BENEFICIAL EFFECTS OF RHIZOBACTERIAL INOCULATION ON NUTRIENT UPTAKE, GROWTH AND YIELD OF BANANA (MUSA SPP. CV. 'BERANGAN')

MD. ABDUL BASET MIA

FP 2002 15
BENEFICIAL EFFECTS OF RHIZOBACTERIAL INOCULATION ON NUTRIENT UPTAKE, GROWTH AND YIELD OF BANANA (MUSA SPP. CV. ‘BERANGAN’)

By

MD. ABDUL BASET MIA

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

June 2002
DEDICATED TO
MY MOTHER, UNCLE ABDUS SHAHID
AND
DEPARTED SOUL OF MY FATHER
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

BENEFICIAL EFFECTS OF RHIZOBACTERIAL INOCULATION ON NUTRIENT UPTAKE, GROWTH AND YIELD OF BANANA (MUSA SPP. CV. ‘BERANGAN’)

By

MD. ABDUL BASET MIA

June 2002

Chairman: Associate Professor Zulkifli Hj. Shamsuddin, Ph.D.

Faculty : Agriculture

Banana, an important fruit crop, requires high amounts of N-fertilizers for commercial cultivation, which is costly and can be hazardous to the environment when used excessively. Biofertilizer is globally accepted as an alternative source of nitrogen fertilizer and can substantially supplement the N requirement while enhancing the uptake of water and mineral nutrients of crop plants.

A series of research study involving six experiments were conducted to observe the effects of PGPR inoculation on root stimulation and colonization, nutrient absorption, growth, yield and fruit quality of bananas (Musa spp cv ‘Berangan’, AA type) under hydroponics condition.

In the preliminary study, the effects of inoculation with two PGPR strains, Sp7 (Azospirillum brasiliense) and UPMB10 (Bacillus sphaericus UPMB10), on plant growth and N accumulation of banana plantlets were observed under N-free hydroponics condition for 45 days. A marked increase in root growth namely length (33-44%), volume
(76-168%) and mass (137-141%) were recorded due to the PGPR inoculation besides a higher shoot growth (123-202%) and N yield (94-144%).

An in vitro electron microscopy study was conducted to observe the pattern of colonization of PGPR strains Sp7 and UPMB10 on roots of banana plantlets. This study demonstrated that both strains could effectively colonize the banana roots and more bacterial cells were present in the root hair proliferation zone.

In the growth study with banana plantlets under hydroponics condition using 4.0 L plastic pots, 150 ppm fertilizer-N was found to be optimum for the 45 days period. Another study with similar conditions was undertaken to observe the synergistic effect of minimal fertilizer-N supply (33% of the total N requirement) and PGPR inoculation on root growth and nutrient uptake of banana plantlets. The results showed that inoculation by UPMB10 with minimal fertilizer-N supply increased the primary root elongation and secondary root initiation and subsequently increased the root mass. The same treatment also increased N concentration in pseudostem and leaves and Ca concentration in roots. The total accumulation of N, P, K, Ca and Mg was increased due to inoculation; a consequence of increased plant growth. Plants with this treatment produced an equivalent total dry matter as those supplied with 100% N.

A subsequent experiment with larger containers (1000 L) was conducted to observe the effect of PGPR inoculation on plant growth, nutrient uptake, yield and fruit quality of bananas at different levels of N-fertilization. The results showed that inoculation together with 33% N improved the bioenhancing activity by increasing root and shoot growth, and photosynthetic rate (25%). As observed in the earlier study with smaller pots (4.0 L), the N concentration in the pseudostem was increased. PGPR inoculation with 33% fertilizer-N also increased the Ca uptake capacity resulting in higher Ca concentration in root, corm and pulp but increased the Mg concentration in the root
only. In addition, the total accumulation of nutrients was heavily influenced by PGPR inoculation due to enhanced root proliferation. PGPR inoculation greatly increased the bunch yield (35-51%) and physical fruit attributes namely finger weight (62-65%), finger length (22-24%), grade and pulp/peel ratio. Plants also flowered three weeks earlier in PGPR-inoculated plants.

PGPR strains Sp7 and UPMB10 were evaluated for their N\textsubscript{2} fixing capacities in association with banana roots by acetylene reduction assay (ARA) and \textsuperscript{15}N isotopic dilution technique using 4 L pots for 45 days. The results conclusively showed that roots of PGPR-inoculated plants produced higher ARA activities (129 \textmu mole plant\textsuperscript{-1} hour\textsuperscript{-1}). Inoculated plants together with the least fertilizer-N supply (3.2 ppm, 2.13% of the total plant N requirement) showed the highest amount of nitrogen derived from atmosphere (Nd\textsubscript{fa} ; 37-39%) while those with higher inorganic-N fertilizer (50 ppm, 33% of the total N requirement) showed the lowest Nd\textsubscript{fa} (5%). However, PGPR inoculation with 20 ppm fertilizer-N (13% of the N requirement) produced a synergistic effect on N\textsubscript{2} fixation with a relatively higher Nd\textsubscript{fa} and amounts of fixed-N\textsubscript{2} (12.4 to 12.5 % Nd\textsubscript{fa}; 10.26-10.85 mg plant\textsuperscript{-1}). However, the nitrogen fixation capacity between the strains was not significantly different.

The findings from the above studies demonstrated that PGPR strains (Sp7 and UPMB 10) inoculation with minimal fertilizer-N supply are effective as a bioenhancer and biofertilizer to fix N\textsubscript{2} and increase plant growth, nutrient uptake, yield and fruit qualities of bananas under hydroponics condition.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN INOKULASI RHIZOBAKTERIA TERHADAP PENGAMBIKAN NUTRIEN, PERTUMBUHAN POKOK DAN HASIL BUAH TANAMAN PISANG (MUSA SPP. CV. ‘BERANGAN’)

Oleh

MD. ABDUL BASET MIA

Jun 2002

Pengerusi : Profesor Madya Zulkifli Hj. Shamsuddin, Ph.D.

Fakulti : Pertanian


Enam siri eksperimen telah dijalankan untuk menilai kesan inokulasi PGPR terhadap perkembangan dan kolonisasi akar, penyerapan nutrien, pertumbuhan hasil dan kualiti buah tanaman pisang berangan (Musa spp. jenis AA) yang ditanam secara hidroponik. Kajian awal melibatkan inokulasi strain PGPR Sp7 (Azospirillum brasilense) dan UPMB10 (Bacillus sphaericus UPMB10) terhadap pertumbuhan pokok dan pengumpulan N pada kultur tisu pisang dalam keadaan hidroponik (tanpa N) selama 45 hari. Inokulasi PGPR tersebut telah meningkatkan pertumbuhan akar terutamanya panjang akar (33-44%), isipadu (76-168%) dan berat akar (137-141%) serta
peningkatan pertumbuhan pucuk (123-202%) dan kandungan jumlah N (94-144%) yang tinggi juga diperhatikan.

Kajian in vitro kolonisasi akar menggunakan mikroskop elektron untuk melihat corak kolonisasi Sp7 dan UPMB10 pada akar pisang kultur tisu telah dijalankan. Kajian ini menunjukkan bahawa kedua-dua strain mampu mengkolonisasi akar terutamanya pada zon pembentukan akar rerambut.

Dalam kajian pertumbuhan pisang kultur tisu menggunakan kaedah hidroponik dan pasu plastik 4.0 L, 150 bsj baja-N didapati optimum untuk tanaman selama 45 hari. Satu lagi kajian inokulasi PGPR dalam keadan yang sama ke atas pisang kultur tisu telah dijalankan bersama pengunaan kandungan N yang rendah (33% dari keperluan N keseluruhan; 150 ppm N) untuk menilai kesan sinergistik di antara kandungan N rendah dan inokulasi PGPR terhadap pertumbuhan akar dan pengambilan nutrien oleh pokok pisang. Keputusan menunjukkan bahawa inokulasi UPMB10 dan kandungan N yang minima dapat meningkatkan kadar pemanjangan akar primer, pencetusan akar sekunder dan seterusnya meningkatkan berat akar. Rawatan yang sama juga dapat meningkatkan kepekatan N di dalam pseudostem dan daun serta kepekatan Ca dalam akar. Jumlah pengumpulan keseluruhan P, K, Ca dan Mg juga meningkat akibat peningkatan pertumbuhan pokok. Berat kering keseluruhan rawatan ini sama seperti aplikasi 100% baja N.

Kajian berikutnya melibatkan penanaman pokok pisang di dalam bekas 1000 L untuk menilai kesan inokulasi PGPR dan paras N yang berbeza terhadap pertumbuhan, pengambilan nutrien, hasil dan kualiti buah pisang. Keputusan eksperimen ini menunjukkan bahawa inokulasi PGPR dengan 33% N dapat meningkatkan aktiviti...
penggalak pertumbuhan biologi tanaman yang meliputi peningkatan pertumbuhan akar dan daun serta kadar fotosintesis (25%). Kesan yang sama dalam pseudostem turut dilihat sama seperti eksperimen sebelum ini yang menggunakan pasu yang lebih kecil (4.0 L). Inokulasi PGPR dan 33%N juga meningkatkan pengambilan Ca dan menyebabkan peningkatan kepekatan Ca di akar, umbi dan pulpa tetapi meningkatkan kepekatan Mg di akar sahaja. Di samping itu, pengumpulan jumlah nutrien juga dipengaruhi oleh inokulasi PGPR yang meningkatkan pembentukan akar. Inokulasi PGPR juga telah merangsang pengeluaran hasil dan kualiti buah terutamanya berat tandan (35-51%) dan sisir (62-65%) serta panjang (22-24%), kualiti dan nisbah pulpa/kulit buah pisang. Pisang yang diinokulasi juga telah mengeluarkan bunga tiga minggu lebih awal daripada rawatan kawalan. Keupayaan strain PGPR Sp7 dan UPMB10 untuk mengikat N2 juga telah dinilai dengan menggunakan teknik asai penurunan asetilin (ARA) dan teknik pencairan isotop 15N di dalam pasu 4 L selama 45 hari. Keputusannya, PGPR menunjukkan aktiviti ARA yang tinggi (129nm/pokok/jam) di dalam akar kultur tisu pisang. Rawatan yang diinokulasi dan diberi bekalan baja inorganik N terendah (3.2 ppm, 2.13% dari keperluan N keseluruhan) menunjukkan kandungan pengikatan N2 (Ndfa) yang tertinggi (37-39%). Rawatan yang dibekalkan dengan kandungan baja nitrogen inorganik yang tinggi sekali (50 ppm, 33% dari keperluan N keseluruhan) pula menunjukkan Ndfa yang terendah (5%). Namun demikian, rawatan dengan bekalan baja inorganik N sebanyak 20 ppm (13% dari keperluan N keseluruhan) menghasilkan kesan sinergistik ke atas pengikatan N2 dengan Ndfa yang tinggi (12.4 hingga 12.5%; 10.26-10.85 mg pokok-1). Walau bagaimana pun
tiada perbezaan yang bermakna diantara keupayaaan pengikatan N\textsubscript{2} kedua-dua strain tersebut.

Kajian ini menunjukkan penggunaan strain PGPR (Sp7 dan UPMB10) dengan penambahan sejumlah kecil baja N adalah berkesan sebagai bio-penggalak dan biobaja (pengikat N\textsubscript{2}) dan seterusnya meningkatkan pertumbuhan perumah, hasil buah serta kualiti fizikal tanaman pisang.
ACKNOWLEDGEMENTS

Praise to Almighty Allah for His blessings Who enabled me to attain this highest academic degree.

I wish to express my deepest gratitude and sincere appreciation to Associate Professor Dr. Zulkifli Hj. Shamsuddin, the Chairman of the Supervisory Committee for his excellent guidance, constant encouragement, invaluable suggestions and generous help throughout the study period and in the preparation of this thesis.

Sincere appreciation also goes to Dr. Zakaria Wahab and Professor Dr. Marziah Mahmood, members of the Supervisory Committee, for their constructive suggestions and guidance in formulation and execution of the research projects and critical review of the manuscript.

I would like to give thanks to Dr. Halimi Mohd. Saud and Dr. Radziah Othman, Department of Land Management, UPM for their suggestions and inspiration during the study.

Special thanks are due to Dr. Gurmit Singh and Madam Ho Yuk Wah, United Plantations Berhad, Teluk Intan, Perak, Malaysia for providing the tissue-cultured banana plantlets. I appreciate the help and cooperation of my laboratory colleagues: Dr. Amir Hamzah bin Ahmad Ghazali, Puan Zarinah Basir, Mr. Neo Sye Peng, Miss Premalatha Pakirisamy and Miss Tan Geok Hun. I also acknowledge the help of technical staff of the Faculty of Agriculture, UPM, especially Mr. Shahril Abdul...
Rahman, Alias Tahar, Mazlan Bangi, Azhar Othman, Aziz Ismail, Rahim Utar and Jamil Omar during the execution of the study. I really appreciate the help from Mrs. Latifah Nordin, Malaysian Institute for Nuclear Technology Research (MINT) in analyzing the $^{15}$N samples.

I would like to say thanks to the Ministry of Science, Technology and Environment for financial support (Graduate Assistantship) (IRPA Grants 51070 and 51386) through the Universiti Putra Malaysia which provided me the opportunity to pursue my Ph.D. degree in Malaysia.

I am grateful to the administrative authority of Bangladesh Institute of Nuclear Agriculture (BINA) for providing deputation and all kinds of help to accomplish my degree. I am also thankful to all my friends and colleagues especially Dr. M.A. Sattar, Chief Scientific Officer, BINA who encouraged me to pursue the Ph. D. at UPM. Special thanks are also due to Dr. A.H.M. Razzaque, Principal Scientific Officer, Dr. M. I. Khalil, Senior Scientific Officer of BINA, Dr. Abul Hossain Molla and Dr. Humayun Kabir for providing me inspiration.

I am grateful to my brothers (Lutfar and Alam), sisters, cousins (Hazrat Ali, Mokbul Hossain, Abul Hossain and Habibur Rahaman), uncle (Abdul Matin), friend (Quddus), father-in-law (Dewan Mojibur Rahman), mother-in-law (Rejia Rahman) and other relatives for their encouragement and moral support. I am forever indebted to my wife, Momena Akter, and my daughter Tasnia Baset for their sacrifice and patience during the course of my graduate study.

xi
I certify that an Examination Committee has met on 19th June 2002 to conduct the final examination of Md. Abdul Baset Mia on his Doctor of Philosophy thesis entitled "Beneficial Effects of Rhizobacterial Inoculation on Nutrient Uptake, Growth and Yield of Banana (Musa spp. cv. 'Berangan')" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

HALIMI MOHD. SAUD, Ph.D.
Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

ZULKIFLI HJ. SHAMSUDDIN, Ph.D.
Associate Professor/ Director
Research Management Centre
Universiti Putra Malaysia
(Member)

ZAKARIA WAHAB, Ph.D.
Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

MARZIAH MAHMOOD, Ph.D.
Professor
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

NANTAKORN BOONKERD, Ph.D.
Professor
Institute of Research and Development
Suranaree University of Technology
Thailand
(Independent Examiner)

SHAMSHER MOHAMAD RAMADILI, Ph.D.
Professor/ Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia
Date: 8 JUL 2002
This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy

AINI IDERIS, Ph.D.
Professor/ Dean
School of Graduate Studies
Universiti Putra Malaysia
Date: 12 SEP 2002
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or currently submitted for any other degree at UPM or other institutions.

__________________________
MD. ABDUL BASET MIA
Date: 8-7-02
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>vi</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>x</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>xii</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xx</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xxiii</td>
</tr>
<tr>
<td>LIST OF PLATES</td>
<td>xxv</td>
</tr>
<tr>
<td>LIST OF APPENDICES</td>
<td>xxvii</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xxviii</td>
</tr>
</tbody>
</table>

CHAPTER

1 INTRODUCTION .................................................................1.1

2 LITERATURE REVIEW .........................................................2.1

2.1 Banana .............................................................................2.1
  2.1.1 Banana Root System ..................................................2.2
  2.1.2 Nutritional Requirement .............................................2.3
    2.1.2.1 Nitrogen .........................................................2.3
    2.1.2.2 Phosphorus .....................................................2.4
    2.1.2.3 Potassium .......................................................2.5
    2.1.2.4 Calcium ........................................................2.6
    2.1.2.5 Magnesium .......................................................2.6
  2.1.3 Yield and Fruit Quality .............................................2.7

2.2 Hydroponics Culture for Root Development Study ..................2.8

2.3 Plant Growth Promoting Rhizobacteria (PGPR) ......................2.9
  2.3.1 Azospirillum ........................................................2.9
  2.3.2 Bacillus .....................................................................2.10
  2.3.3 Colonization of PGPR on Roots of Crop Plants .................2.11
  2.3.4 Plant Growth and Development ....................................2.13
  2.3.5 Nitrogen Fixation and Utilization .................................2.16
  2.3.6 Quantification of BNF ..............................................2.18
  2.3.7 Nutrient Uptake .....................................................2.21
  2.3.8 Enhancement of Physiological Properties in the Host Plants 2.24

2.4 Conclusion .......................................................................2.25

xv
3 GENERAL MATERIALS AND METHODS

3.1 Plant Nutrient Solution ................................................. 3.1
3.2 PGPR Strains .............................................................. 3.2
3.3 Bacterial Culture ......................................................... 3.3
3.4 Tissue-Cultured Banana Plantlets .................................... 3.3
3.5 Data Collection ............................................................. 3.3
3.5.1 Morphological Parameters of Shoots ............................ 3.3
3.5.2 Root Parameters ....................................................... 3.4
3.5.3 Primary and Secondary Roots ..................................... 3.4
3.5.4 Chlorophyll Content ................................................ 3.4
3.5.5 Physiological Parameters .......................................... 3.6
3.6 Plant Analysis for N, P, K, Ca and Mg ............................ 3.7
3.7 Statistical Analysis ..................................................... 3.7

4 EFFECTS OF RHIZOBACTERIAL INOCULATION ON GROWTH AND NITROGEN INCORPORATION IN BANANA PLANTLETS UNDER NITROGEN-FREE HYDROPONICS CONDITION

4.1 Introduction ............................................................... 4.1
4.2 Materials and Methods ................................................ 4.2
4.3 Results ................................................................. 4.5
4.3.1 Root Growth .......................................................... 4.5
4.3.2 Shoot Growth ......................................................... 4.8
4.3.3 Nitrogen Accumulation ............................................. 4.9
4.4 Discussion .............................................................. 4.10
4.5 Conclusion ............................................................ 4.11

5 RHIZOBACTERIAL COLONIZATION PATTERN ON ROOT-SURFACE OF BANANA PLANTLETS

5.1 Introduction ............................................................ 5.1
5.2 Materials and Methods ................................................ 5.2
5.3 Results ................................................................. 5.6
5.4 Discussion ............................................................ 5.13
5.5 Conclusion ............................................................ 5.15

6 NITROGEN REQUIREMENT FOR GROWTH OF BANANA PLANTLETS

6.1 Introduction ............................................................ 6.1
6.2 Materials and Methods ................................................ 6.2
6.3 Results ................................................................. 6.3
6.3.1 Plant height ......................................................... 6.3

xvi
6.3.2 Chlorophyll Content .............................................. 6.3
6.3.3 Total Dry Matter ................................................. 6.6
6.4 Discussion ............................................................. 6.6
6.5 Conclusion ............................................................. 6.8

7  ROOT STIMULATION, NUTRIENT ACCUMULATION AND GROWTH OF BANANA PLANTLETS INOCULATED WITH RHIZOBACTERIA UNDER LOW FERTILIZER-NITROGEN REGIME

7.1 Introduction ......................................................... 7.1
7.2 Materials and Methods ............................................. 7.2
7.3 Results ............................................................... 7.3
  7.3.1 Root Growth ..................................................... 7.3
    7.3.1.1 Primary Root Elongation .................................. 7.3
    7.3.1.2 Secondary Root Initiation ................................ 7.4
    7.3.1.3 Root Number ............................................... 7.5
    7.3.1.4 Total 1° Root Length .................................... 7.7
    7.3.1.5 Root Base Diameter ...................................... 7.7
    7.3.1.6 Root Volume ............................................... 7.8
    7.3.1.7 Root Dry Weight .......................................... 7.8
  7.3.2 Shoot Growth .................................................. 7.10
    7.3.2.1 Plant Height ............................................... 7.10
    7.3.2.2 Leaf Development ......................................... 7.10
    7.3.2.3 Leaf Chlorophyll Content ................................ 7.14
    7.3.2.4 Net Leaf Photosynthesis (Pn) ............................. 7.15
    7.3.2.5 Shoot Dry Weight ......................................... 7.15
  7.3.3 Root to Shoot Ratio (R/S) .................................... 7.16
  7.3.4 Nutrient Uptake ............................................... 7.17
7.4 Discussion .......................................................... 7.21
7.5 Conclusion ........................................................ 7.26

8  GROWTH, NUTRIENT ACCUMULATION, YIELD AND FRUIT QUALITY OF BANANAS INOCULATED BY RHIZOBACTERIA

8.1 Introduction ....................................................... 8.1
8.2 Materials and Methods ............................................ 8.2
8.3 Results ............................................................. 8.5
  8.3.1 Root Growth .................................................. 8.5
    8.3.1.1 Root Number .............................................. 8.5
    8.3.1.2 Total Root Length ........................................ 8.5
    8.3.1.3 Root Volume ............................................... 8.6
    8.3.1.4 Root Dry Weight .......................................... 8.7
8.3.2 Shoot Growth ....................................................... 8.9
8.3.2.1 Plant Height ................................................... 8.9
8.3.2.2 Pseudostem Base Circumference ............................. 8.11
8.3.2.3 Total Leaf Chlorophyll Content .............................. 8.11
8.3.2.4 Net Leaf Photosynthesis ..................................... 8.14
8.3.2.5 Shoot Dry Weight .............................................. 8.14
8.3.2.6 Dry Matter Partitioning ...................................... 8.16
8.3.2.7 Root to Shoot Ratio .......................................... 8.18
8.3.3 Plant Nutrient Uptake ........................................... 8.18
8.3.3.1 Nitrogen ....................................................... 8.18
8.3.3.2 Phosphorus .................................................... 8.23
8.3.3.3 Potassium ...................................................... 8.25
8.3.3.4 Calcium ........................................................ 8.25
8.3.3.5 Magnesium .................................................... 8.27
8.3.4 Days to Flowering ............................................... 8.27
8.3.5 Fruit Yield ....................................................... 8.31
8.3.6 Number of Hand .................................................. 8.31
8.3.7 Finger Weight .................................................... 8.31
8.3.8 Finger Length and Diameter .................................... 8.33
8.3.9 Pulp/Peel Ratio .................................................. 8.33
8.3.10 Sugar Content ................................................... 8.34
8.3.11 Titratable Acidity .............................................. 8.34
8.3.12 Ascorbic Acid Content ........................................ 8.34
8.4 Discussion .......................................................... 8.35
8.5 Conclusion ........................................................ 8.41

9 ESTIMATION OF N$_2$-FIXED BY RHIZOBACTERIA IN ASSOCIATION WITH ROOTS OF BANANA PLANTLETS UNDER LOW FERTILIZER-N REGIMES

9.1 Introduction ...................................................... 9.1
9.2 Materials and Methods ........................................... 9.2
9.2.1 Inoculum Preparation and Plant Inoculation ................. 9.3
9.2.2 Acetylene Reduction Assay .................................... 9.4
9.2.3 Sample Preparation for $^{15}$N Determination ................ 9.4
9.2.4 Statistical Analysis ........................................... 9.6
9.3 Results ............................................................ 9.6
9.3.1 Acetylene Reduction Assay .................................... 9.6
9.3.2 Dry Matter Production ........................................ 9.8
9.3.3 Nitrogen Yield .................................................. 9.10
9.3.4 $^{15}$N Isotope Dilution ........................................ 9.11
9.3.5 Nitrogen Fixation .............................................. 9.12
9.4 Discussion ........................................................ 9.15
9.5 Conclusion ........................................................ 9.19
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Composition of nutrient solution for hydroponics banana cultivation</td>
</tr>
<tr>
<td>4.1</td>
<td>Root growth of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 grown under hydroponics condition for 45 days</td>
</tr>
<tr>
<td>4.2</td>
<td>Shoot growth of hydroponically-grown banana plantlets inoculated with PGPR strains Sp7 and UPMB10 for 45 days</td>
</tr>
<tr>
<td>4.3</td>
<td>Concentration and total N yield of hydroponically grown banana plantlets inoculated with PGPR strains Sp7 and UPMB10 grown for 45 days</td>
</tr>
<tr>
<td>7.1</td>
<td>Root growth of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 grown under low fertilizer-N regimes</td>
</tr>
<tr>
<td>7.2</td>
<td>The N, P, K, Ca and Mg concentrations in root, pseudostem and leaves of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 grown under hydroponics condition under low fertilizer-N regimes for 45 days</td>
</tr>
<tr>
<td>7.3</td>
<td>Total accumulation of N, P, K, Ca and Mg in banana plantlets inoculated with PGPR strains Sp7 and UPMB10 at low fertilizer-N regimes for 45 days</td>
</tr>
<tr>
<td>8.1</td>
<td>Root growth of mature banana plants inoculated with PGPR strains Sp7 and UPMB10 grown under hydroponics condition</td>
</tr>
<tr>
<td>8.2</td>
<td>Net photosynthesis of youngest expanded leaf of bananas inoculated with PGPR strains Sp7 and UPMB10 grown under hydroponics condition</td>
</tr>
<tr>
<td>8.3</td>
<td>The N, P, K, Ca and Mg concentration in root and corm of bananas inoculated with PGPR strains Sp7 and UPMB10 at different levels of fertilizer-N</td>
</tr>
<tr>
<td>8.4</td>
<td>The N, P, K, Ca and Mg concentration in pseudostem and leaf of bananas inoculated with PGPR strains Sp7 and UPMB10 at different levels of fertilizer-N</td>
</tr>
</tbody>
</table>
8.5 The N, P, K, Ca and Mg concentration in banana pulp inoculated with PGPR strains Sp7 and UPMB10 at different levels of fertilizer-N ................................................................. 8.23

8.6 Total accumulation of N, P, K, Ca and Mg in banana plants inoculated with PGPR strains Sp7 and UPMB10 at different levels of fertilizer-N ................................................................. 8.24

8.7 Yield, yield contributing characters and fruit quality of bananas inoculated with PGPR strains Sp7 and UPMB10 grown under hydroponics condition ......................................................... 8.30

9.1 Total dry matter production of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 and supplied with various levels of fertilizer-N under hydroponics condition .................. 9.9

9.2 Dry matter in root, pseudostem and leaves of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 and supplied with various levels of fertilizer-N grown under hydroponics condition .................. 9.9

9.3 Total N yield of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 and supplied with various levels of fertilizer-N grown under hydroponics condition .................. 9.10

9.4 Nitrogen yield in root, pseudostem and leaves of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 and supplied with various levels of fertilizer-N grown under hydroponics condition .................. 9.11

9.5 Distribution of % $^{15}$N atom excess in root, pseudostem and leaves of PGPR inoculated plants at various levels of fertilizer-N ......................................................................................... 9.12

9.6 Percentage of N derived from atmosphere (%Ndfa) in banana plantlets (total plant basis) inoculated with PGPR strains Sp7 and UPMB10 and supplied with various fertilizer-N grown under hydroponics condition ................................................................. 9.13

9.7 Total N$_2$-fixed in banana plantlets inoculated with PGPR strains Sp7 and UPMB10 and supplied with various levels of fertilizer-N grown under hydroponics condition .......................... 9.14
9.8 Distribution of percentage of N derived from atmosphere (%Ndfa) in root, pseudostem and leaves of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 and supplied with various levels of fertilizer-N grown under hydroponics condition................................................................. 9.14

9.9 Total N₂-fixed in root, pseudostem and leaves of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 and supplied with various levels of fertilizer-N grown under hydroponics condition................................................................. 9.15
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Standard curve for total leaf chlorophyll content (Chl. mg cm(^{-2})) compared to CM value (Chlorophyll meter measurement of leaf greenness) for bananas.</td>
<td>3.5</td>
</tr>
<tr>
<td>6.1</td>
<td>Relationship between plant growth and fertilizer-N, A: plant height, B: chlorophyll content and C: TDM production grown under hydroponics condition for 45 days.</td>
<td>6.4</td>
</tr>
<tr>
<td>7.1</td>
<td>Primary root elongation of banana plantlets inoculated with PGPR strains Sp7 and UPMB10.</td>
<td>7.4</td>
</tr>
<tr>
<td>7.2</td>
<td>Secondary root initiation of banana plantlets inoculated with PGPR strains Sp7 and UPMB10.</td>
<td>7.5</td>
</tr>
<tr>
<td>7.3</td>
<td>Plant height of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 grown for 45 days.</td>
<td>7.11</td>
</tr>
<tr>
<td>7.4</td>
<td>Leaf number of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 grown for 45 days.</td>
<td>7.12</td>
</tr>
<tr>
<td>7.5</td>
<td>Leaf area, photosynthetic rate and shoot dry weight of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 grown for 45 days.</td>
<td>7.13</td>
</tr>
<tr>
<td>7.6</td>
<td>Leaf chlorophyll content of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 grown for 45 days.</td>
<td>7.14</td>
</tr>
<tr>
<td>7.7</td>
<td>Root:shoot ratio of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 grown under hydroponics condition at low fertilizer-N regimes for 45 days.</td>
<td>7.16</td>
</tr>
<tr>
<td>8.1</td>
<td>Plant height of bananas inoculated with PGPR strains Sp7 and UPMB10 at different levels of fertilizer-N grown for 210 days.</td>
<td>8.10</td>
</tr>
<tr>
<td>8.2</td>
<td>Pseudostem base circumference of banana plants inoculated with PGPR strains Sp7 and UPMB10 at different levels of fertilizer-N grown for 210 days.</td>
<td>8.12</td>
</tr>
<tr>
<td>8.3</td>
<td>Chlorophyll content of banana leaves inoculated with PGPR strains Sp7 and UPMB10 at different levels of fertilizer-N grown for 210 days.</td>
<td>8.13</td>
</tr>
<tr>
<td>Section</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>8.4</td>
<td>Shoot dry weight of mature banana plants inoculated with PGPR strains Sp7 and UPMB10 at different levels of fertilizer-N application</td>
<td>8.16</td>
</tr>
<tr>
<td>8.5</td>
<td>Dry matter partitioning to root, corm, pseudostem, leaf and bunch of mature banana plants inoculated with PGPR strains Sp7 and UPMB10 at different levels of fertilizer-N</td>
<td>8.17</td>
</tr>
<tr>
<td>8.6</td>
<td>Root: shoot ratio of banana matured plants inoculated with PGPR strains Sp7 and UPMB10 at different levels of fertilizer-N</td>
<td>8.19</td>
</tr>
<tr>
<td>9.1</td>
<td>Acetylene reduction activity (ARA) of hydroponically-grown banana plantlets inoculated with PGPR at different fertilizer-N levels, A: main effect of inoculation; B: interaction of fertilizer-N and inocula</td>
<td>9.7</td>
</tr>
</tbody>
</table>