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 BALOUEI

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

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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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By

FATEMEH BALOUEI

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 nmol C$_2$H$_4$ plant$^{-1}$ h$^{-1}$. Highest amounts of MDA and proline were recorded at 92.98 µg groot$^{-1}$ and 15.91 mg groot$^{-1}$, respectively, and POX was 2.75 EU 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) PENGGALAK 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 (N₂), dengan mewujudkan hubungan simbiotik dengan bakteria nodul tanah dari order Rhizobiales. Hubungan rapat dengan bakteria pengikat N₂ ini membolehkan pertumbuhan normal dan perkembangan pokok kacang dalam tanah yang kekurangan nitrogen di bawah tekanan abiotik. Pengikatan N₂ oleh soya adalah mekanisma utama yang bertanggungjawab untuk memenuhi permintaan tumbuhan terhadap N ketika perkembangan pokok. Pengikatan nitrogen telah dikenalpasti sebagai mekanisma yang sensitif terhadap kemarau, terutama dalam tindakbalas terhadap kekurangan nitrogen 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 terutama 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, 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 C₂H₄ pokok⁻¹ j⁻¹. Jumlah tertinggi MDA dan proline direkodkan pada 92.98 µg 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 pengenaan 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 menyiapkan 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 metabolisma 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.

iv
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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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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>ABSTRACT</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td></td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td>v</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>vi</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xiii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xvi</td>
</tr>
<tr>
<td>ABBREVIATIONS OF STATISTIC ANALYSIS</td>
<td>xvii</td>
</tr>
</tbody>
</table>

## CHAPTER

### 1 INTRODUCTION

1.1 General Introduction
1.2 Objectives
1.3 Hypothesis

### 2 LITERATURE REVIEW

2.1 Origin of Soybean
2.2 Environmental Conditions for Soybean Growth
2.3 Importance of Soybean to be Cultivated
  2.3.1 Soybean Production
  2.3.2 Uses of Soybean
2.4 Soybean Growth and Development
  2.4.1 Soybean Morphology
  2.4.2 Soybean Development
  2.4.3 Soybean Vegetative and Reproductive Phase
    2.4.3.1 Vegetative Phase
    2.4.3.2 Reproductive Phase
  2.4.4 Development and Yield
2.5 Plant Growth-Promoting Rhizobacteria (PGPR)
  2.5.1 Beneficial Mechanisms of PGPR
    2.5.1.1 Nitrogen Fixation
    2.5.1.2 Phytohormone Production
    2.5.1.3 Siderophore Production
  2.5.2 Application of PGPR
    2.5.2.1 Nitrogen Fixation
    2.5.2.2 Bacillus
2.6 Effects of Water Stress on Plant Growth
  2.6.1 Water Stress
    2.6.1.1 Effects of Water Stress on Plant Growth
    2.6.1.2 Effects of Water Stress on N2-fixation
    2.6.1.3 Effects of Water Stress on Pigment Composition
    2.6.1.4 Effects of Water Stress on Water Relation
    2.6.1.5 Effects of Water Stress on Biochemical
Parameters

2.6.1.6 Effects of Water Stress on Soybean Growth Stages

2.7 Tolerance to Drought by PGPR

2.7.1 Use of Plant Growth-Promoting Rhizobacteria as Inoculant

3 GENERAL MATERIALS AND METHODS

3.1 Experiment Site

3.2 Planting materials

3.2.1 Soil Sampling and Analysis

3.2.2 Soybean Seed

3.2.3 Bacterial Inoculums

3.2.4 Bacterial Preparation

3.3 Planting Soybean

3.4 Fertilization

3.5 Water Treatment Application

3.6 N₂-fixing Activity of Indigenous Isolates

3.7 Phytohormone Production (Indole-3-Acetic Acid)

3.8 Iron Sequestration (Siderophore Production)

3.9 Root Length and Root Dry Weight

3.10 N₂-fixing Activity of Inoculated Soybean With Indigenous Isolates

3.11 Plant Height

3.12 Water Relation Attributes (LWP and LRWC)

3.13 Malondialdehyde Assay (MDA)

3.14 Proline Content

3.15 Enzyme Assays, Peroxidase (POX) activity and Harvest Index (HI) of POX

3.16 Treatments, Experimental Design and Statistical Analysis

4 EFFECT OF WATER STRESS TREATMENTS ON PHYSIOLOGICAL AND BIOCHEMICAL ANALYSES OF INOCULATED SOYBEAN (Glycine max L.) WITH PGPR

4.1 Introduction

4.2 Materials and Methods

4.2.1 Experimental Site

4.2.2 Planting materials

4.2.2.1 Soil Sampling and Analysis

4.2.2.2 Soybean Seed

4.2.2.3 Bacterial Inoculums

4.2.2.4 Bacterial Preparation

4.2.3 Planting and Fertilization of Soybean

4.2.4 Water Treatment Application

4.2.5 N₂-fixing Activity of Indigenous Isolates

4.2.6 Phytohormone Production (Indole-3-Acetic Acid)

4.2.7 Iron Sequestration (Siderophore Production)

4.2.8 Root Length and Root Dry Weight

4.2.9 N₂-fixing Activity of Inoculated Soybean With Indigenous Isolates

xi
4.2.10 Plant Height 27
4.2.11 Water Relation Attributes (LWP and LRWC) 28
4.2.12 Malondialdehyde Assay (MDA) 28
4.2.13 Proline Content 28
4.2.14 Enzyme Assays, Peroxidase (POX) activity and Harvest Index (HI) of POX 28
4.2.15 Treatments, Experimental Design and Statistical Analysis 28

4.3 Results 29
4.3.1 Laboratory Experiment 29
4.3.2 Glasshouse Experiment 30
  4.3.2.1 Root Length and Root Dry Weight 30
  4.3.2.2 N2-fixing Activity (Acetylene Reduction Assay (ARA)) 31
  4.3.2.3 Plant Height 32
  4.3.2.4 Water Relation Attributes (LWP and LRWC) 33
  4.3.2.5 Malondialdehyde (MDA-Lipid Peroxidation) 35
  4.3.2.6 Proline Content 37
  4.3.2.7 Peroxidase (POX) Activity 39
  4.3.2.8 Harvest Index of Peroxidase (POX) Activity 41

4.4 Discussion 43
4.5 Conclusion 48

5 IMPACT OF WATER STRESS IMPOSITION ON GROWTH AND DEVELOPMENT OF SOYBEAN (Glycine max L.) WITH PGPR AT FLOWERING AND POD-FILLING STAGES 49
5.1 Introduction 49
5.2 Materials and Methods 50
  5.2.1 Experimental Site 50
  5.2.2 Materials and Methods 50
    5.2.2.1 Soil Sampling and Analysis 50
    5.2.2.2 Soybean Seed 50
    5.2.2.3 Bacterial Inoculums 50
    5.2.2.4 Bacterial Preparation 50
  5.2.3 Planting and Fertilization of Soybean 50
  5.2.4 Water Treatment Application 51
  5.2.5 Root Length and Root Dry Weight 51
  5.2.6 N2-fixing Activity of Inoculated Soybean With Indigenous Isolates 51
  5.2.7 Plant Height 51
  5.2.8 Water Relation Attributes 51
  5.2.9 Malondialdehyde Assay (MDA) 51
  5.2.10 Proline Content 51
  5.2.11 Enzyme Assays, Peroxidase (POX) activity and Harvest Index (HI) of POX 52
  5.2.12 Treatments, Experimental Design and Statistical Analysis 52
5.3 Results
5.3.1 Root Length and Root Dry Weight
5.3.2 N2-fixing Activity (Acetylene Reduction Assay (ARA))
5.3.3 Plant Height
5.3.4 Water Relation Attributes (LWP and LRWC)
5.3.5 Malondialdehyde (MDA-Lipid Peroxidation)
5.3.6 Proline Content
5.3.7 Peroxidase (POX) Activity
5.3.8 Harvest Index of Peroxidase (POX) Activity
5.3.9 Correlation between Physiological and Biochemical Parameters of Soybean Inoculated with PGPR and Bradyrhizobium at Flowering and Pod-filling Stages

5.4 Discussion
5.5 Conclusion

6 EFFECT OF WATER STRESS ON N2-FIXING ACTIVITY AT ONSET OF POD AND ONSET OF SEED STAGES OF SOYBEAN INOCULATED WITH PGPR
6.1 Introduction
6.2 Materials and Methods
6.2.1 Experimental Sites
6.2.2 Planting materials
6.2.2.1 Soil Sampling and Analysis
6.2.2.2 Soybean Seed
6.2.2.3 Bacterial Inoculums
6.2.2.4 Bacterial Preparation
6.2.3 Planting and Fertilization of Soybean
6.2.4 Water Treatment Application
6.2.5 Root Length and Root Dry Weight
6.2.6 N2-fixing Activity of Inoculated Soybean Indigenous Isolates
6.2.7 Plant Height
6.2.8 Water Relation Attributes
6.2.9 Malondialdehyde Assay (MDA)
6.2.10 Proline Content
6.2.11 Enzyme Assays, Peroxidase (POX) activity and Harvest Index (HI)
6.2.12 Treatments, Experimental Design and Statistical Analysis

6.3 Results
6.3.1 Root Length and Root Dry Weight
6.3.2 N2-fixing Activity (Acetylene Reduction Assay (ARA))
6.3.3 Plant Height
6.3.4 Water Relation Attributes (LWP and LRWC)
6.3.5 Malondialdehyde (MDA-Lipid Peroxidation)
6.3.6 Proline Content
6.3.7 Peroxidase (POX) Activity
6.3.8 Harvest Index of Peroxidase (POX) Activity

6.4 Discussion
6.5 Conclusion
## SUMMARY, CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCHES

<table>
<thead>
<tr>
<th>Section</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.1 Summary</td>
<td>96</td>
</tr>
<tr>
<td>7.2 General Conclusion</td>
<td>98</td>
</tr>
<tr>
<td>7.3 Recommendations For Future Studies</td>
<td>99</td>
</tr>
</tbody>
</table>

### REFERENCES

100

### APPENDICES

125

### BIODATA OF STUDENT

132
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Soybean Reproductive Stages as Defined by Fehr and Caviness</td>
</tr>
<tr>
<td>3.1</td>
<td>Characteristics of Clay-loam Soil (Serdang series)</td>
</tr>
<tr>
<td>3.2</td>
<td>Indigenous PGPR and Bradyrhizobium Isolates Inoculated with Soybean Seeds</td>
</tr>
<tr>
<td>4.1</td>
<td>Bacterial Inoculation and Water Treatments Used in This Experiment</td>
</tr>
<tr>
<td>4.2</td>
<td>Assessment of N2-fixing Activity, IAA and Siderophore Production of Selected Isolates</td>
</tr>
<tr>
<td>5.1</td>
<td>Bacterial Inoculation and Water Treatments Used in This Experiment</td>
</tr>
<tr>
<td>5.2</td>
<td>Pearson's Correlation Coefficients between Root Dry Weight, N2-fixing Activity, Plant height, HI, LWP, MDA content, Proline content and POX Activities of Soybean Inoculated with Different Strains of Bacteria under Water Treatments at Flowering and Pod-filling Stages</td>
</tr>
<tr>
<td>6.1</td>
<td>Bacterial Inoculation and Water Treatments Used in This Experiment</td>
</tr>
</tbody>
</table>
**LIST OF FIGURES**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Mechanism of Plant Growth Promoting Rhizobacteria</td>
<td>9</td>
</tr>
<tr>
<td>4.1</td>
<td>Effect of water treatments on root length of soybean inoculated with bacteria</td>
<td>31</td>
</tr>
<tr>
<td>4.2</td>
<td>Effect of water treatments on root dry weight of soybean inoculated with bacteria</td>
<td>32</td>
</tr>
<tr>
<td>4.3</td>
<td>Effect of water treatments on N2-fixing activity of soybean inoculated with bacteria</td>
<td>33</td>
</tr>
<tr>
<td>4.4</td>
<td>Effect of water treatments on Plant height of soybean inoculated with bacteria</td>
<td>34</td>
</tr>
<tr>
<td>4.5</td>
<td>Effect of water treatments on LWP of soybean inoculated with bacteria</td>
<td>35</td>
</tr>
<tr>
<td>4.6</td>
<td>Effect of water treatments on MDA content in root of soybean inoculated with bacteria</td>
<td>36</td>
</tr>
<tr>
<td>4.7</td>
<td>Effect of water treatments on MDA content in leaves of soybean inoculated with bacteria</td>
<td>37</td>
</tr>
<tr>
<td>4.8</td>
<td>Effect of water treatments on proline content in roots of soybean inoculated with bacteria</td>
<td>38</td>
</tr>
<tr>
<td>4.9</td>
<td>Effect of water treatments on proline content in leaves of soybean inoculated with bacteria</td>
<td>39</td>
</tr>
<tr>
<td>4.10</td>
<td>Effect of water treatments on POX activity in roots of soybean inoculated with bacteria</td>
<td>40</td>
</tr>
<tr>
<td>4.11</td>
<td>Effect of water treatments on POX activity in leaves of soybean inoculated with bacteria</td>
<td>41</td>
</tr>
<tr>
<td>4.12</td>
<td>Effect of water treatments on HI (POX activity) in roots of soybean inoculated with bacteria</td>
<td>42</td>
</tr>
<tr>
<td>4.13</td>
<td>Effect of water treatments on HI (POX activity) in leaves of soybean inoculated with bacteria</td>
<td>43</td>
</tr>
<tr>
<td>5.1</td>
<td>Effect of water treatments on root length of soybean inoculated with bacteria at flowering and pod-filling stages.</td>
<td>53</td>
</tr>
</tbody>
</table>
5.2 Effect of water treatments on root dry weight of soybean inoculated with bacteria at flowering and pod-filling stages.

5.3 Effect of water treatments on N2-fixing activity of soybean inoculated with bacteria at flowering and pod-filling stages.

5.4 Effect of water treatments on plant height of soybean inoculated with bacteria at flowering and pod-filling stages.

5.5 Effect of water treatments on LWP of soybean inoculated with bacteria at flowering and pod-filling stages.

5.6 Effect of water treatments on LRWC of soybean inoculated with bacteria at flowering and pod-filling stages.

5.7 Effect of water treatments on MDA content of soybean's roots inoculated with bacteria at flowering and pod-filling stages.

5.8 Effect of water treatments on MDA content of soybean's leaves inoculated with bacteria at flowering and pod-filling stages.

5.9 Effect of water treatments on proline content of soybean's roots inoculated with bacteria at flowering and pod-filling stages.

5.10 Effect of water treatments on proline content of soybean's leaves inoculated with bacteria at flowering and pod-filling stages.

5.11 Effect of water treatments on POX activity of soybean's roots inoculated with bacteria at flowering and pod-filling stages.

5.12 Effect of water treatments on POX activity of soybean's leaves inoculated with bacteria at flowering and pod-filling stages.

5.13 Effect of water treatments on HI (POX activity) of soybean's roots inoculated with bacteria at flowering and pod-filling stages.

5.14 Effect of water treatments on HI (POX activity) of soybean's leaves inoculated with bacteria at flowering and pod-filling stages.

6.1 Effect of water treatments on root length of soybean inoculated with bacteria at pod-filling, onset of pod and seed stages.

6.2 Effect of water treatments on root dry weight of soybean inoculated with bacteria at pod-filling, onset of pod and seed stages.

6.3 Effect of water treatments on N2-fixing activity of soybean inoculated with bacteria at pod-filling, onset of pod and seed stages.

6.4 Effect of water treatments on plant height of soybean inoculated with bacteria at pod-filling, onset of pod and seed stages.
6.5 Effect of water treatments on LWP of soybean inoculated with bacteria at pod-filling, onset of pod and seed stages.

6.6 Effect of water treatments on LRWC of soybean inoculated with bacteria at pod-filling, onset of pod and seed stages.

6.7 (IIHFW RI ZDWHU WUHDWPHQWV RQ 0"$ FRQWHQW RI VR\ inoculated with bacteria at pod-filling, onset of pod and seed stages.

6.8 (IIHFW RI ZDWHU WUHDWPHQWV RQ 0"$ FRQWHQW RI VR\ inoculated with bacteria at pod-filling, onset of pod and seed stages.

6.9 Effect of water treatments on proline content RI VR\EHDOIV UR\ inoculated with bacteria at pod-filling, onset of pod and seed stages.

6.10 (IIHFW RI ZDWHU WUHDWPHQWV RQ SUROLQH FRQWHQW inoculated with bacteria at pod-filling, onset of pod and seed stages.

6.11 Effect of ZDWHU WUHDWPHQWV RQ 32; DFWLYLW\ RI VR\ inoculated with bacteria at pod-filling, onset of pod and seed stages.

6.12 (IIHFW RI ZDWHU WUHDWPHQWV RQ 32; DFWLYLW\ RI VR\ inoculated with bacteria at pod-filling stage, onset of pod and seed stages.

6.13 (IIHFWRIZDWHU WUHDWPHQWV RQ+, 32; DFWLYLW\RI VR\ inoculated with bacteria at pod-filling, onset of pod and seed stages.

6.14 (IIHFWRIZDWHU WUHDWPHQWV RQ+, 32; DFWLYLW\RI VR\ inoculated with bacteria at pod-filling stage, onset of pod and seed stages.
## LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>°C</td>
<td>Degree centigrade</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>g/L</td>
<td>gram per liter</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>DAI</td>
<td>day after inoculation</td>
</tr>
<tr>
<td>L</td>
<td>liter</td>
</tr>
<tr>
<td>M</td>
<td>mole</td>
</tr>
<tr>
<td>EU</td>
<td>enzyme unit</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>ml</td>
<td>milliliter</td>
</tr>
<tr>
<td>mM</td>
<td>milimole</td>
</tr>
<tr>
<td>mm</td>
<td>millimeter</td>
</tr>
<tr>
<td>nmol C(_2)H(_4)/mL/h</td>
<td>nanomol methanol per milliliter per hour</td>
</tr>
<tr>
<td>nm</td>
<td>nanometer</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>rpm</td>
<td>Round per minute</td>
</tr>
<tr>
<td>RT</td>
<td>Retention Time</td>
</tr>
<tr>
<td>m</td>
<td>meter</td>
</tr>
<tr>
<td>J</td>
<td>microgram</td>
</tr>
<tr>
<td>µg/ mL</td>
<td>microgram per milliliter</td>
</tr>
<tr>
<td>JO</td>
<td>microgram per litter</td>
</tr>
<tr>
<td>JJ</td>
<td>microgram per gram</td>
</tr>
<tr>
<td>O</td>
<td>microliter</td>
</tr>
<tr>
<td>P</td>
<td>micromilimeter</td>
</tr>
<tr>
<td>0</td>
<td>Micromol</td>
</tr>
<tr>
<td>g/plant</td>
<td>gram per plant</td>
</tr>
<tr>
<td>mL/min</td>
<td>millilitre per minute</td>
</tr>
<tr>
<td>CFU</td>
<td>colony forming unit</td>
</tr>
<tr>
<td>MPa</td>
<td>Mega-Pascal</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter</td>
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</table>
ABBREVIATIONS OF STATISTIC ANALYSIS

ANOVA  Analysis of Variance
P       Probably
CRD     Complete Randomized Design
RCBD    Randomized Complete Block Design
SAS     Statistical Analysis Software
SD      Standard Deviation
SEM     Standard Error of Means
CHAPTER 1

INTRODUCTION

1.1 General Introduction

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 N$_2$-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 stimul ated 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 N$_2$-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.
1.2 Objectives

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 pheonological 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.

1.3 Hypothesis

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 nodulaing 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.


Giordano, W. and Hirsch, A.M. (2004). The expression of MaEXP1, a melilotus alba expansin gene, is upregulated during the sweet clover-sinorhizobium meliloti interaction: MPMI. 17: 613-622.


119


