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EFFECTS OF SINGLE AND COMBINED INOCULATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA ON GROWTH PERFORMANCE OF PADDY (ORYZA SATIVA L.) UNDER GLASSHOUSE AND FIELD CONDITIONS

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By

MOHAMAD HALID BIN A RAZAK

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for Degree of Master of Science

March 2018

DEDICATION

I dedicate this thesis to my beloved Wife, Mrs. Nursyaza Bt Zulkifli and my parents for their patience, love, understanding and encouragement.

Thank you very much for everything

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

EFFECTS OF SINGLE AND COMBINED INOCULATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA ON GROWTH PERFORMANCE OF PADDY (*Oryza sativa L.*) UNDER GLASSHOUSE AND FIELD CONDITIONS

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Supervisor : Associate Prof. Halimi Bin Mohd Saud, PhD
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Rice (*Oryza sativa* L.) is a staple food in Malaysia grown over 708,148 hectares in various granary areas of Malaysia. In 2016, the average rice production in Malaysia was 2.252 million tons. As the population of Malaysia increases from the current number of 31.2 million, the rice demand also increases. However, Malaysia is only able to achieve 71.2% self-sufficiency level (SSL). Thus, Malaysia has to import rice from other countries, requiring higher productivity in limited granary areas to fulfill domestic demand. A potential solution to this problem is by increasing the usage of plant growth promoting rhizobacteria (PGPR) as a biofertilizer to enhance the productivity and reduced environmental problems caused by application of chemical fertilizers. The objectives of this study were to study the interaction effects of single and combined inoculations of PGPR using *in vitro* assay studies, effects on rice growth and development in glasshouse experiments and effects on rice yield, biomass and nutrient uptake in a field experiment. Determination of panicle breaking strength to minimize panicle grain shattering under field experiments was also conducted in this study. PGPR used in this study were *Bacillus pumilus* GM118 and *Bacillus subtillis* UPMB10 which act as phosphate solubilizing bacteria (PSB) and nitrogen fixing bacteria (NFB), respectively. *B. pumilus* GM118 and *B. subtillis* UPMB10 growth population trends, ability to fix nitrogen and solubilize phosphate on three different selective media and production of indole-3-acetic acid (IAA) were conducted *in vitro* assay. Four treatments of PGPR with different fertilizers treatments (including control) were laid out in a randomized complete block design (RCBD) with four replications

conducted in a glasshouse at Ladang 10, Faculty of Agriculture, Universiti Putra Malaysia. The treatments were as follows: T1: control N0K0; T2: N0K90; T3: N40K30; T4: N40K60; T5: N80K30; T6: N80K60; T7: N120K0 and T8: N120K90 in the present/absent of single and combined inoculations of *B. pumilus* GM118 and *B. subtilis* UPMB10. N and K in the treatments refer to the amount of nitrogen and potassium, respectively. The paddy variety used in glasshouse experiment was MR263 and it was planted using 35 L buckets containing 15 kg of paddy soil. Meanwhile, the field experiment was conducted in Kemasin-Semerak Integrated Agricultural Development Area (IADA), Kelantan. Four treatments were applied in IADA Kemasin-Semerak: T1: control (without bacterial inoculation); T2: single inoculation of *B. pumilus* GM118; T3: single inoculation of *B. subtilis* UPMB10 and T4: combined inoculations of *B. pumilus* GM118 and *B. subtilis* UPMB10. Normal fertilization practices were used in these plots. All data were analyzed statistically using SAS Software Program (Version 9.3) and treatment means were compared using Duncan Multiple Range Test ($P < 0.05$). *In vitro* assay indicated that combined inoculations of *B. pumilus* GM118 and *B. subtilis* UPMB10 produced the shortest generation times (14.4 minutes) for cells doubling compared to single inoculations of *B. pumilus* GM118 (36.1 minutes) and *B. subtilis* UPMB10 (24.1 minutes). However, single inoculation of *B. subtilis* UPMB10 is able to produce more IAA (63.8 µg/mL) compared to combined inoculations of *B. pumilus* GM118 and *B. subtilis* UPMB10 (50.1 µg/mL). Combined inoculations of *B. pumilus* GM118 and *B. subtilis* UPMB10 produced the highest grain yield (33%), followed by single inoculations of *B. subtilis* UPMB10 (29%) and *B. pumilus* GM118 (23%) as compared to the control in the glasshouse experiment. *B. pumilus* GM118 showed the highest N contents (15%) while combined inoculations of *B. pumilus* GM118 and *B. subtilis* UPMB10 showed the highest K content (16%) compared to control. Detachment of the grain panicle from the pedicle required the highest force for paddy inoculated with single inoculation of *B. subtilis* UPMB10 (11%) and followed by combined inoculations of *B. pumilus* GM118 and *B. subtilis* UPMB10 (8%) as compared to the control. In the field experiment, both single and combined inoculations of *B. pumilus* GM118 and *B. subtilis* UPMB10 showed significant difference in grain yield and biomass. In grain yield, *B. pumilus* GM118 produced 40%, *B. subtilis* UPMB10 (61%) and combined inoculations (61%) higher compared to the control. For biomass production, *B. pumilus* GM118 produced 33%, *B. subtilis* UPMB10 (42%) and combined inoculations of both (40%) higher compared to the control. IADA Kemasin-Semerak study showed that single inoculation of *B. pumilus* GM118 was able to increase 44% and 126% of plant N and P uptake while *B. subtilis* UPMB10 was able to increase 30% of plant K uptake compared to control, respectively.

This study indicated that co-inoculation having beneficial multi-mechanism of fixing nitrogen, solubilizing phosphate and producing IAA, gave the optimum performance in both glasshouse and field studies. This study proves that inoculation of selected PGPR can enhance rice plant yield, biomass, nutrient uptake and reduce postharvest losses due to grain shattering. The results showed the potential use of combined inoculations of *B. pumilus* GM118 and *B. subtilis* UPMB10 as biofertilizer with multiple beneficial characteristics that can increase rice productivity in the granary areas of Malaysia.

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

KESAN INOKULASI TUNGGAL DAN GABUNGAN BAKTERIA PENGGALAK PERTUMBUHAN POKOK TERHADAP PRESTASI PADI (*Oryza sativa* L.) DALAM KAJIAN RUMAH KACA DAN LAPANGAN

Oleh

MOHAMAD HALID BIN A RAZAK

Mac 2018

Penyelia : Prof. Madya Halimi Bin Mohd Saud, PhD
Fakulti : Pertanian

Beras (*Oryza sativa* L.) merupakan makanan ruji di Malaysia yang ditanam melebihi 708,148 hektar di kawasan jelapang padi yang berbeza di Malaysia. Secara puratanya, penghasilan padi di Malaysia pada tahun 2016 adalah sebanyak 2.252 juta tan. Bilangan terkini penduduk di Malaysia adalah kira-kira 31.2 juta dan apabila kadar populasi penduduk semakin meningkat, permintaan terhadap beras juga kian meningkat. Walau bagaimanapun, Malaysia hanya mampu mencapai 71.2% kadar keberhasilan padi (SSL). Oleh itu, Malaysia harus mengimport beras dari negara luar dan meningkatkan lagi produktiviti di kawasan jelapang padi yang terhad ini untuk memenuhi kehendak domestik. Potensi untuk menangani masalah ini adalah dengan meningkatkan penggunaan bakteria penggalak pertumbuhan pokok (PGPR) sebagai biobaja untuk meningkatkan produktiviti serta mengurangkan masalah pencemaran akibat penggunaan baja kimia. Objektif kajian ini adalah untuk mengkaji kesan interaksi di antara inokulasi tunggal dan gabungan inokulasi PGPR dalam kajian *in vitro*, kesannya terhadap pertumbuhan dan perkembangan padi di dalam kajian rumah kaca dan kesannya terhadap hasil padi, biomass dan pengambilan nutrien di kajian lapangan. Penentuan kekuatan pemutusan panikel untuk meminimumkan leraian buah padi di bawah kaedah kawalan dan lapangan juga turut dijalankan. PGPR yang digunakan di dalam kajian ini adalah *Bacillus pumilus* GM118 yang bertindak sebagai bakteria pelarut fosfat (PSB) dan *Bacillus subtillis* UPMB10 yang bertindak sebagai bakteria pengikat nitrogen (NFB). Pertumbuhan populasi, kebolehan untuk mengikat nitrogen dan mengurai fosfat oleh *B. pumilus* GM118 dan *B. subtillis* UPMB10 pada tiga

media terpilih yang berbeza serta penghasilan asid indole-3-asetik (IAA) dijalankan secara *in vitro*. Empat rawatan PGPR dengan rawatan baja yang berbeza (termasuk kawalan) telah dijalankan menggunakan reka bentuk blok lengkap rawak (RCBD) dengan empat replikasi di dalam rumah kaca Ladang 10, Fakulti Pertanian, Universiti Putra Malaysia. Rawatan tersebut adalah seperti berikut: T1: kawalan N0K0; T2: N0K90; T3: N40K30; T4: N40K60; T5: N80K30; T6: N80K60; T7: N120K0 and T8: N120K90 dengan kehadiran atau tanpa kehadiran inokulasi tunggal dan gabungan inokulasi *B. pumilus* GM118 dan *B. subtillis* UPMB10. N dan K di dalam rawatan merujuk kepada jumlah nitrogen dan kalium. Varieti padi yang digunakan adalah MR263 dan ditanam di dalam baldi bersaiz 35 L mengandungi 15 kg tanah sawah. Kajian lapangan pula dijalankan di Kemasin-Semerak Kawasan Pembangunan Bersepadu (IADA), Kelantan. Empat rawatan telah diaplikasikan di IADA Kemasin Semerak (IADA KS) iaitu: T1: Kawalan (tanpa inokulasi bakteria), T2: inokulasi tunggal *B. pumilus* (GM118), T3: inokulasi tunggal *B. subtilis* (UPM B10) dan T4: gabungan inokulasi *B*. *pumilus* GM118 dan *B. subtilis* UPMB10. Kesemua data yang diperoleh telah dianalisis menggunakan Program Perisian SAS (Versi 9.3), dan purata rawatan telah dibandingkan menggunakan Ujian Pelbagai Duncan (DMRT) ($P<0.05$). Kajian *in vitro* menunjukkan gabungan inokulasi *B*. *pumilus* GM118 dan *B. subtilis* UPMB10 menghasilkan sel berganda dua dalam masa yang paling singkat (14.4 minit) untuk sel berganda dua berbanding inokulasi tunggal *B. pumilus;* GM118 (36.1 minit) dan *B. subtilis;* UPMB10 (24.1 minit). Walau bagaimanapun, inokulasi tunggal *B. subtilis* UPMB10 telah menghasilkan lebih banyak IAA (63.8 µg/mL) berbanding gabungan inokulasi *B*. *pumilus* GM118 dan *B. subtilis* UPMB10 (50.1 µg/mL). Gabungan inokulasi *B*. *pumilus* GM118 dan *B. subtilis* UPMB10 menghasilkan hasil padi yang paling tinggi (33%) dan diikuti oleh inokulasi tunggal *B. subtilis* UPMB10 (29%) dan *B*. *pumilus* GM118 (23%) berbanding kawalan di dalam kajian rumah kaca. Inokulasi tunggal *B*. *pumilus* GM118 menunjukkan kandungan N yang tertinggi (15%) sementara gabungan inokulasi *B*. *pumilus* GM118 dan *B. subtilis* UPMB10 menunjukkan kandungan K yang tertinggi (16%) berbanding kawalan. Penginokulatan dengan inokulasi tunggal *B. subtilis* UPMB10 memerlukan daya yang paling tinggi (11%) untuk meleraikan panikel padi daripada tangkai diikuti dengan gabungan inokulasi yang memerlukan 8% lebih daya berbanding kawalan. Inokulasi tunggal dan gabungan inokulasi *B*. *pumilus* GM118 dan *B. subtilis* UPMB10 menunjukkan perbezaan yang ketara untuk hasil padi dan biojisim di dalam kajian lapangan. Untuk hasil padi, *B*. *pumilus* GM118 menghasilkan 40%, *B. subtilis* UPMB10 (61%) dan gabungan inokulasi kedua-dua bakteria (61%) lebih tinggi berbanding kawalan. Kajian IADA Kemasin-Semerak menunjukkan inokulasi tunggal *B*. *pumilus* GM118 mampu meningkatkan pengambilan N sebanyak 44% dan pengambilan P oleh tumbuhan sebanyak 126% sementara *B. subtilis* UPMB10 mampu meningkatkan pengambilan K oleh tumbuhan sebanyak 30% berbanding kawalan.

Oleh yang demikian, kajian menunjukkan gabungan inokulasi mekanisma pelbagai manfaat untuk mengikat nitrogen, melarut fosfat dan menghasilkan IAA dapat memberikan kesan optimum di dalam kajian rumah kaca dan lapangan. Kajian ini membuktikan bahawa inokulasi dengan PGPR terpilih dapat meningkaktkan hasil padi, biojisim, pengambilan nutrien dan mengurangkan kerugian pasca-tuai akibat peleraian buah. Hasil kajian menunjukkan potensi penggunaan gabungan inokulasi *B. pumilus* GM118 dan *B. subtilis* UPMB10 sebagai biobaja yang bercirikan pelbagai manfaat untuk meningkatkan produktiviti padi di kawasan jelapang padi di Malaysia.

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

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LIST OF GLOSSARY OF ABBREVIATIONS AND SI UNITS

g	Gram
g/L	Gram per liter
H	Hour
L	Liter
mg	Milligram
mg/L	Milligram per liter
mm	Millimeter
min	Minute
M	Molar
μM	Micro molar
NA	Nutrient Agar
NB	Nutrient Broth
RPM	Revolution per minutes
UPM	Universiti Putra Malaysia
IADA	Integrated Agricultural Development Area
SOM	Soil Organic Matter
IAA	Indole-3-acetic acid

CHAPTER 1

INTRODUCTION

Rice (*Oryza sativa* L.) is the main crop in Asia and it covers half of the arable land used for agriculture in most countries (Cantrell and Hettel, 2004). Rice is the second most widely grown cereal crop and it is a primary food which serves food calories and protein as main part of daily diets for most of the world's population. Based on FAOSTAT (2017) database, world rice production in 2013 was 741.9 million tons and average yield production was 4.51 tons/ha. However, world rice production in 2016 was 740.9 million tons and average yield production was 4.64 tons/ha. The demand for rice is correlated with an increase in population. The Food and Agriculture Organization (FAO) rice market in 2012 stated that the current supply of rice outpaces consumption. However, an increase in cereal crop supply is necessary to overcome the future rice demand in world population. Sheehy and Mitchell (2013) reported that it requires an increase of 50% in rice yield to fulfill the predicted world population of 9.3 billion by 2050.

In Malaysia, rice is the staple food and the third most widely planted crop after oil palm and rubber. Based on FAOSTAT database, area harvested of rice and rice production in Malaysia in 2016 are 708,148 hectare and 2.252 million tons, respectively (www.fao.org) and population in Malaysia is about 31.2 million (www.worldometers.info). However, Malaysia is only able to achieve 71.2% of self-sufficiency level (SSL) in order to produce its own rice to fulfill local rice demand (DOA, 2015). Hence, Malaysia has to import rice from other countries such as Thailand (41%) and Vietnam (32%) to support the rice domestic demand as Malaysia is unable to achieve 100% self-sufficiency level (SSL) of rice production. Besides, to meet the targeted level of SSL, continuous R&D is needed to produce new high yielding varieties and improved conventional method (productivity target needed to fulfill the gap between the actual and the desired production). In conventional method, farmers commonly apply more than the recommended rate of fertilizer to increase rice yield as a short-term solution.

Arshad et al. (2011) stated that Malaysia could sustain 70% SSL in a long-term target and fulfill the agreement on agriculture (AoA) of the World Trade Organization (WTO). They also mentioned that a productivity target based on SSL target was recommended for higher rice production. Therefore, Malaysia needs to propose continues R&D to increase rice production, cultivation area and crop productivity in order to fulfill the demand of a rising population. Hence, one way to overcome this problem is by promoting the use of Plant Growth Promoting Rhizobacteria (PGPR).

PGPR are heterogeneous clusters of microorganisms that have the ability to colonize roots rhizosphere and benefit the crop plants. PGPR are considered as bacteria which originated within rhizosphere or endophytic, free-living or in association with plant roots (Maksimov et al. 2011). Rhizosphere is the zone or area in the soils surrounding plant root and has the highest microbial activity area which have the capability to exchange essential nutrient elements to usable forms by biological stress activator (Muraleedharan et al., 2010).The microbial populations in the rhizosphere are varied compared with the other areas due to root exudates that act as nutrient sources for the microbes (Burdman et al., 2000). According to Lugtenberg et al. (2001), PGPR need to colonize the root surface effectively and compete well with other microbes for nutrient sources and spaces by invading the root in order to give advantage to the plants.

PGPR can act as an essential part in improving plant growth via various modes of mechanisms. The mode of PGPR action which encourages plant growth are biotic and abiotic stress tolerance, enhance nutrient uptake, phytohormones production and siderophore production (Choudhary et al., 2011; García-Fraile et al., 2015). Commonly, a single PGPR inoculation such as *Bacillus* sp., *Pseudomonas* sp. and *Azospirillum* sp. used as inoculants, showed capability to enhance shoot growth, root density and yield. These improvements in plant growth caused by PGPR are due to the potential of fixing and solubilizing mineral fertilizer and production of phytohormones resulting in the increased of nutrients availability and roots permeability (Enebak and Carey, 2000). Combine inoculation of PGPR can also increase plant growth and yield, but compared to single inoculation, combine inoculation provides the plants with more balanced nutrition, and improved the absorption of mineral nutrients (Bashan and Holguin, 1997). Thus, plant growth can be increased by dual inoculation of nitrogen-fixing bacteria and phosphate-solubilizing bacteria which can produce more phytohormones when grown in mixed culture. The combination of microbes that interact synergistically and promotes plant growth is currently an on-going study in many other countries. In Malaysia, similar studies need to be conducted to determine the effectiveness of locally isolated PGPR and their effect on the growth and yield of local rice varieties.

Hence, the present study was focused on the effect of nitrogen fixing bacteria (NFB) and/or phosphate solubilizing bacteria (PSB) strains to improve uptake, growth and yield of rice.

The main objectives of the study are:

i) To differentiate the response of single and combined inoculations of PGPR bacteria *in vitro* assay studies focusing on population growth and its mechanisms.
ii) To determine the growth response of inoculated rice plant (*Oryza sativa L.*) with different ratio of chemical fertilizer with single and combined inoculations of PGPR bacteria under glasshouse condition.
iii) To measure the breaking panicle strength at different applications of chemical fertilizer to minimize panicle grain shattering habit under glasshouse condition.
iv) To evaluate the growth response of rice plant inoculated with single and combined inoculations of PGPR bacteria under field condition.

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Abstract

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