



**UNIVERSITI PUTRA MALAYSIA**

***THE EFFECT OF N<sub>2</sub>- FIXING BACTERIA AND SPLIT TIME  
APPLICATION ON GROWTH OF *Oryza sativa****

**TUAN SYARIPAH NAJIAH TUAN MOHD RAZALI**

**FP 2013 53**

**THE EFFECT OF N<sub>2</sub>- FIXING BACTERIA AND SPLIT TIME  
APPLICATION ON GROWTH OF *Oryza sativa***

**TUAN SYARIPAH NAJIHAH TUAN MOHD RAZALI**

**DEPARTMENT OF AGRICULTURE TECHNOLOGY**

**FACULTY OF AGRICULTURE**

**UNIVERSITI PUTRA MALAYSIA**

**2012/2013**

**THE EFFECT OF N<sub>2</sub>- FIXING BACTERIA AND SPLIT TIME  
APPLICATION ON  
GROWTH OF *Oryza sativa***

**By**

**TUAN SYARIPAH NAJIHAH TUAN MOHD RAZALI**

**A project report submitted to Faculty of Agriculture  
Universiti Putra Malaysia**

**In fulfilment of the requirement of PRT4999 (Project)  
for the award of the degree of  
Bachelor of Agricultural Science**

**DEPARTMENT OF AGRICULTURE TECHNOLOGY  
FACULTY OF AGRICULTURE  
UNIVERSITI PUTRA MALAYSIA**

**2012/2013**

## CERTIFICATION FORM

This project paper entitled “The effect of N<sub>2</sub>-fixing bacteria on rice plant and split time application on *Oryza Sativa*” is prepared by Tuan Syaripah Najihah Tuan Bt Mohd Razali and submitted to the Faculty of Agriculture in the fulfilment of the requirement of PRT4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science.

Student's name:

Student's signature:

**TUAN SYARIPAH NAJIHAH BT  
TUAN MOHD RAZALI**

.....

Certified by:

\_\_\_\_\_  
(PROF. MADYA. DR HALIMI BIN MOHD SAUD)

**Project Supervisor**

**Department of Agriculture Technology,**

**Faculty of Agriculture,**

**Universiti Putra Malaysia.**

**Date:** \_\_\_\_\_

## ACKNOWLEDGEMENT

In the name of Allah The Most Gracious, The Most Merciful..

Alhamdulillah, with His wills and blessing I had successfully accomplished this project paper.

First of all, I would like to extend my sincere appreciation to Assoc.Prof Dr Halimi Mohd Saud for his brilliant guidance, suggestion and advice during planning until the end of my project.

Secondly, I would like to thank the laboratory staff of Department of Agriculture Technology and Department of Land Management especially Mr. Azali and Mdm. Fauziah for their guidance and help during this project.

A special thank to my family and my friends for their understanding and continuous support that made my work go smoothly and perfectly.

Last but not least, I am thankful to all those helping me to finish up this project. Without them, it would have been difficult for me to accomplish this project. Only God can repay for your kindness. Thank you very much.

## TABLE OF CONTENTS

Page

ACKNOWLEDGMENT	i
TABLE OF CONTENTS	ii
LIST OF TABLE	iv
LIST OF FIGURES	v
LIST OF APPENDICES	vi
LIST OF PLATES	vii
ABSTRACT	viii
ABSTRAK	ix
 <b>CHAPTER 1: INTRODUCTION</b>	
1.1 Introduction	1
1.2 Objectives	3
 <b>CHAPTER 2: LITERATURE REVIEW</b>	
2.1 <i>Oryza sativa</i> L.	4
2.2 MR219	7
2.3 Plant Growth Promoting Rhizobacteria	7
2.4 Rhizosphere	9
2.5 <i>Bacillus sphaericus</i> (UPMB10)	10
 <b>CHAPTER 3: METHODOLOGY</b>	
3.1 Location of study	12
3.2 Soil preparation	12
3.3 Seed preparation	13
3.4 <i>Bacillus sphaericus</i> (UPMB10) inoculations	13

3.5 Data collection	13
3.5.1 Chlorophyll content (SPAD meter)	13
3.5.2 Leaves dry weight (g)	14
3.5.3 Plant height (cm)	14
3.5.4 Root analysis	14
3.5.5 Plant tissue analysis	14
3.6 Statistical analysis	15
 <b>CHAPTER 4: RESULTS AND DISCUSSION</b>	
4.1 Leaves chlorophyll content (ppm)	16
4.2 Leaves dry weight (g)	17
4.3 Leaves height (cm)	18
4.4 Root volume (cm <sup>3</sup> )	19
4.5 Root length (cm)	20
4.6 Root surface area (cm <sup>2</sup> )	21
4.7 Nitrogen content (mg/plant)	22
4.8 Phosphorus content (mg/plant)	23
4.9 Potassium (mg/plant)	24
 <b>CHAPTER 5: CONCLUSION</b>	25
REFERENCES	26
APPENDICES	30
PLATES	32

## LIST OF TABLE

Table	Title	Page
3.1	List of treatments and time application of <i>Bacillus sphaericus</i> (days).	12





## LIST OF FIGURES

Figure	Title	Page
2.1	Growth stage of rice plant	6
4.1	Leaves Chlorophyll Content	16
4.2	Leaves Dry Weight	17
4.3	Leaves Height	18
4.4	Root Volume	19
4.5	Root Length	20
4.6	Root Surface Area	21
4.7	Nitrogen Content	22
4.8	Phosphorus Content	23
4.9	Potassium Content	24

## LIST OF APPENDICES

Appendices	Page
Appendix 1: ANOVA for Leaves Chlorophyll Content	29
Appendix 2: ANOVA for Leaves Dry Weight	29
Appendix 3: ANOVA for Leaves Height	29
Appendix 4: ANOVA for Nitrogen Content	30
Appendix 5: ANOVA for Phosphorus Content	30
Appendix 6: ANOVA for Potassium Content	30

## LIST OF PLATES

Plate	Title	Page
1	Application of <i>Bacillus sphaericus</i> (UPMB10) on the 15 <sup>th</sup> day	32
2	Taking chlorophyll content on the 69 <sup>th</sup> day using SPAD meter	32
3	Roots that have been washed	33
4	Leaves that have been cut on the 70 <sup>th</sup> day	33
5	Root analysis using a scanner (Expression 1680, Epson)	33

## ABSTRACT

Rice plant or its scientific name, *Oryza sativa* is the staple food of Malaysia. Rice (*Oryza sativa* L.) is one of the most important food crops in the world and more than 90% of rice is produced and consumed in Asia. In order to increase the production of this crop, various methods have been done including the use of plant growth-promoting rhizobacteria (PGPR). Plant growth-promoting rhizobacteria (PGPR) are defined as root-colonizing bacteria that exert beneficial effects on plant growth and development. Nitrogen fertilizer is also used widely in rice plant production. But, only little nitrogen is absorbed by the plant and the rest is loss. The efficiency of the urea-N in rice culture is very low, generally around 30–40%, in some cases even lower. It is such a waste of input for not applying this fertilizer and bacteria at the correct time. Therefore this experiment is conducted to observe the effect of N<sub>2</sub>-fixing bacteria on rice plant and split time application on rice plant and to determine if there is any interaction between nitrogen fertilizer and split time application of N<sub>2</sub>-fixing bacteria on the growth of rice plant. Five treatments including one control with 6 replications were used in this experiment and are arranged by using RCBD. The nitrogen fertilizer was applied at the beginning of experiment while the N<sub>2</sub>-nitrogen fixing bacteria (UPMB10) was applied on 15<sup>th</sup>, 30<sup>th</sup>, 45<sup>th</sup>, 60<sup>th</sup> day after planting. The results showed that *Bacillus sphaericus* (UPMB10) increase the rice plant's dry weight by 10.81%, root volume by 23.18%, root length by 9.93%, root surface area by 13.7% and nitrogen content by 4.36%. The most effective time to inoculate this bacteria is on the 45<sup>th</sup> day after planting. An early inoculation time did not give any advantage to the growth response of *O. Sativa*.

## ABSTRAK

Padi atau nama saintifiknya, *Oryza sativa* merupakan makanan ruji rakyat Malaysia. Padi (*Oryza sativa* L.) adalah salah satu tanaman makanan yang paling penting di dunia dan lebih daripada 90% daripada beras yang dihasilkan dan digunakan di Asia. Dalam usaha untuk meningkatkan pengeluaran tanaman ini, pelbagai kaedah telah dilakukan termasuk penggunaan rhizobakteria penggalak pertumbuhan tanaman (PGPR). Penggalak (PGPR) merupakan bakteria yang memberi kesan baik terhadap pertumbuhan dan pembangunan tumbuhan. Baja nitrogen juga digunakan secara meluas dalam pengeluaran tanaman padi. Tetapi, hanya sedikit nitrogen diserap oleh tumbuhan dan selebihnya hilang. Keberkesanan baja urea dalam penanaman padi adalah sangat rendah, umumnya sekitar 30-40%. Ini merupakan satu pembaziran input jika baja dan bakteria ini tidak digunakan dalam masa yang betul. Eksperimen ini dijalankan untuk melihat kesan bakteria pengikat nitrogen terhadap tanaman padi dan pengaplikasian waktu yang berbeza dan interaksi antara baja nitrogen dan bakteria pengikat nitrogen terhadap pertumbuhan padi. Lima rawatan termasuk satu kawalan dengan 6 replikasi digunakan dalam eksperimen ini dan disusun dengan menggunakan RCBD. Baja nitrogen digunakan pada awal eksperimen manakala bakteria pengikat nitrogen (UPMB10) akan digunakan pada 15, 30, 45, 60 hari selepas menanam. Keputusan menunjukkan bahawa *Bacillus sphaericus* (UPMB10) meningkatkan berat kering daun padi sebanyak 10.81%, isipadu akar sebanyak 23.18%, panjang akar sebanyak 9.93%, luas permukaan akar sebanyak 13.7% dan kandungan nitrogen sebanyak 4.36%. Masa yang paling efektif untuk menyuntik bakteria ini adalah pada hari ke 45 selepas menanam. Penginokulatan pada peringkat awal tumbesaran tidak memberi kelebihan kepada tumbesaran *O. Sativa*.

# CHAPTER 1

## INTRODUCTION

### 1.1 Introduction

*Oryza Sativa* or its commonly known as rice plant is a staple food for many countries including Malaysia. It is one of the world's most important crops that is widely planted in Asia. Rice plant can be planted in the lowland or highland using terraces. In Malaysia, it is commonly planted in lowland because it produces a higher yield compared to highland rice. According to DOA (2011), paddy cultivation area in 2011 for Malaysia is 683,677 hectare, but Malaysia is still importing 960,000 metric tonnes of rice from the neighbouring countries to meet the total needs for our nation's rice. It shows that Malaysia self-sufficiency for rice is still not enough. Therefore, it is very important to increase our rice production by increasing the technology, research and development.

Of all the farming practices, rational fertilization and management of soil fertility is one of the most important measures to improve yield and quality of rice toward a sustainable production (Lee *et al.*, 2008). One of the ways to improve the rice production is by using bio fertilizer. To maintain high yield of oil production, continual input of chemical fertilizer has been practiced in many paddy field. Alternatively, plant growth-promoting rhizobacteria (PGPR) can be applied to the rice plant to reduce environmental problems caused by the use of chemical fertilizers.

Biofertilizer has been identified as an alternative to chemical fertilizer to increase soil fertility and crop production in sustainable farming (Wu *et al.*, 2004). It is also called beneficial microorganisms or is known as plant growth promoting rhizobacteria. PGPR have been applied to various crops to enhance growth, seed emergence and crop yield, and some have been commercialized (Dey *et al.*, 2004; Herman *et al.*, 2008; Minorsky, 2008). The examples of PGPR are *Bacillus*, *Acetobacter*, *Pseudomonas*, *Klebsiella* and many more.

As we know nitrogen is the most important nutrient to the rice plant. According to Ladha and Reddy (2003), nitrogen is the major limiting nutrient for rice production. Nitrogen is required for cellular synthesis of enzymes, proteins, chlorophyll, DNA and RNA, and is therefore important in plant growth and the production of food and feed (Matiru and Dakora, 2003). But not all nitrogen available in soil is absorbed by the root of rice plant. Most of the nitrogen is loss during flooding, leaching and so on.

Nitrogen fixing bacteria have been used for many years to improve the fertility of soils. This kind of bacteria gives a positive effect and enhances the plant growth by increasing the ability of root to absorb nitrogen. Moreover, PGPR can reduce the cost of using chemical fertilizer especially when it is applied at the right time. The beneficial effects of plant growth promoting rhizobacteria (PGPR) have been attributed to biological N<sub>2</sub> fixation (Boddy *et al.*, 1995; Meunchang *et al.*, 2004).

This experiment was conducted to study the effect of N<sub>2</sub>-fixing bacteria on rice plant and split time application on rice plant and if there is any interaction between nitrogen fertilizer and split time application of N<sub>2</sub>-fixing bacteria on the growth of rice plant.

## 1.2 Objectives

Therefore, the objectives of the study are:

1. To study the effect of split time application of N<sub>2</sub>-fixing bacteria on rice plant.
2. To identify the interaction between nitrogen fertilizer and split time application of N<sub>2</sub>-fixing bacteria on the growth of rice plant.



## REFERENCES

- Agency, P. M. (2006, April 7). *Bacillus sphaericus Strain 2362*. Retrieved from <http://www.publications.gc.ca/>
- Amir, H.G., Shamsuddin, Z.H., Halimi, M.S., Ramlan, M.F. and Marziah, M. (2002). N<sub>2</sub> fixation, plant growth enhancement and root-surface colonization by rhizobacteria in association with oil palm plantlets under in vitro conditions. *Malaysian J Soil Sci* , 6:75–82.
- Amir, H.G. (2001). Nitrogen fixation and plant growth enhancement of beneficial rhizobacteria in association with oil palm seedlings. Ph.D Thesis, Universiti Putra Malaysia, Serdang, Selangor, Malaysia
- Amir, H.G., Shamsuddin, Z.H., Halimi, M.S., Ramlan, M.F. and Marziah, M. (2002) N<sub>2</sub> fixation, plant growth enhancement and root-surface colonization by rhizobacteria in association with oil palm plantlets under in vitro conditions. *Malaysian J Soil Sci* , 6:75–82.
- Antoun, H. and Prevost, D. (2005). Ecology of plant growth promoting rhizobacteria. In Siddiqui, *PGPR: Biocontrol and Biofertilization* , pp. 1-38.
- Ashrafuzzaman, M., Hossen, F.A., Ismail, M.R., Hoque, M.A. and Zahurul, M. (2009). Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth. *African Journal of Biotechnology*, pp. 1247-1252.
- Azmi, T. (2012, September 18). Inisiatif Nestle 'tingkat' pendapatan pesawah. Sinar Harian, <http://www.sinarharian.com.my/rencana/inisiatif-nestle-tingkat-pendapatan-pesawah-1.86250>.
- Bakker, P.A.H.M., Raaijmakers, J.M., Bloemberg, G.V., Hofte, M., and Cooke, M. (2007). New Perspectives and Approches in Plant Growth-Promoting Rhizibacteria Research. Dordrecht: Springer.

- Baset, M.M.A., Zulkifli, H.S., Zakaria, W. and Marziah, M. (2000). Rhizobacteria colonization pattern and their influence on root stimulation of hydroponically grown tissue-culture Banana plantlet. Crowne Plaza Johor Bahru, Malaysia: Abstract Soil Science Conference of Malaysia.
- Boddey, R.M. and Co-workers. (1995). Biological nitrogen fixation associated with sugar cane and rice: contributions and prospects for improvement. *Plant Soil*, 174:195-209.
- Chang, T. (1964). Present knowledge of rice. *Internatl. Rice Res. Inst*, 1: 1-96.
- Chaudhary, R.C. and Tran, D.V. (2001). Speciality rices of the world: a prologue. In R. Duffy, *Speciality rices of the world*. Plymouth, UK: Science Publishers, pp. 3.
- Choudhary, D.K. Prakash, A. and Johri, B.N. (2007). Induced systemic resistance (ISR) in plants: mechanism of action. *Indian J Microb*, 47:289-297.
- Department of Agriculture. (2011). Food Statistic 2011. *Maklumat utama padi dan beras, 2006-2011*, pp. 33.
- Dey, R., Pal, K.K., Bhatt, D.M. and Chauhan, S.M. (2004). Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. *Microbiol. Res.*, 159: 371-394.
- Enebak, S.A. and Carey, W.A. (2000). Evidence of induced systemic protection to fusiform rust in loblolly pine by plant growth promoting rhizobacteria. *Plant Dis*, 84: 306-308.
- Fageria, N. (2007). Yield physiology of rice. *J Plant Nutr*, 30:843-879.
- Hamdy, E.L., Czarnes, S., Hallet, P.D., Alamercury, S., Bally, R. and Monrozier, L.J. (2007). Early changes in root characteristics of maize (*Zea mays*) following seed inoculation with the PGPR *Azospirillum lipoferum* CRT1. *Plant Soil*, 291: 109-118.
- Herman, M.A.B., Nault, B.A. and Smart, C.D. (2008). Effects of plant growth promoting rhizobacteria on bell pepper production and green peach aphid infestations in New York. *Crop Protect.*, 27: 996-1002.
- Ladha, J.K. and Reddy, P.M. (2003). Nitrogen fixation in rice systems: state of knowledge and future prospects. *Plant Soil*, 252:151-167.

- Lee, C.H., Kang, U.G., Park, K.D. and Lee, D.K. (2008). Long-Term Fertilization Effects on Rice. *Journal of Plant Nutrition*, 31: 1496–1506.
- Liew, Y.A., Syed Omar, S.R. and Husni, M.H.A. (2010). Effects of Micronutrient Fertilizers on the Production of MR 219 Rice (*Oryza sativa* L.). *Malaysian Journal of Soil Science* , pp. 71-82.
- Liu, M., Liu, X., Zhong, Z. and Cao, S. (2010). Estimating Leaf Chlorophyll Content of Rice under Heavy Metal Stress using Neural Network Model. *Seventh International Conference on Fuzzy Systems and Knowledge Discovery*. Beijing, China: China University of Geosciences, pp. 1896.
- Lynch, J. (1990). *The rhizosphere*. London, UK: Wiley.
- MARDI. (2002). *Manual penanaman padi*. Selangor: Malaysian Agricultural Research and Development Institute.
- Matiru, V.N. and Dakora, F.D. (2003). Potential use of rhizobial bacteria as promoters of plant growth for increased yield in landraces of African cereal crops. *African Journal of Biotechnology*, pp 1-7.
- Meunchang, S. and Co-workers. (2004). Phylogenetic and physiological characterization of indigenous Azospirillum isolates in Thailand. *Soil Sci. Plant Nutr*, 50: 413-421.
- Mia, M. (2002). *Ph.D Thesis*. UPM.
- Mia, M.A.B. and Shamsuddin Z.H. (2010). Rhizobium as a crop enhances and biofertilizer for increased cereal production. *Afr.J.Biotechnol*.
- Minorsky, P. (2008). On the inside. *Plant Physiol.*, 146: 323-324.
- NARI. (2001). *Morphology and Growth of Rice*. Morobe Province: National Agricultural Research Institute.
- Padi, L. (2013, April 21). *LKPP PADI SDN. BHD*. Retrieved from Varieti-varieti padi: <http://www.lkpppadi.com.my/varieti.html>
- Pemila, E.C. and David, P.R. (2012). Ribotyping of Plant Growth Promoting Bacteria (PGPR) from rhizosphere soils of Siruvani region for crop growth enhancement. *Int.J Adv. Life Sci*, 5: 43-50.

- Peterson, T.A., Blackmer, T.M., Francis, D.D. and Schepers, J.S. (1993). *Using a chlorophyll meter to improve N management*. Nebguide G93-1171A: Coop. Ext. Ser., Univ. of Nebraska, Lincoln.
- Raaijmakers, J. (2001a). Rhizosphere and rhizosphere competence. In O. M. Maloy, *Encyclopedia of plant pathology*. Wiley, USA, pp. 859–860.
- Raaijmakers, J. (2001b). Rhizosphere and rhizosphere competence. In M. TD, *Encyclopedia of plant pathology*. Wiley, USA: Maloy OC, pp. 859–860.
- Raaijmakers, J.M., Paulitz, T.C., Steinberg, C., Alabouvette, C. and Moënne-Loccoz, Y. (2009). The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant Soil*, pp 341–361.
- Ramesh, M., Murugiah. V., Gupta, A.K. (2009). Efficient in vitro plant regeneration via leaf base segments of indica rice (*Oryza sativa* L.). *Ind J Exp Biol*, 47: 68–74.
- Sturz, A.V., Christie, B.R., and Nowak, J. (2000). Bacterial endophytes: potential role in developing sustainable systems of crop production. *Crit. Rev. Plant. Sci*, 19: 1-30.
- Wu, S.C., Cao, Z.H., Li, Z.G., Cheung, K.C. and Wong M.H. (2004). Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. *Geoderma*, pp 155–166.