



UNIVERSITI PUTRA MALAYSIA

***IMPACT OF WATER STRESS ON CHARACTERISTICS OF SOYBEAN
(Glycine max L.) INOCULATED WITH PLANT GROWTH-PROMOTING
RHIZOBACTERIA AT DIFFERENT GROWTH STAGES***

FATEMEH BALOUEI



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By

FATEMEH BALOU EI

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

September 2015



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DEDICATIONS

This thesis is dedicated to my:

Lovely mother and father (Effat and Esffandiyar)

And

Adorable sister and brother (Fahima and Ali)

With innermost and everlasting affection and love



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

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September 2015

Chairman : Associate Professor Hawa ZE Jaafar, PhD
Faculty : Agriculture

In this study, the effect of water stress on physiological characteristics and biochemical changes of soybean inoculated with PGPRs were considered. Soybean (*Glycine max* (L) Merr.) like most other legumes is able to fix nitrogen (N_2), once it can establish a symbiotic relationship with soil nodulating bacteria belonging to the Rhizobiales order. This close association with N_2 -fixing bacteria makes possible for normal growth and development of legumes in nitrogen-poor soil under abiotic stresses. Soybean N_2 -fixation is a primary plant mechanism responsible for meeting plant-N demand during plant development. Nitrogen fixation is recognized as a drought-sensitive mechanism, especially in response to water deficit at different growth stages. Information about the effects of indigenous PGPRs (UPMB10 and UPMB12) and *Bradyrhizobium* (UPMR19) isolates as nitrogen fixer and their potential for soybean under drought, and their biological influence in tropical areas are still scarce; such data would be useful to provide information on availability of nitrogen for soybeans growth and development under abiotic stress particularly drought in tropical region. A factorial design experiment 1 was designed to determine the effect of water stress (well-watered, high and severe water stress) on nitrogenase activity, Malondialdehyde (MDA) and proline contents as well as Peroxidase (POX) activities of inoculated soybean with UPMB10, UPMB12 and UPMR19; and to inspect any potential of incremented tolerance of inoculated soybean to water stress. The average value of nitrogenase activity ranged from 22.25 to 51.3 $\text{nmol C}_2\text{H}_4 \text{ plant}^{-1} \text{ h}^{-1}$. Highest amounts of MDA and proline were recorded at 92.98 $\mu\text{g root}^{-1}$ and 15.91 mg root^{-1} , respectively, and POX was 2.75 EU $\text{mg protein leaf}^{-1}$ in un-inoculated soybean at severe stress level. In second experiment, the effect of water stress imposition at flowering (R2) and pod-filling (R6) stages (well-watered and high water stress) on MDA, proline and POX was designed in a factorial manner. The results showed values of mentioned parameters in ascending manner at flowering stage, but lightly decreasing at pod-filling and sharply diminishing at well-watered treatment. The results from experiment 2 showed that soybean exposed to high stress levels at pod-filling stage had best performance compared to flowering stage. In experiment 3, a factorial RCBD study was carried out to determine and investigate the most sensitive reproductive stage to stress during pod-filling (R6), either at the onset of pod (R3) stage or the onset of seed (R5) stage, as demonstrated by

the nitrogenase enzyme production, MDA, proline and POX activities. The results showed that soybean at onset of pod stage is more sensitive to water stress compared to the onset of seed stage; and that, PGPRs+*Bradyrhizobium* (co-inoculated) protected the soybean plant more than inoculated and un-inoculated soybean under water deficit condition. Taken together, these results indicated that inoculated and co-inoculated of indigenous PGPRs and *Bradyrhizobium* with soybean are able to protect plant against oxidative damage generated by water stress due to their symbiosis that can directly alleviate the stress effect through nodule metabolism and N₂-fixation.



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

**KESAN TEKANAN AIR TERHADAP CIRI-CIRI KUALITATIF DAN
KUANTITATIF KACANG SOYA (*Glycine max* L.) DENGAN
RHIZOBAKTERIA (PGPR) PENGALAK TUMBUHAN RHIZOBAKTERIA
(PGPR) PADA PERINGKAT PERTUMBUHAN BERBEZA**

Oleh

FATEMEH BALOUEI

September 2015

Pengerusi : Profesor Madya Hawa ZE Jaafar, PhD
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Dalam kajian ini, kesan tekanan air terhadap ciri-ciri fizikal dan perubahan biokimia kacang soya berinokulasi dengan PGPR telah dipertimbangkan. Kacang soya (*Glycine max* (L) Merr.) seperti pokok kekacang yang lain mampu untuk mengikat nitrogen (N_2), dengan mewujudkan hubungan simbiotik dengan bakteria nodul tanah dari order Rhizobiales. Hubungan rapat dengan bakteria pengikat N_2 ini membolehkan pertumbuhan normal dan perkembangan pokok kekacang dalam tanah yang kekurangan nitrogen di bawah tekanan abiotik. Pengikatan N_2 oleh soya adalah mekanisme utama yang bertanggungjawab untuk memenuhi permintaan tumbuhan terhadap N ketika perkembangan pokok. Pengikatan nitrogen telah dikenalpasti sebagai mekanisme yang sensitif terhadap kemarau, terutamanya dalam tindakbalas terhadap kekurangan air pada peringkat pertumbuhan yang berbeza. Maklumat mengenai kesan asal pemencilan PGPR asli (UPMB10 dan UPMB12) dan *Bradyrhizobium* (UPMR19) sebagai pengikat nitrogen dan potensi mereka terhadap kacang soya pada musim kemarau, dan terhadap pengaruh biologi kawasan tropikal masih terhad; data seperti ini amat berguna untuk memberi maklumat mengenai ketersediaan nitrogen bagi pertumbuhan dan perkembangan kacang soya di bawah tekanan abiotik terutamanya pada musim kemarau di rantau tropika. Kajian faktorial telah dijalankan untuk menentukan kesan tekanan air (disiram, ketegasan air yang tinggi dan teruk) terhadap aktiviti nitrogenase, MDA dan kandungan proline serta aktiviti-aktiviti POX kacang soya berinokulasi dengan UPMB10, UPMB12 dan UPMB19; dan untuk menentukan mana-mana potensi peningkatan toleransi inokulasi kacang soya terhadap ketegasan air. Nilai purata aktiviti nitrogenase adalah di antara 22.25 hingga 51.3 nmol C_2H_4 pokok⁻¹ j⁻¹. Jumlah tertinggi MDA dan proline direkodkan pada 92.98 $\mu\text{g g root}^{-1}$ dan 15.91 mg g root⁻¹, masing-masing, dan POX adalah 2.75 EU mg protein daun⁻¹ dalam kacang soya yang tidak berinokulasi pada tahap ketegasan yang teruk. Pada eksperimen kedua, kesan pengenalan ketegasan air pada peringkat berbunga (R2) dan pengisian pod (R6) (disiram dan ketegasan air yang tinggi) keatas MDA, proline dan POX telah direka dalam kajian faktorial. Hasil kajian menunjukkan bahawa nilai-nilai parameter yang disebut meningkat pada peringkat berbunga, tetapi berkurangan sedikit pada pengisian pod dan menurun dengan ketara

pada rawatan yang disiram. Hasil daripada Eksperimen 2 menunjukkan bahawa kacang soya yang terdedah kepada tahap ketegasan yang tinggi pada peringkat pengisian pod mempunyai prestasi yang terbaik berbanding peringkat berbunga. Kajian faktorial yang dijalankan dalam Eksperimen 3 adalah untuk menyiasat sensitiviti dalam peringkat pra-pengisian pod (R6), sama ada pada permulaan pod (R3) atau permulaan benih (R5) terhadap enzim nitrogenase, MDA, proline dan aktiviti-aktiviti POX di bawah tahap ketegasan yang tinggi. Merujuk kepada hasil keputusan, kacang soya lebih sensitif kepada kemarau pada permulaan pod berbanding permulaan benih dan juga, kacang soya yang berinokulasi bersama dengan PGPR+*Bradyrhizobium* didapati mendapat perlindungan yang lebih daripada kacang soya berinokulasi dan tidak berinokulasi di bawah keadaan yang kekurangan air. Bersama-sama ini, hasil keputusan menunjukkan bahawa inokulasi dan inokulasi bersama PGPR dan *Bradyrhizobium* dengan kacang soya boleh melindungi tumbuhan daripada kerosakan oksidatif yang dihasilkan oleh kemarau kerana simbiosis mereka boleh mengurangkan tekanan kemarau dengan mempengaruhi metabolisme nodul dan pengikatan N₂ secara langsung dengan meningkatkan kadar fotosintesis, meningkatkan penghidratan daun, menangguhkan penurunan potensi air daun dan mempertingkatkan ciri-ciri kualitatif dan kuantitatif kacang soya pada peringkat pertumbuhan yang berbeza.

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I certify that a Thesis Examination Committee has met on 25 February 2016 to conduct the final examination of Fatemeh Balouei on her thesis entitled "Impact of Water Stress on Characteristics of Soybean (*Glycine max* L.) Inoculated with Plant Growth-Promoting Rhizobacteria at Different Growth Stages" 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

°C	Degree centigrade
g	gram
kg	kilogram
g/L	gram per liter
h	hour
DAI	day after inoculation
L	liter
M	mole
EU	enzyme unit
mg	milligram
ml	milliliter
mM	milimole
mm	millimeter
nmol C ₂ H ₄ /mL/h	nanomol methanol per millileter per hour
nm	nanometer
ppm	parts per million
rpm	Round per minute
RT	Retention Time
m	meter
J	microgram
µg/ mL	microgram per milliliter
JO	microgram per litter
JJ	microgram per gram
O	microliter
P	micromilimeter
0	Micromol
g/plant	gram per plant
mL/min	millilitre per minute
CFU	colony forming unit
MPa	Mega-Pascal
cm	Centimeter

ABBREVIATIONS OF STATISTIC ANALYSIS

ANOVA	Analysis of Variance
<i>P</i>	Probably
CRD	Complete Randomized Design
RCBD	Randomized Complete Block Design
SAS	Statistical Analysis Software
SD	Standard Deviation
SEM	Standard Error of Means



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* H Q H U D O , Q W U R G X F W L R Q

Soybean (*Glycine max*) is a legume crop; perform nitrogen fixation and establishing a symbiotic relationship with the bacterium *Bradyrhizobium japonicum* (as an aerobic microbe) in soil. It can break down nitrogen gas from the atmosphere into ammonia, which is a nitrogen product that is usually low in the soil (Van wyk, 2005). Soybean needs much more water by increasing plant development, peaking during the flowering and pod filling stages and decreasing thereafter (Embrapa, 2011, Farias et al., 2007). The loss of productivity under water deficit conditions depends on the soybean phenological stage, duration and intensity of water shortages (Doss and Thurlow, 1974).

In Malaysia, same as other tropical areas, most of their soils are deficient in available N and also microbial activities are diminished because of high temperature, direct and high light intensity, rainfall and leaching of nutrients. The enhancement of available N in these areas involves the using and improving indigenous bacterial isolates from tropical Malaysian soils which could be directly attributed to the beneficial effects from biological N₂-fixation (BNF). BNF is one way of converting elemental nitrogen into plant usable form. A number of microbes are involved in the process of BNF, which contains nitrogenase enzyme responsible for fixing atmospheric dinitrogen into soil or establishing a symbiotic relationship with legume. Indigenous bacterial isolates required in this research as plant growth-promoting rhizobacteria (PGPR) includes UPMB10 and UPMB12 (*Bacillus sp.*) isolated from oil palm roots and UPMR19 (*Bradyrhizobium japonicum*) isolated from soybean root that cultivated and grown in Malaysian soils. All PGPR indigenous isolates stimulated plant growth through increasing root distribution, phytohormone production (IAA), increasing nodulation leading to increasing BNF and enhancing access to N. PGPRs symbiosis can alleviate drought stress by affecting nodule metabolism and N₂-fixation directly, enhancing of root growth and plant height, postpone declined leaf water potential and protection plants against the oxidative damage generated by drought.

In developing of indigenous bio-inoculants, native PGPRs and *Bradyrhizobium* isolates from tropical Malaysian soils played an important role as biofertilizer. The fact that environmental conditions can impact on the activity of indigenous isolates of PGPRs and *Bradyrhizobium*, may give an insight how inoculated (PGPRs and *Bradyrhizobium*) and co-inoculated (PGPR+*Bradyrhizobium*) soybean under greenhouse could be manipulate to produce high quality harvest of soybean at different growth stages in Malaysia. However, there has been no detail study to relate this, and hence need further investigation.

2 E M H F W L Y H V

A project consisting of three major experiments hence, was carried out with the overall objectives:

- 1) To investigate the effects of indigenous PGPRs isolates as nitrogen fixer under drought stress condition on soybean growth and development.
- 2) To determine the role of PGPR on drought alleviation of soybean at different phenological stages (Flowering and pod-filling stages).
- 3) To determine the critical impact of nitrogenase enzyme as the mechanism to the tolerate against stress condition on pre-podfilling stages.

+ \ S R W K H V L V

This study investigates the hypothesis that native PGPRs isolates as biofertilizer can protect soybean (*Glycine max* L. cv. Williams) co-inoculated and inoculated against drought condition. Soybean can continue growth and development under water deficit through increasing nitrogen fixing activity and close association with soil nodulating bacteria, increased uptake of nutrient specially nitrogen, augmenting root distribution, increasing chlorophyll content and postpone declines in leaf water potential by reducing oxidative damage cause by the reactive oxygen species (ROS) generated during drought condition. Furthermore, PGPRs and *Bradyrhizobium* would improve accumulation of proline, MDA and POX activities under drought and this correlated to plant protection against environmental stresses. In the same way, soybean co-inoculated and inoculated with PGPRs and *Bradyrhizobium* subjected to drought had lower oxidative damage to lipids (MDA) and proline in roots (nodules + roots) than un-inoculated soybean, and this was linked to protection against nodule senescence and would enhance process of nodulation and nitrogen fixation synergistically in order to boosting growth and development of soybean in tropical regions. Thus, bio-inoculants as biofertilizers are gaining prominence role in the maintenance of soil fertility

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