UNIVERSITI PUTRA MALAYSIA

EFFECTS OF *Bacillus subtilis* UPMB10 INOCULATION ON GROWTH AND YIELD OF IRRIGATED RICE GROWN IN SEMARAK-KEMASIN PADDY SOILS AND ITS CLAY MINERALOGY

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By

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

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Faculty : Agriculture

Rice is a main diet for half of the global human population and there is an urgent need to increase the rice yield to meet the ever-increasing demand, nationally and globally. Rice cultivation requires more potassium (K) fertilizer compared to nitrogen (N) and phosphorus (P). The challenges are due to the high cost of fertilizer-K and especially due to a majority of the soil K which presents in the insoluble form of mineral rocks and the concentrations of soil solution K are very low. Potassium solubilizing bacteria (KSB) are commonly utilized as plant growth promoters and capable to solubilize different forms of inorganic potassium and improve soil conditions, especially the microbiological and chemical properties, for increased crop production. A sequences of experiments were conducted under laboratory and field conditions with the subsequent objectives; i) to isolate and characterize the KSB from flooded rice, ii) to determine the effects of selected KSB inoculation with different fertilizer-K rates on growth and yield of rice, iii) to determine various forms of soil K concentration as affected by KSB inoculation. Twelve KSB strains were isolated from flooded rice. Four of these strains were selected for their high K solubilization activities namely RS5, RS11, RS13 and ES3, renamed UPMB22, 23, 24 and 25 respectively, along with a reference strain UPMB10, *Bacillus subtilis* originally isolated from oil palm (*Elaeis guineensis* Jacq.) roots. These five strains, especially UPMB10, were able to solubilize K *in-vitro* at the rates between 12.41 to 13.77 mg L^{-1} in 5 days of incubation and showed beneficial PGPR characteristics (N_2 fixer and P solubilizer). Results from the XRD (X-Ray Diffraction) analysis showed that the soil sampled from Kemasin-Semerak Integrated Agricultural Development Project consisted of a small proportion of 2:1 clay minerals (mica) and behaved as a reservoir for soil-K, critically important to supply the short term plant-K requirements and conserve the soil-K in a long term productive crop ecosystem by reducing K loses. The clay fraction of the soil was dominated by kaolinite

while the other minerals which included quartz, mica, gibbsite and magnetite occurred in minor quantities. Results from the field study indicated that inoculation with *Bacillus subtilis* UPMB10 significantly increased the plant height, tiller numbers and leaf chlorophyll content (SPAD). This significant increase indicates the possible beneficial effects of UPMB10 inoculation on vegetative growth of rice under irrigated system. The maximum grain yield obtained were equivalent to 8.93 and 5.94 t ha^{-1} with and without UPMB10 inoculation, respectively. Increased rates of K fertilization, complemented with KSB inoculation, increased grain yield production until the 80 kg ha^{-1} K fertilization rate; equivalent to the grain yield production with the highest rate of uninoculated fertilizer-K (120 kg ha^{-1}). This implies that the usage of UPMB10 at 80 kg ha^{-1} K fertilization could save 40 kg ha^{-1} and yet achieve the maximum yield. The total K in soil was observed to significantly decrease after the bacterial inoculation process. The cation exchange capacity (CEC), exchangeable and water soluble K in soil did not show a significant change with UPMB10 application. CEC and water soluble K significantly increased with the application of fertilizer-K (without inoculation) until 80 kg ha^{-1} while the exchangeable K is significantly decreased. It indicates that 80 kg K ha^{-1} is sufficient for optimum rice uptake and K balance under irrigated lowland rice system. The study also showed that effective bacterial inoculation could successfully produce rice plants with improved growth and increased yield.

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sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN PENGINOKULASIAN *Bacillus subtilis* UPMB10 TERHADAP TUMBESARAN DAN HASIL PADI SAWAH YANG DITANAM DI TANAH PADI SEMARAK-KEMASIN DAN MINERAL LEMPUNGNYA

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Padi adalah makanan ruji untuk separuh populasi dunia dan hasilnya perlu ditingkatkan untuk permintaan tempatan dan juga dunia yang semakin meningkat. Penanaman padi memerlukan lebih potasium (K) berbanding nitrogen (N) dan fosforus (P). Ini disebabkan oleh kos yang tinggi bagi baja K dan juga kerana majoriti, ataupun lebih daripada 90% K dalam tanah wujud dalam bentuk tidak terlarut yakni batuan mineral manakala kepekatan terlarutnya di dalam tanah lazimnya adalah sangat rendah. Bakteria pelarut potasium (KSB) biasanya digunakan sebagai penggalak tumbesaran tumbuhan dan dikenali mampu untuk melarutkan potasium yang tidak organik dalam pelbagai bentuk dan memperbaiki kondisi tanah, terutamanya secara mikrobiologi dan juga kandungan kimia dalam tanah untuk meningkatkan pengeluaran tanaman. Beberapa eksperimen telah dijalankan di makmal dan juga ladang dengan tujuan seperti berikut; i) untuk mengasingkan dan mengkarekterisasi KSB daripada padi sawah, ii) untuk menentukan keberkesanan KSB yang terpilih pada kadar baja K yang berbeza terhadap pertumbuhan dan hasil padi, iii) untuk menentukan kepekatan bentuk K dalam tanah yang dipengaruhi oleh penginokulasian KSB. Dua belas strain KSB diisolasi dari padi sawah. Empat strain terpilih untuk diuji kerana potensi keterlarutan K yang tinggi iaitu RS5, RS11, RS13 dan ES3, dinamakan semula sebagai UPMB22, 23, 24 dan 25, di samping strain rujukan UPMB10, *Bacillus subtilis* yang diisolasikan daripada akar kelapa sawit (*Elaeis guineensis* Jacq.). Kelima-lima strain tersebut terutamanya UPMB10 dapat melarutkan K dalam keadaan *in-vitro* dengan kadar antara 12.41 to 13.77 mg L^{-1} dalam masa 5 hari inkubasi. Analisis XRD (X-Ray Diffraction) menunjukkan sampel tanah daripada Projek Pembangunan Integrasi Pertanian Kemasin-Semerak terdiri daripada 2:1 lempung mineral (mica) dan bertindak sebagai simpanan untuk K dalam tanah, terutamanya untuk keperluan tumbuhan dalam masa yang singkat. Pecahan lempung tersebut didominasi oleh kaolinite dan

mineral lain termasuklah quartz, mica, gibbsite and magnetite yang terdapat dalam kuantiti yang rendah. Keputusan daripada kajian ladang menunjukkan inokulasi dengan *Bacillus subtilis* UPMB10 secara signifikannya meningkatkan tinggi tanaman, bilangan rumpun dan kandungan klorofil daun (SPAD). Peningkatan yang signifikan ini menunjukkan keberkesanan inokulasi UPMB10 pada pertumbuhan padi secara sistem pengairan. Hasil bijirin maksimum yang diperolehi adalah bersamaan dengan 8.93 and 5.94 t ha^{-1} dengan dan juga tanpa UPMB10 inokulasi. Pembajaan K yang tinggi, dengan penginokulasian KSB, meningkatkan lagi pengeluaran hasil bijirin sehingga pada kadar 80 kg ha^{-1} pembajaan K; bersamaan dengan kadar yang paling tinggi iaitu (120 kg ha^{-1}) tanpa penginokulasian KSB. Ini menunjukkan aplikasi UPMB10 pada 80 kg ha^{-1} pembajaan K mampu menjimatkan 40 kg ha^{-1} baja K dan dapat mencapai hasil yang maksimum. Jumlah kandungan keseluruhan K dalam tanah berkurang secara signifikan selepas diinokulasi dengan bakteria. Kadar pertukaran kation (CEC), pemboleh-tukaran K dan K terlarut dalam tanah tidak menunjukkan perubahan dengan inokulasi UPMB10. Terdapat peningkatan yang signifikan pada CEC dan K terlarut dengan pembajaan K sehingga 80 kg ha^{-1} (tanpa inokulasi) manakala keboleh-tukaran K menurun secara signifikan. Ini membuktikan yang 80 kg K ha^{-1} adalah cukup untuk pertumbuhan padi yang optimum dan keseimbangan K di kawasan padi sawah yang dijaga sistem pengairannya. Kajian juga membuktikan bahawa dengan penginokulasian bakteria yang efektif mampu menghasilkan tanaman padi dengan pertumbuhan serta hasil yang lebih tinggi.

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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 Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

N	nitrogen
P	phosphorus
K	potassium
pgpr	plant growth-promoting rhizobacteria
NFB	nitrogen fixing bacteria
PSB	phosphorus fixing bacteria
KSB	potassium solubilizing bacteria
KSM	potassium solubilizing microorganism
mg kg^{-1}	milligram per kilogram
mg L^{-1}	milligram per litre
^{o}C	degree Celcius
%	percentage
m	meter
kg	kilogram
g	gram
mg	miligram
t	tonne
ha	hectare
pH	potential different of hydrogen ion
CEC	cation exchange capacity
mL	mililitre
XRD	X-ray Diffraction
AAS	atomic absorption spectrophotometer
AA	autoanalyzer
m^2	meter square
cmolc kg^{-1}	centimol charge per kilogram
s	second
min	minute
h	hour
TSA	tryptic soy agar
sdH_2O	sterile distilled water
rpm	revolution per minute
TC	total carbon
TN	total nitrogen
vol	volume
wt	weight
DAI	day after incubation
DAS	day after sowing
DAT	day after transplanting
cfu	colony forming unit
HCl	hydrochloric acid
HNO_3	nitric acid
H_2SO_4	sulfuric acid
H_2O_2	hydrogen peroxide
SAS	statistical analysis system
LSD	least significant different
ANOVA	analysis of variance
DOA	Department of Agriculture

NH_4OAC	ammonium acetate
K_2SO_4	potassium sulfate
$Ca(H_2PO_4)_2$	calcium hydrogen phosphate
$MgSO_4.7H_2O$	magnesium sulfate heptahydrate
$CaCO_3$	calcium carbonate
$FeCl_3.6H_2O$	iron chloride hexahydrate
KOH	potassium hydroxide
K_2HPO_4	potassium hydrogen phosphate
NaCl	sodium chloride
$CaCl_2$	calcium chloride
Fe-EDTA	ferric ethylenediaminetetraacetic acid
H_3BO_3	boric acid
$MnSO_4.H_2O$	managanese sulfate hydrate
$NaMoO_4$	sodium molybdate
$ZnSO_4.7H_2O$	zinc sulfate heptahydrate
$CuSO_4.5H_2O$	copper sulfate pentahydrate
$Ca_3(PO_4)_2$	calcium phosphate
$(NH_4)_2SO_4$	ammonium sulfate
KCl	potassium chloride
$FeSO_4.7H_2O$	ferrous sulfate heptahahydrate

CHAPTER 1

INTRODUCTION

According to United Nations, the world population is expected to reach 8.9 billion by 2050, with the developing countries of Asia and Africa to absorb the vast majority of the growth (Wood, 2001). The demands placed upon agriculture to supply future food will be one of the greatest challenges due to the rise of world populations. To meet this challenge, greater efforts with focus on the soil-plant-microbe interactions and the agro-ecosystem are needed to enable better perception of the intricate processes which govern the stability of agricultural lands. Soil is a dynamic natural body on the earth's crust. There are several minerals containing essential elements in the soil, most importantly are nitrogen (N), phosphorus (P), and potassium (K). K is the third essential element and it plays a part in the growth, metabolism, and development of plant. Plants with inadequate supply of potassium, will produce unhealthy roots, stunted growth and poor quality seeds (White and Karley, 2010; McAfee 2008).

Half of the world populations consume rice as their staple diet. Its growth is limited by various environmental conditions such as soil and climatic conditions. In Malaysia, there is an urgent need to increase the rice yield to meet the national food requirement. The major challenges for rice cropping are limited arable land, unpredictable water supply and climatic conditions as well as pest and disease problems. Its production requires more fertilizer-K input compared to nitrogen and phosphorus. This is because K is a mobile nutrient and easily leached when not absorbed by plant roots. In rice, K is important especially for the formation of tillers and grains. The K nutrient absorbed by roots can increase the lignification in culms and subsequently enhance the number of tillers and stem strength. The increase in numbers of strong tillers will consequently produce higher yields with improved quality.

Plants uptake K from the soil and its availability is influenced by K dynamics and also the soil K content (Parmar and Sindhu, 2013). Soil K can be classified into four available forms based on the degree of availability to crops, i.e. soil solution, exchangeable, non-exchangeable and mineral K (Darunsontaya et al., 2012). The soil solution K is the crucial source for plant root uptake. This readily available K is usually relatively small and does not provide a good indication of the long term ability of a soil to supply K to plants. Exchangeable-K which is held by the negative charges on soil clay minerals and organic matter exchange sites is in related with water soluble K and collectively known as the available K pool (Meena et al., 2016). Soil-fixed (non-exchangeable) K is an important contribution to plant K supply (Mengel et al., 1993) and is held as fixed ion in the lattice structure of clay minerals.

The use of microorganisms in agriculture is a vital practice to enhance plant nutrient availability. The application of plant growth-promoting rhizobacteria

(PGPR) has grown immensely in the recent years. It is well established for its beneficial effect towards plant growth and development. These bacteria become beneficial when they can fix or solubilize the three essential plant nutrient elements (N, P, K) in soil. This is because some of the elements are in unavailable or bound forms for plant uptake such as being adsorbed by rocks and soil minerals. Nitrogen Fixing Bacteria (NFB), for example, are able to fix the abundantly available atmospheric-N. Some other bacteria can solubilize insoluble P and K in soil and are known as Phosphate Solubilizing Bacteria (PSB) and Potassium Solubilizing Bacteria (KSB), respectively (Tan, 2015). Bacteria will become more beneficial to plant growth when they are also able to produce phytohormones which are undoubtedly vital for its growth and development especially the root system. These bacteria can be grouped as plant growth-promoting rhizobacteria (PGPR) due to these beneficial characteristics.

Plant growth-promoting rhizobacteria also enact a significance function in K mineral cycle and, thus, they can become a green alternative technology to solubilize K minerals for plant uptake. These bacteria which possess potassium solubilizing abilities are termed KSB and they can solubilize the insoluble K into available forms in soil as soil solution K and render them as available K to the plants (Zeng et al., 2012). The major mechanism of mineral K solubilization is due to organic acids synthesized by rhizospheric microorganisms. Organic acids production leads to acidification of the microbial cell and its surrounding environment which promote the solubilization of mineral K.

An imbalanced or surplus application of chemical fertilizers can produce negative environmental impacts and increase the cost of crop production. So, there is an urgent need to apply cost effective and eco-friendly alternatives to effectively improve crop production. Therefore, the application of KSB is viewed a reliable approach in enhancing the agricultural land productivity. This new practice is also asserted to be able to ameliorate the degraded, marginally productive and unproductive agricultural soils (Basak and Biswas, 2012). Currently, minimal information is available on soil potassium dissolution by rhizobacteria and their effects on growth and yield of rice. Hence, the objectives of this study are:

1. To isolate and characterize the KSB from flooded rice.
2. To determine the effects of selected KSB inoculation with different fertilizer-K rates on growth and yield of rice.
3. To determine various forms of soil K concentration as affected by KSB inoculation.There may be a preamble at the beginning of a chapter. The purpose may be to introduce the themes of the main headings.

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Conference/Symposium:

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Nur Farhana, C. H., Roslan, I. Radziah, O. and Shamsuddin, Z. H. 2016. Application of Potassium Solubilizing Bacterial Inoculation on Soil Potassium Content, Growth and Yield of Irrigated Rice. *In Proceedings of the Soil Science Conference of Malaysia 2016*. (Paper Presenter)

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