Malaysia, 2015). Malaysian rice industry typically depended on wetland varieties such as MR219 and MR220 to meet the consumption demand. However, due to increasing population, it is insufficient and Malaysia needed to import 1031.4 thousand metric tonnes of rice (Din et al., 2016). In 2015, the self-sufficiency level of rice in Malaysia is just about 71.4% (Economic Planning Unit, Prime Minister's Department, 2015), which is much below the Malaysia government target of 100% to be achieved in 2020. Globally, annual rice production is around 600 million tons from cultivation on more than 150 million hectares (Guimaraes, 2009; Kondo et al., 2003). Upland rice comprises less than 15 percent of global rice production and is cultivated on around 14 million hectares of land (Hynes, 2008). Although upland rice might have a small role in total rice production, it is a major source of food in some Asian countries (Thanh et al., 1999). Bangladesh, Indonesia, Laos and the Philippines are the countries that plant the most upland rice, unfortunately its yield is low, about 1 t/ha only (Reuveni, 2011; Musa et al., 2009).

Upland rice (O*ryza sativa*) refers to rice planted under dry conditions and usually grown on either flat or sloping land. Water source for upland rice during its planting season is rainfall only. According to Wang et al., (2008), the planting of upland rice is always restricted by its lower and unstable yield because it solely depends on nutrients that are dissolved in the soil moisture for growth. When soil moisture is low, limited nutrients are available (Hynes, 2008).

In Malaysia, upland rice is planted in Sabah and Sarawak regions with about 165,888 ha land and the natives grow this rice for their subsistence (Sohrabi et al., 2012). It is an important agricultural activity for home consumption and sometimes the farmers sell surplus rice to earn some money (Musa et al., 2009). Previously, upland rice was typically grown without added fertilizers and accompanied by a long fallow period. Due to increased population pressure, such lengthy fallow periods are no longer practicable making upland rice planting as a major cause of land degradation and nutrient mining because of slash-and-burn technique on sloping land (Mutert and Fairhurst, 2002).

Nitrogen (N) is one of the macronutrients that are needed by all plants and N fertilizer is an essential input for crop production including upland rice. A balanced N fertilizer input can ensure the maximum growth and yield of crops. There are several factors that contribute to the production of high-yielding rice, such as N supply pattern, plant uptake process, and absolute amount of absorbed N. The amount of N supply is proportional to the formation of yield component, for example the number of panicles at each crucial stage. At early growth stage, the amount of N absorbed closely corresponds to the number of tillers because N is crucial in tillers formation. Thus, the potential number of panicles can be determined by tiller number (Mae and Shoji, 1984).

Improving the uptake and utilization efficiency of crop would be a great contribution to agriculture. Nitrogen use efficiency (NUE) can be simply expressed as the yield of N per unit of available N in the soil. NUE can be studied using stable N isotope technique (Harmsen, 2003). Several approaches can be followed in achieving this goal or at least optimally maintaining crop productivity. These approaches include biotechnology, plant breeding and adopt the best N management strategies. Increased cereal crop NUE is environmentally beneficial along with economically benefits the crop producer (Beatty et al., 2010). Genetic variability might cause the differences in NUE. The differences in NUE are the results of differences in absorption, translocation, shoot demand, dry matter production per unit of nutrient absorbed by plants as well as environmental interactions (Baligar et al., 2001). The NUE is combinations of effective ion transportation start from the soil to the root surface before entering roots and transported to the shoots and then, remobilize to plant organs.

Plant growth is usually limited by nitrogen supply. In agriculture; N fertilizer is applied to optimize yield. Plant growth and development correspond with plant N metabolism and carbon regulation. If there are any changes in N supply, it could trigger genes alteration resulting in modifications in root morphology and growth rate development (Miller, 2010). Plant response to P limitation is different from that to N limitation. This difference in plant response could be explained by their respective role in a plant system. Besides that, plant response also might be affected by the relatively higher accumulation of inorganic phosphate (Pi) compared to nitrate. Plant N concentration could be affected by inadequate P supply. When P is limited, plant N concentration decreases (de Groot et al., 2003).

The concern on human health and the environment caused by poor N fertilization management and excessive application of N fertilizer has also arisen. The leached out N can harm the environment as well as human as it can contaminate the water source in the soil that is used as drinking water. Besides that, applying more fertilizers to a crop can reduce the profit margin due to the high cost of fertilizers and their applications.

This research is focused on comparing the NUE of selected upland rice landraces. Since the roots are the uptake organ for N, thus a systematic analysis of N uptake directly from the root is necessary. The most important aspect of this research is to understand some of the factors that contribute to differences in NUE of upland rice.

Therefore, this research would help generate some important information that is lacking in understanding the nitrogen uptake in upland rice. The objectives of this study were:

i. To determine the NUE of selected upland rice landraces as influenced by P fertilization.
ii. To evaluate the root characteristics of upland rice landraces as influenced by N fertilization.
iii. To study the expression of a high-affinity ammonium (NH_4^+) transporter that is expressed on landraces with different NUE at different N levels.

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LIST OF PUBLICATIONS

Adibah, M. A., Zaharah A. R., Hanafi, M. M., SitiNorAkmar, A. Variation in Nitrogen Uptake Efficiency of Upland Rice Landraces as Influenced by P Fertilization. Australian Journal of Crop Science. (Accepted).

Adibah, M. A., Zaharah A. R., Hanafi, M. M., SitiNorAkmar, A. Root Characterization of Selected Upland Rice Landraces as Influenced by Nitrogen Fertilization. Journal of Agricultural Science and Technology. (In review).

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