ผลของราอาบัสคิวลาร์ไมคอร์ไรซาต่อผักสลัด *Lactuca sativa* L. ‘Butterhead’
และจุลชีพในไรโซสเฟียร์

นางสาวเพทาย จรูญนารถ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาวิทยาศาสตร์ชีวภาพ
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2558

EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI ON LETTUCE *Lactuca sativa* L. 'Butterhead' AND RHIZOSPHERE MICROBES

Miss Patai Charoonnart

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Biological Sciences
Faculty of Science
Chulalongkorn University
Academic Year 2015

Thesis Title	EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI ON LETTUCE *Lactuca sativa* L. 'Butterhead' AND RHIZOSPHERE MICROBES
By	Miss Patai Charoonnart
Field of Study	Biological Sciences
Thesis Advisor	Teerada Wangsomboondee, Ph.D.
Thesis Co-Advisor	Assistant Professor Kanogwan Seraypheap, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

.....Dean of the Faculty of Science
(Associate Professor Polkit Sangvanich, Ph.D.)

THESIS COMMITTEE

.....Chairman
(Assistant ProfessorTosak Seelanan, Ph.D.)

.....Thesis Advisor
(Teerada Wangsomboondee, Ph.D.)

.....Thesis Co-Advisor
(Assistant Professor Kanogwan Seraypheap, Ph.D.)

.....Examiner
(Assistant Professor Boonthida Kositsup, Ph.D.)

.....Examiner
(Associate Professor Alisa Vangnai, Ph.D.)

.....External Examiner
(Associate Professor Nantana Angkinand)

เพทาย จรูญนารถ : ผลของราอาบัสคิวลาร์ไมคอร์ไรซาต่อผักสลัด *Lactuca sativa* L. 'Butterhead' และจุลชีพในไรโซสเฟียร์ (EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI ON LETTUCE *Lactuca sativa* L. 'Butterhead' AND RHIZOSPHERE MICROBES) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: อ. ดร. ธีรดา หวังสมบูรณ์ดี, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ. ดร. กนกวรรณ เสรีภาพ, 156 หน้า.

การปลูกพืชในระบบเกษตรอินทรีย์มีพื้นที่เพิ่มขึ้นมากอันเป็นผลมาจากความใส่ใจในสุขภาพที่มากขึ้นของผู้บริโภค โดยผักสลัด (*Lactuca sativa* L.) เป็นผักที่มีความสำคัญและได้รับความต้องการจากผู้บริโภคสูงตลอดทั้งปี ดังนั้นจึงทำการศึกษาชนิดและปริมาณที่เหมาะสมในการใช้จุลชีพที่พบในธรรมชาติ ได้แก่ ราอาร์บัสคิวลาร์ไมคอร์ไรซา ในการปรับปรุงคุณภาพและเพิ่มปริมาณผักสลัดบัตเตอร์เฮด (Butterhead) ที่ปลูกในระบบเกษตรอินทรีย์ การแยกเชื้อราอาร์บัสคิวลาร์ไมคอร์ไรซาจากดินที่ปลูกผักสลัดในระบบเกษตรอินทรีย์ 3 แห่ง ได้ราอาร์บัสคิวลาร์ไมคอร์ไรซาที่สามารถเพิ่มปริมาณได้ทั้งสิ้น 23 ไอโซเลท (จังหวัดนครราชสีมา; N1-N7, จังหวัดราชบุรี; R1-R12, และจังหวัดปทุมธานี; P1-P4) จากนั้นนำ ราอาร์บัสคิวลาร์ไมคอร์ไรซาแต่ละไอโซเลทจำนวนละ 20 สปอร์มาปลูกเชื้อลงในกระถางเปรียบเทียบกับต้นที่ไม่ได้รับราอาร์บัสคิวลาร์ไมคอร์ไรซาเป็นเวลา 60 วัน ทำการเก็บตัวอย่างเพื่อวัดการเติบโต ผลการทดลองพบว่าราอาร์บัสคิวลาร์ไมคอร์ไรซาไอโซเลท N3 สามารถเพิ่มผลผลิตของบัตเตอร์เฮดแตกต่างจากชุดการทดลองควบคุมอย่างมีนัยสำคัญ ตามด้วยไอโซเลท N2, N4, N5 และ R5 ตามลำดับ จากนั้นนำราทั้ง 5 ไอโซเลทที่ได้รับคัดเลือกไปศึกษาผลของปริมาณสปอร์ราอาร์บัสคิวลาร์ไมคอร์ไรซาต่อการเติบโตของบัตเตอร์เฮด โดยทำการเปรียบเทียบการปลูกเชื้อในบัตเตอร์เฮดโดยใช้ราอาร์บัสคิวลาร์ไมคอร์ไรซาจำนวน 50, 100 และ 200 สปอร์ต่อหนึ่งต้น พบว่าบัตเตอร์เฮดที่มีการติดเชื้อโดยไอโซเลท N2, N4 และ R5 มีการเติบโตเพิ่มมากขึ้นเมื่อมีปริมาณสปอร์สูงมากขึ้น ในขณะที่บัตเตอร์เฮดที่มีการติดเชื้อไอโซเลท N3 และ N5 ให้ผลตรงกันข้าม โดยชุดการทดลองที่สามารถเพิ่มการเติบโตของผักสลัดได้ที่สุดคือการใช้ราอาร์บัสคิวลาร์ไมคอร์ไรซาไอโซเลท N5 จำนวน 50 สปอร์ และไอโซเลท R5 จำนวน 200 สปอร์ โดยไอโซเลท N5 จำนวน 50 สปอร์ซึ่งได้รับการระบุชนิดเป็น *Glomus mosseae* นั้นถูกเลือกมาศึกษาต่อในแปลงทดลองเนื่องจากความคุ้มทุนจากการที่ใช้ปริมาณสปอร์ที่น้อยกว่า ผลจากการทดลองในแปลงแสดงให้เห็นว่าการให้ *G. mosseae* สามารถเพิ่มการเติบโตของพืชได้ โดยพิจารณาจากการเพิ่มขึ้นของจำนวนใบและน้ำหนักสดอย่างมีนัยสำคัญ คลอโรฟิลด์เอของชุดการทดลองที่ได้รับ *G. mosseae* มีปริมาณมากกว่าชุดการทดลองควบคุมอย่างมีนัยสำคัญ ในขณะที่คลอโรฟิลด์บีไม่มีความแตกต่างระหว่างชุดการทดลอง ปริมาณคลอโรฟิลด์เอยังมีความเกี่ยวข้องกับการสังเคราะห์ด้วยแสงและปริมาณคาร์บอนไดออกไซด์ภายนอกเซลล์ คือการสังเคราะห์ด้วยแสงในชุดการทดลองที่ได้รับ *G. mosseae* มีค่าเพิ่มขึ้นอย่างมีนัยสำคัญ ในขณะที่ปริมาณคาร์บอนไดออกไซด์ภายนอกเซลล์มีค่าลดลง นอกจากนี้ ดินบริเวณรากที่มีการอยู่อาศัยของ *G. mosseae* มีปริมาณอินทรีย์วัตถุ ฟอสฟอรัสและโพแทสเซียมที่พืชสามารถนำไปใช้ได้สูงที่สุด แต่แคลเซียมและแมกนีเซียมในดินบริเวณรากของชุดการทดลองนี้มีปริมาณลดลง ปริมาณไนโตรเจน ฟอสฟอรัส โพแทสเซียมทั้งหมดในใบและรากบัตเตอร์เฮดที่มี *G. mosseae* เข้าอยู่อาศัยมีแนวโน้มสูงกว่าชุดการทดลองควบคุม แต่ไม่มีความแตกต่างกันทางสถิติ เอนไซม์คาตาเลสและแอสคอร์เบทเปอร์ออกซิเดสของทั้งสองชุดการทดลองไม่มีความแตกต่างกัน เช่นเดียวกับบริมาณวิตามินซีและสารประกอบฟีนอลิก แต่เป็นที่น่าสนใจว่าบัตเตอร์เฮดที่ได้รับ *G. mosseae* มีค่าความสามารถในการกำจัดอนุมูลอิสระ DPPH มากกว่าชุดการทดลองควบคุมถึงสองเท่า ดินที่มีการให้ *G. mosseae* มีการเปลี่ยนแปลงความหลากหลายทางชีวภาพและปริมาณแบคทีเรียและราบางชนิดในบริเวณดังกล่าว การมีอยู่ของ *G. mosseae* ส่งผลให้ปริมาณ Proteobacteria เพิ่มขึ้นในขณะที่ Acidobacteria มีปริมาณลดลง ซึ่งทั้งสองไฟลัมนี้เป็นไฟลัมที่มีปริมาณมากที่สุดในชุมชนแบคทีเรีย จากการศึกษาชุมชนรา พบว่าราในไฟลัม Ascomycota มีปริมาณมากที่สุด ซึ่งสกุลของราที่พบมากที่สุดในชุมชนได้แก่ *Thielavia* และ *Cochliobolus* ซึ่งการเข้าอยู่อาศัยของ *G. mosseae* ส่งผลให้มีการลดลงของราทั้งสองสกุลในปริมาณมาก ผลการทดลองทั้งหมดนี้ได้แสดงถึงประโยชน์ของการใช้ราอาร์บัสคิวลาร์ไมคอร์ไรซาในการปลูกบัตเตอร์เฮดในแปลงปลูกจริง และผลการทดลองยังสามารถนำไปสู่การศึกษาเพิ่มเติมเพื่อหาปัจจัยหรือวิธีการอื่นๆที่สามารถเพิ่มปริมาณผลผลิตที่ปลูกในระบบเกษตรอินทรีย์ได้

สาขาวิชา วิทยาศาสตร์ชีวภาพ ลายมือชื่อนิสิต .....

ปีการศึกษา 2558 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

5273899523 : MAJOR BIOLOGICAL SCIENCES
KEYWORDS: AMF / BUTTERHEAD / ORGANIC FARMING / MICROBIAL COMMUNITY

PATAI CHAROONNART: EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI ON LETTUCE *Lactuca sativa* L. 'Butterhead' AND RHIZOSPHERE MICROBES. ADVISOR: TEERADA WANGSOMBOONDEE, Ph.D., CO-ADVISOR: ASST. PROF. KANOGWAN SERAYPHEAP, Ph.D., 156 pp.

Recently, organic crop production areas are increasing due to health conscious consumers. Lettuce (*Lactuca sativa* L.) is the most important vegetable which has high demand all year round. Therefore, in order to improve quality and quantity of lettuce in organic farming system, natural microbe, arbuscular mycorrhizal fungi (AMF), were studied to select the potentially appropriate isolate and amount for increasing yield of lettuce var. 'Butterhead'. Twenty-three AMF isolates were obtained from three organic farms planted lettuce (Nakhon Ratchasima; N1-N7, Ratchaburi; R1-R12, Pathum Thani; P1-P4). Potting experiments were conducted by inoculating 20 spores of each isolate into butterhead comparing to uninoculated plant used as a control. Sixty days after planting, lettuces were harvesteded and growth was measured. The result showed that isolate N3 was the best isolate that could significantly raise yield of lettuce comparing to control treatment, followed by N2, N4, N5 and R5, respectively. These five isolates were selected and number of spore of each isolate was varied for 50, 100, and 200 spores per plant as inoculum then their effects on butterhead growth were evaluated. Growth of butterhead inoculated with isolate N2, N4 and N5 were greater corresponding with increased number of spore while isolate N3 and N5 were inversed. The best isolates that could improve butterhead growth were 50 spores of N5 and 200 spores of R5. For cost-effective use, 50 spores of isolate N5 identified as *Glomus mosseae* was chosen for applying in trial field. Growth induction of 60 days-old butterhead in field experiment was presented in *G. mosseae* treatment by significant increase leaf number and leaf fresh weight. Chlorophyll a content of *G. mosseae* inoculation showed significantly higher while chlorophyll b content of the two treatments were not difference. The result of chlorophyll a content indicated correlation with net photosynthesis and intercellular CO_2 in which net photosynthesis of 60 days-old *G. mosseae* inoculated butterhead was significantly higher than uninoculation while intercellular CO_2 was lower. Organic matters, available phosphorous and exchangeable potassium were the highest in *G. mosseae* inoculated rhizosphere soil. Calcium and magnesium in rhizosphere soil decreased after butterhead cultivation. Total nitrogen, phosphorous and potassium tended to be higher in leaf and root of butterhead but were not significant difference. Catalase and Ascorbate peroxidase were not significant difference between treatments as well as vitamin C and phenolic content. Interestingly, scavenging of DPPH free radical revealed two folds higher in *G. mosseae* treatment than uninoculated treatment. Bacterial and fungal communities in *G. mosseae* inoculated soil were changed in diversity and abundance of some specific species. Proteobacteria was found to be increased and Acidobacteria was decreased in *G. mosseae* inoculated soil. Both of them were among the highest bacterial phyla abundance. Highest relative abundance fungal phylum was Ascomycota and the highest genera were *Thielavia* and *Cochliobolus* which exhibited dramatically decrease in *G. mosseae* inoculation soil. These results presented the practical application of AMF in field and they can be used to contribute many more experiments for finding other relevance microbes and methods which can improve plant cultivation in an organic farming system.

Field of Study: Biological Sciences
Academic Year: 2015

Student's Signature .....
Advisor's Signature .....
Co-Advisor's Signature .....

ACKNOWLEDGEMENTS

First, I would like to acknowledge with pleasure to my advisor, Dr. Teerada Wangsomboondee, for her invaluable help, guidance and support not only for academic research methodologies but also for many other methodologies in life. Furthermore, my appreciative thanks to my co-advisor, Assistant Professor Dr. Kanogwan Seraypheap, for important guidance and helps on the improvement of the quality of my thesis.

I would like to express my appreciation to Assistant Professor Dr. Tosak Seelanan, Associate Professor Dr. Alisa Wangnai, Assistant Professor Dr. Boonthida Kositsup, and Associate Professor Nantana Angkinand for serving on my thesis committees, and giving valuable suggestions to complete my thesis.

I would like to give special thanks to owner of organic farms for allowing me to collect soil sample. Also, my special thanks to Mr.Anusorn Pansook and Mr.Tanet Jirapornprasert, as well as all the staff at Center of Learning Network for the Region of Chulalongkorn University, Keang Koi District, Saraburi Province, for all helps, warmly welcome, and all the supports during field experiment.

I would like to thank Chulalongkorn university Graduate scholarship to commemorate the 72nd anniversary of His Majesty King Bhumibol Adulyadej for giving me a personal financial support for my PhD study. This research was financially supported by 90th Year Chulalongkorn Scholarship and partially supported by the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission (FW656B and WCU-019-FW-57).

I would like to thank to, KS, NK, TU, PJ, PP, PD, CP, BW, PC, NK, CL and others who does not mention here, for helping me in my works. Sincere thanks are also extended to all members of Center of Excellence in Environment and Plant Physiology for lending a helping hand whenever needed, giving warm environment and being friendship throughout the period of this research, at the Department of Botany, Chulalongkorn University. An appreciation is also expressed to friends from Department of Biology, Faculty of Sciences, Chulalongkorn University.

Nevertheless, I would like to express the deepest appreciation to all teachers and staffs of Department of Botany, Chulalongkorn University for their grateful teaching, advising and also helping during my academic life. Finally, I most gratefully acknowledge my family for their infinite love, care, understanding, encouragement and support during my study and life that push me to accomplish my work.

CONTENTS

Page

Page

Page

Page

List of Figures

List of Tables

CHAPTER I

INTRODUCTION

1.1 Rationale

About 80% of terrestrial plant species were found to have ability to form symbiosis with arbuscular mycorrhizal fungi (AMF) (Quilambo, 2003). Most of them are in phylum Glomeromycota which currently contains 10 genera with approximately 200 identified species (Redecker and Raab, 2006). Arbuscular mycorrhiza (AM) are symbiotic interaction between plants and fungi which could extend hyphae into plant root and often form a unique structure, arbuscules, in plant root cells (Hata et al., 2010). AMF hyphae could also spread into rhizosphere and uptake phosphorous in both fixed form, such as Fe-phosphate or Al-phosphate, and water-insoluble form, such as rock phosphate (Satter et al., 2006). The tree-like structure of arbuscule increases surface areas between hyphae and plant cell resulting in higher nutrients exchanging between the plant and fungi. This structure also contains large amount of phosphorous which is the important nutrient that affects growth of plant (Smith and Read, 1997a). So, inoculated plant roots has higher absorption of water and some essential nutrients such as phosphorous and nitrogen than those of uninoculated plants (Govindarajulu et al., 2005; Karandashov and Bucher, 2005). AMF symbiosis increases nutrient's absorption not only nitrogen and phosphorous, but also K, Mg, Cu, Zn, Fe, Ca, Cd, Ni, and U (Quilambo, 2003). Tian et al. (2002) reported that approximate 300-400 spores of *Glomus aggregatum* could enhance growth of *Hippophae tibetana* by increasing shoot fresh and dry weight and numbers of root. Moreover, inoculated *H. tibetana* had higher dry nodule weight than uninoculated one which refered to more ability for nitrogen-fixation from atmosphere. Likewise, Piquin pepper (*Capsicum annuum* var. *aviculare*) inoculated with *G. mosseae* and *G. intraradice* showed 61% of plant height higher than control (Medina et al., 2002).

Safir et al. (1972) suggested that increase in P uptake by AMF symbiosis probably caused indirect water relation improvement in osmotic stress. For direct

alleviation, the accumulation of proline, an osmotic adjustment, and aquaporin, protein channel on plasma membrane, were higher in plants inoculated with AMF (Ruiz-Lozano, 2003). Some isolates of *Glomus* spp. could absorb and accumulate metal ions, such as Cd, Zn and Al, from environment into their extraradical mycelia. So, they were used in bioremediation of metal ion contaminated soil (Liao et al., 2003). Besides, AM had an influence on the presence of many microorganism species including pathogenic microorganisms and plant growth promoting rhizobacteria (PGPR). They could act as biological control which was known to develop resistance in plant to various pathogenic species such as *Fusarium* spp. and *Phytophthora* spp. (Azcón-Aguilar and Barea, 1997b). The increase in PGPR also played an important role in biological control in both biotic and abiotic resistance plants (Kohler et al., 2007). In addition, the secretion of a hydrophobic substance, glomalin, from AMF hyphae into soil led to more stability of soil and retention of water (Gianinazzi et al., 2010).

Recently, all countries around the world especially in Europe and North America are focusing on organic farming system and attempt to enlarge organic agricultural areas (Rigby and Cáceres, 2001). In organic farming system, all synthetic fertilizers and chemical substances including pesticide, herbicide, fungicide, and hormone are excluded. The exclusion of these substances will decrease rate of plant growth and lead to higher prices which come from limitation of culturing method and maintenance. However, recent researches suggested that the AMF biodiversity in organic farming was higher when comparing to conventional agriculture (Oehl et al., 2004). The improvement of nutrition uptake and soil quality should be done for enhancing product yield in organic farming system. According to the benefit of AM as discussed above, they are considered to be used in this system as a bio-fertilizer for improving plant growth (Gosling et al., 2006). In another way, organic farming or low input agriculture also gives an advantage to AMF inoculation because the high level of P in conventional agriculture had an adverse effect to root colonization (Ijdo et al., 2011).

One of the important dietary leafy vegetables is lettuce (*Lactuca sativa* L.) which belongs to family Asteraceae (Liu et al., 2007). Lettuce contains high amount of various nutrients and vitamins including others beneficial components such as fiber,

carotenoid, phenolic compounds, and antioxidant agents (Llorach et al., 2008). It is accepted to be 'healthier food'. Generally, lettuce is consumed fresh or raw thus the consumer can have all the nutrients and also the toxic substances. So, lettuce cultivation should be promoted to grow in organic farming system to reduce the accumulation of the toxicity in the plant. The application of AMF in lettuce were studied and proposed to use for many aspects. Lettuce plant inoculated with *Glomus* spp. was reported to increase foliar P and N content under drought stress (Ruiz-Lozano and Azcón, 1996). Under salt stress, AMF inoculated lettuce also showed higher peroxidase, catalase, proline, and total soluble sugar content that caused plant tolerance to the stress more than uninoculated one (Kohler et al., 2009). AM could also improve percentage of soil aggregation under salt stress by releasing glomalin-related soil protein (Kohler et al., 2010). However, there is no report related to the use of AMF for promoting growth of lettuce in organic farming system.

In accordance with the application of AMF as mentioned above, AMF tend to have many advantages for elevating growth including other physiological changes on lettuce under organic agriculture. We hypothesized that AMF have potential to improve soil quality and raise growth parameters of lettuce by increasing nutrients uptake. Some antioxidant enzyme activities and non-enzymatic antioxidant contents were studied at harvesting time in field experiments. Moreover, bacterial and fungal communities in the field experiment were observed. The results of this study could be developed to be commercial bio-fertilizer with cost-effective for organic farming application.

1.2 Objectives

1. To select an appropriate isolate of arbuscular mycorrhizal fungi capable of infecting lettuce root.
2. To study the effect of various amount of arbuscular mycorrhizal fungal spores on lettuce.
3. To study the quantitative changes of photosynthesis performance and antioxidant substances in lettuce inoculated with arbuscular mycorrhizal fungi.

4. To evaluate the quantitative changes of rhizosphere microbes in arbuscular mycorrhizal fungus inoculated soil.

1.3 Expecting benefits

An appropriate treatment will be applied to use in practical agriculture for supporting organic farming system and increase nutrition to the consumer. The effect of AMF on growth of butterhead will be used to decide the use of AMF for maximizing efficiency of AMF application in agriculture.

1.4 Contents of the thesis:

1. Literature review
2. The study of effect of isolates and amount of AMF spore density on growth of butterhead lettuce
    2.1 Identification of spore collected from organic farm cultivated lettuce
    2.2 Effect of AMF isolates on growth of butterhead
    2.3 Effect of spore number on growth of butterhead
3. The application of selected AMF spore amount and isolate on yield and quality of butterhead in trial field cultivated under organic farming system
    3.1 Plant growth measurement
    3.2 Photosynthesis measurement
    3.3 Soil properties and plant nutrients analysis
    3.4 Determination of antioxidant enzyme activities and contents
4. Bacterial and fungal community of arbuscular mycorrhizal fungus (*Glomus mosseae*) inoculated soil planting butterhead lettuce
    4.1 Bacterial community in rhizosphere soil of butterhead inoculated with *G. mosseae*
    4.2 Fungal community in rhizosphere soil of butterhead inoculated with *G. mosseae*
5. Discussion
6. Conclusions

CHAPTER II

LITERATURE REVIEWS

2.1 Lettuce

2.1.1 *Lactuca sativa* L. (Prohens and Nuez, 2008)

Lettuce (*Lactuca sativa* L.) is self-pollinated annual plant in family Asteraceae. It is mostly cultivated and leaves are often consumed as fresh vegetable. Some less common use of lettuce can produce nicotine which is used in cigarette production, while seed of them is used to produce edible oil. Lettuce is cool-prefer season crop but it is presently grown widely around the world including many countries in temperate zone. About 2 in 3 of total lettuce production in the world are in Asia with the largest lettuce production is China following by USA, Western Europe, India, and Japan etc. Lettuce can form a deep taproot with a lot of long lateral root. For nutrients and water adsorption convenience, roots of lettuce are generally dense near soil surface. Leaves rosette are spirally and densely arrangement on the shortened stem. Lettuce is diverse group which containing many cultivars with various in form, color, shape, leaf margin, leaf surface, and leaf texture. Leaf margin can be in many shape such as entire, lobed, indented, or undulated while leaf surface is possibly smooth, savoy, or crinkled. The range of colors can be vary from yellow to dark green with various in degree of shininess. Stem usually form single and elongation of stem is the beginning of reproductive stage. Leaves per plant can be from 7 to 35 and each floret produces single-seeded achene which are ribbed and topped with pappus hair. Freshly harvested seeds usually present short-period dormancy with different levels of thermodormancy can be found in different cultivars.

According to their diversity in appearances, they can be classified into different types such as romain, crisphead, Leaf (green or red), stem, and butterhead which have variety in nutritions, as shown in Table 1, and appearance as follow

Table 1 Nutrients content of different lettuce types, per 100 g of edible product (Prohens and Nuez, 2008).

Nutrient	Crisp head	Butter head	Red leaf	Green leaf	Romaine	Stem
Water (g)	95.6	95.6	95.6	95.1	94.6	-
Energy (kcal)	14	13	16	15	17	-
Protein (g)	0.90	1.35	1.33	1.36	1.23	0.60
Total lipid (fat) (g)	0.14	0.22	0.22	0.15	0.30	0.10
Carbohydrate (g)	2.97	2.23	2.26	2.79	3.28	1.90
Dietary fiber (g)	1.2	1.1	0.9	1.3	2.1	-
Total sugar (g)	1.76	0.94	0.48	0.78	1.19	-
Calcium (mg)	18	35	33	36	33	7
Iron (mg)	0.41	1.24	1.20	0.86	0.97	2.00
Magnesium (mg)	7	13	12	13	14	-
Phosphorus (mg)	20	33	28	29	30	31
Potassium (mg)	141	238	187	194	247	-
Sodium (mg)	10	5	25	28	8	-
Zinc (mg)	0.15	0.20	0.20	0.18	0.23	-
Copper (mg)	0.025	0.016	0.028	0.029	0.048	-
Manganese (mg)	0.125	0.179	0.203	0.250	0.155	-
Selenium	0.1	0.6	1.5	0.6	0.4	-
Vitamin A (IU*)	502	3312	7492	7405	5807	33
Vitamin B-6 (mg)	0.042	0.082	0.100	0.090	0.074	-
Vitamin C (mg)	2.8	3.7	3.7	18.0	24.0	1.0
Vitamin E (mg)	0.18	0.18	0.15	0.29	0.13	-

Nutrient	Crisp head	Butter head	Red leaf	Green leaf	Romaine	Stem
γ-Tocopheral (mg)	0.09	0.27	0.24	0.37	0.36	-
Vitamin K (µg)	24.1	102.3	140.3	173.6	102.5	-
Folate (µg)	29	73	36	38	136	-
Lutein+zeaxanthin (µg)	277	1223	1724	1730	2312	-
Niacin (mg)	0.123	0.357	0.321	0.375	0.313	0.500
Pantothenic acid (mg)	0.091	0.150	0.144	0.134	0.142	-
Riboflavin (mg)	0.025	0.062	0.077	0.080	0.067	0.020
Thiamine (mg)	0.041	0.057	0.064	0.070	0.072	0.030

*IU, International Unit.

Romaine: or in common name “Cos lettuce” has elongated, coarse, and crispy texture leaves. Head tends to have upright stature and form loaf-shape after rosette stage. Top of the head can be either close or open. Usually, outer leaves are light to dark green with yellowish ornament. Its taste is strongly sweeter than other lettuce types. Some cultivars in this group may present red leaves.

Crisphead: or in common name “Iceberg” produces spherical firm head. Rossette stage exhibits elongation of early leaves and continuously increases in width. The mature leaves have width wider than length. Leaves have changed to be cup-shape for forming a head structure when number of leaf is up to 10-12 leaves. New leaves grow from inside and fill up the head to become larger and firmer head. Outer leaves have brighter color with light green to creamy yellow ornament.

Leaf: lettuce group has been considered variation in size, shape, margin, texture, and color of leaf. Leaves rosette shape in this group can be broad, elongated, or lob. Colors can be varying from various shade of yellow, green or

red. Texture can be in range of soft leaf to crispy leaf. Usually, leaf lettuce form loose head when they reach mature stage.

Stem: or can be called celtuce, atalk, or asparagus lettuce. They are grown mainly for erecting thickened stem (4-10 cm in diameter and 50-60 cm in length). Their leaves usually long and narrow and mature leaves produce high latex content and have bittersweet in taste, so only young foliage is harvested.

Butterhead: is small and loose head form with broad, crumpled, thin, and tender leaf. It is commonly cultivated in winter under shelter and prefers partial sun with often watering. The taste of butterhead is sweet flavor and leaf contains high amount of vitamin A and K when comparing to others plant in lettuce group. Moreover, a hundred gram of butterhead contains a gram of iron. Butterhead is in the top three among all lettuce cultivar which most cultivated around the world.

2.1.2 Butterhead lettuce cultivation

Usually, butterhead seed takes 6 days for germination with 55-60 days for cultivation. Nowadays, there are 2 popular and commercial methods for cultivated butterhead which are conventional and hydroponic cultivation. Plant production in hydroponic system is very popular according to the success in quality and quantity of lettuce production to market demand. Butterhead are favor to be grown with hydroponic technique due to the system is suitable with loosely head form lettuce. In this system, plants are grown in their most appropriated nutrient solution recipes circulating in container such as pipe or tray, so plants can get higher nutrients and water retention capacity. Thus, weed management is not needed to perform while the system need to cover material for pest management (Lages Barbosa et al., 2015). Besides, there is no need to be concerned on soil-borne disease incidence. However, there are

many disadvantages of performing lettuce cultivation in this system. First, it is cost-production method according to chemicals and materials used and grower needs knowledge and experience as well as technical skill. Second, the nutrients solution used lacks of soil buffering capacity, then pH of solution as well as O_2 were continuously changed along the cultivation period. Even though plant cultivated in hydroponic system can alleviate soil-borne diseases, but there are also some water mold such as *Pythium* or *Phytophthora* that can damage hydroponic crop which need specific fungicide to control these pathogens (Zinnen, 1988). Ultimately, hydroponic production is not considered as organic farming production.

Conventional agriculture is soil-based cultivation and can be adjusted to suit organic farming system. Lettuce is best grown at temperature 7-18^{o}C. Soil texture should be sandy-loam with well-supplied organic matter. The optimum soil pH for butterhead cultivation is low acidic in the range of pH 6.2-6.8. Seeds should be placed at 3-5 cm soil depth. Each plant should have 15-20 cm space for leafy lettuce. In hot weather area, the cover material for filtering sunshine should be placed and indoor seedling preparation is needed due to the germination is difficult in hot weather (Lu, 2000). In comparing with hydroponic system, conventional agriculture needs larger area for performing plant cultivation with a lot of laborious grower. Likewise, fertilizers, pesticide, and fungicide are needed to be used for improving crop yield (Lages Barbosa et al., 2015). However, lettuce in particularly soft leaf plant such as butterhead is a delicate vegetable so it can easily absorb any chemical. Chemical residue in lettuce is a major concerned by consumers because lettuce mostly consumed as fresh. Therefore, lettuce are best cultivated in the conventional agriculture system which have less input of chemicals like organic farming system.

2.1.3 Organic farming system

According to definition from Organic Agriculture Certification Thailand (ACT) certified by International Federation of Organic Agriculture Movement (IFOAM), organic agriculture is a farming system in which the use of artificial fertilizer and synthetic pesticides and in compliance indicated in ACT standard are prohibited (ACT organic agriculture standard, 2011). Others prohibited input are such as hormones and GMOs seed. For any additive aids, all ingredients are needed one step traced back in the process of production whether they must not derive from genetic engineering in both direct and indirect way. The example of additive aids allowed in organic farming for improving soil and facilitating plant growth is shown in Table 2. Land for organic farming must be separated and converted from chemical farming at least 12 months and the conversion back and forth between these 2 types are prohibited. Primary ecosystem must not be cleared and it needs to be maintained and enhanced for conserving at least 5% of the field to be habitats for other plants and animals.

Table 2 The example of input fertilizers and soil conditioners approved for using in organic farming (ACT organic agriculture standard, 2011).

Input		Details/Restriction
Molasses	✓	Use in composting green fertilizer as food for microorganisms
Vegetable seed cake	!	Organic waste from oil extraction such as peanut, soybean, neem, custard seed. The seed cake is rich with nitrogen. Best used after composting. Direct soil application could harm crops.
Rice husks	!	It can be used to improve clay soil but should be mixed with other materials because it is low in

Input		Details/Restriction
		nutrients, has poor water retention, and is slow to decompose.
Industrial waste	!	The source is needed to be reported and shall not come from chemical industrial. Analysis to confirm no contamination with chemicals/prohibited substances should be available.
Saw dust	!	Saw dust from treated wood is not allowed. Mix with soil to improve aeration and water retention but slow to decompose. Should be composed before using.
Microorganisms	✓	Allow to use with compost, plants, seeds and soil. Except from GMOs
Bio-fertilizer	✓	Organic Matter rich in micro-organisms that help to breakdown nutrients and make them available to plants.
Micro nutrients	!	Synthetic nutrients such as copper, cobalt, sulphate, selenium, boron, manganese, molybdenum, zinc, iron, iodine. Allow to use when necessary or when there is a clear sign of lack. Nitrate and chloride form are not permitted.
Nitrogen fertilizer	!	Use only those from natural sources, e.g. blood meal, blue-green algae, vegetable meals, and neem cake, green manure, and chicken manure.
Green Manure	✓	Such as sesbania, sun hemp, legume crop
Potassium fertilizer	!	Use only those from natural sources, e.g wood ash, and rock dust.
Phosphorous fertilizer	!	Use only those from natural sources, e.g bone meal, dried sea weed, chicken manure, bat manure, wood ash, and vegetable seed meal.

Input		Details/Restriction
Animal manure	!	• Application of non-aging manure is prohibited, except when the manure is treated with heat and should be in dry condition. Do not expose manure to sun light as nitrogen is lost through volatilization. Store in shade and put dried leaf or straw underneath. Add rock phosphate can increase nutrients.
Compost	✓	Compost help to kill weed seeds in animal manure. Allow only compost with ingredients of organic materials listed as approved. *Urban waste compost is prohibited.*
Crop Rotation	✓	Rotations should use different crop types to a balanced nutrient uptake. Crop with low to high nutrient uptake is as follow: (1) legumes, (2) root, (3) leaf, (4) fruit and (5) cereal.
Straw and natural mulching materials	✓	Use of natural materials such as rice straw, dried leaves and grass. Straw from conventional farm may be used.
Rhizobium	✓	Mixed with legume seed before planting. Dissolve in a 30% sugar solution of water or water with milk powder and soak with the seed before planting.
Soil improvement materials	✓	• Natural materials used to improve physical, chemical, or biological characteristics of the soil. Includes rice husk and straw, corn stalks, sawdust, and bean pods etc.
Compost bacteria	✓	Except GMOs
Plant and vegetable residue	✓	Use for composting

✓ is representing allowing to use and ! is representing used with caution.

These organic farming rules are aims for many advantages. The system contributes to development of plant production for integrating farming in order to maintain ecosystem and diversity of all organisms. Species richness of all organisms including birds and insects is usually 30% higher in organic farming than conventional farming. Also, the abundance of most organisms in organic farm presented 50% higher than conventional farm (Bengtsson et al., 2005). However, the abundance of weed plants is increased as well because herbicides were not used. Organic material in soil can be enhanced by organic farming practice resulting in the increase of soil animals such as earthworm and other general soil faunas found in agriculture (Andrén and Lagerlöf, 1983; Zwart et al., 1994). According to the prohibition of many chemical inputs, this practice produces good quality of crop plant which does not contain any pollution which has an impact on both consumer and environment. The system can be considered as sustainable agriculture due to the maintaining of ecology, plant production and environment (Rigby and Cáceres, 2001).

Not only biological factors which are facilitated with organic farming practice, but also soil fertility which are improved as well (Maeder et al., 2002). The study of Maeder et al. suggested 10-60% increase of soil aggregate stability in an organic plots and this increase has been reported the significant correlation with microbial and earthworm biomass. Consequently, soil enzyme activities, dehydrogenase, protease, and phosphatase, were found to be higher than conventional system which can be indicated that the higher of microbial was active and had capacity to digest some proteins and organic phosphorous (Oehl et al., 2001). Long-term organic farming has been reported to show higher organic matter without adding any additive when comparing with conventional method. The higher organic matter led to granulation encouraging, water storage enhancement, nutrients supplement enhancement, and microbial activity induction resulting in long-term soil fertility improvement (Reganold et

al., 1987). The comparison of nutritional level between conventional and organic farming practice presented higher in various types of antioxidant substances of many leafy vegetables such as Chinese mustard, Chinese kale, lettuce, and spinach. Vitamin C particularly in lettuce had higher level in organic farm than conventional farm while riboflavin could be observed only in Chinese kale and spinach planted in organic farm (Ismail and Cheah, 2003).

2.2 Arbuscular Mycorrhizal Fungi (AMF)

2.2.1 Arbuscular mycorrhizal fungi symbiosis

Mycorrhiza means a type of mutualistic symbiosis between soil fungi and root of plant without having pathogenic impact (Quilambo, 2003). Mycorrhiza can be mainly divided in 2 major groups which are ectomycorrhiza and endomycorrhiza. Over 5,000 species of ectomycorrhiza have been identified and they are mostly in phylum Basidiomycota and little are in Ascomycota and Zygomycota. Ectomycorrhiza can form a multi-layer hyphal structure cover root tip called mantle sheath (Taylor and Alexander, 2005). Its hyphae can penetrate between cells in root cortex and form a complex structure called Hartig net. This structure allows exchange of many metabolites between plant and fungi. The major plants that can be formed symbiosis with ectomycorrhizal fungi are woody perennials (Fitter and Moyersoen, 1996). Meanwhile, endomycorrhizal fungi can form symbiosis with about 80% of terrestrial plant (Smith and Read, 1997a).

Endomycorrhiza or arbuscular mycorrhizal fungi (AMF) is a group of fungi in phylum Glomeromycota (Schüβler et al., 2001). They are obligately symbiosis and asexual organisms. Spore of AMF is varying in size (50-500 μm) but generally larger than spore of other fungi. AMF spore can be found in soil and their hyphae do not have septum. AMF can germinate their hyphae under specific preferable condition and penetrate into root cortex. Unlike

ectomycorrhizal fungi, AMF hyphae form a unique tree-like structure inside root cells called arbuscule. Besides, Vesicle forming in root cortex is another unique character of symbiosis between plant and AMF which can be found in all of AMF genus except Gigasporaceae. The comparison of symbiosis character between ectomycorrhizal fungi and endomycorrhizal fungi is shown in Figure 1.

After spore germination, hyphae grow through soil to find their host. In case of there is no host, AMF spore get in resting and hyphae are retracted back to spore (Logi et al., 1998). This situation has not been found much due to AMF can symbiosis with very wide host-range. Growing hyphae is in direction to chemical secreted from plant root called root exudate which has been characterized as strigolactones (Besserer et al., 2006). This strigolactones promote respiration of hyphae and consequently increases branching and stimulate their growth pattern of hyphae to encounter with host root (Buee et al., 2000). These steps occur before AMF hyphae adhere to host cell and call presymbiotic phase. The chemical secreting from AMF is also perceived by plant and calcium spiking in root was stimulated (Navazio et al., 2007). The trigger signal leads to broad aggregation of cytoplasm of root cells and turns trigger cell to be prepenetration apparatus (PPA) which is tunnel-like shape (Nagahashi and Douds Jr, 1997). The first step of symbiosis between plant and fungi is adhesion of specialize hypha, hyphopodium, to root surface. Then, root colonization is emerged by root penetration strictly follows PPA route from epidermis to inner cortex and forming intracellular structure (Genre et al., 2005). Hyphae grow along root axis continuously trigged cells and PPA mechanism are then repeated along to growing hyphae. Eventually, plant is colonized with AMF nearly all cell volume.

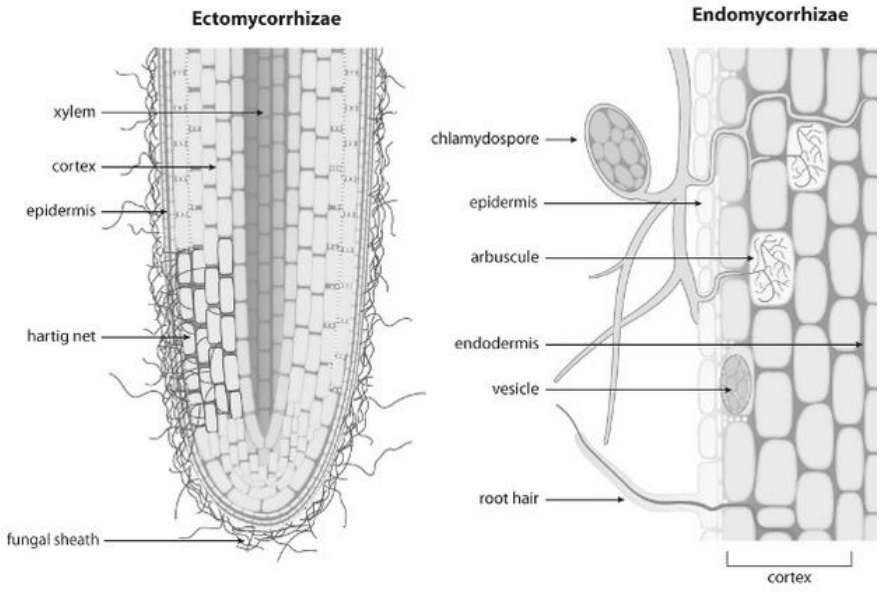

Figure 1 Symbiosis characters of ectomycorrhizal fungi (left) and endomycorrhizal fungi (right) (McNear Jr, 2013).

AMF are unable to complete their life cycle without having symbiosis with root of host plant as described above. In the symbiotic phase, AMF need carbon source from host plant via hexose transporter of fungi. Meanwhile, AMF facilitate nutrients uptake to plant by absorbing inorganic phosphate, ammonium, nitrate, and amino acid through selective channel on fungal membrane and translocate to plant (Figure 2) (Bonfante and Genre, 2010; Parniske, 2008). Before translocation, the inorganic phosphate is hydrolyzed by phosphatase secreted from AMF, thus AMF are known to be a key microbe that can increase plant growth from this significant symbiosis character.

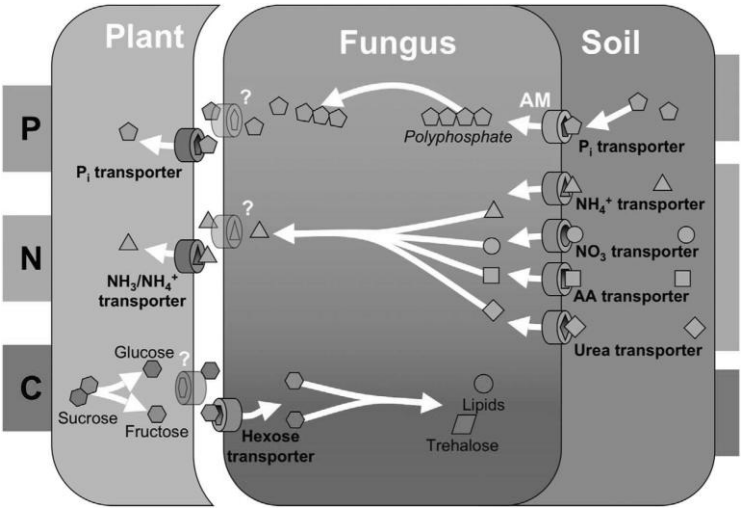

Figure 2 Scheme of nutrients translocation from soil to AMF and between AMF and host plant (P: phosphorous, N: nitrogen, C: carbon) (Bonfante and Genre, 2010).

Therefore, AMF were introduced to compensate in organic farming practice according to prohibited synthetic chemicals usages. This was natural and biological processes to take place instead of chemical function (Galvez et al., 2001). In organic farm with no additive chemical, the diversity and abundance of AMF were obviously greater than conventional farming (Galvez et al., 2001). Colonization percentage of AMF colonizing root was higher in organic farm (Bending et al., 2004). Generally, AMF play an important role by increase phosphorous in organic farm which is low input agriculture. Kahiluoto and Vestberg (1998) suggested applying AMF in organically cultivated soil could provide available phosphorous more than adding superphosphate in conventionally cultivation.

2.2.2 Application of AMF in agriculture

Increasing nutrients uptake

AMF are soil-borne microorganism that can provide significant advantages in agricultural performing especially soil nutrients. Usually, the rate

of phosphorous uptake of plant root is higher than diffusion in the soil, so phosphate depletion zone generally occur around plant root (Bucher, 2007). The symbiosis of AMF in plant root has great advantage with this problem according to their extraradical hyphae elongate beyond the depletion zone and absorb phosphate as well as other nutrients such as nitrogen, and finally transfer through hyphae into intraradical hyphae and arbuscule (Govindarajulu et al., 2005). Particularly in legume plant, high phosphorous is required for nodulation and N_2 fixation process. The symbiosis of AMF can improve phosphorous in soil and leads to increase early growth and nutrients uptake (Dodd, 2000). The status of phosphorous can also be controlling factor in the plant-AMF symbiosis and finally leads to growth and yield induction (Koide, 2000).

Nitrogen is another important nutrient which can be absorbed by AMF in many forms such as ammonium (NH_{4+}) and its transport to plant is resulted in higher biomass of maize production with low level of potassium, calcium, and magnesium in soil (Liu et al., 2002). However, many reports suggested high input of fertilizer, especially phosphorous and nitrogen, exhibited negative effects on AMF symbiosis by suppressing root colonization level and decreasing nutrients uptake ability of AMF. Micronutrients uptake such as Zn, Cu, and Fe were also reported to show an enhancement in AMF symbiosis plant (Smith and Read, 1997a).

Environmental stress alleviation

Osmotic stress, salt and drought stress, has been reported that can be overcome by many AMF symbiosis mechanisms (Smith and Read, 1997a). Many studies presented the enhancement of AMF in root hydraulic conductivity, stomatal regulation, and recovery of hosts osmotic adjustment. Soil particles were also aggregated by AMF hyphae which allowing water extraction from

smaller soil pores (Augé, 2004). However, the level of osmotic stress was major factor of stress alleviation efficiency by AMF in which they were capable to ameliorate plant from only moderate stress level (Ruiz-Lozano and Azcón, 1995). Ruiz-Sánchez et al. (2010) presented higher accumulation of proline content which is osmoprotectant compound in plant root symbiosis with AMF. Moreover, glutathione which is one of the antioxidant substance was also enhanced by AMF and thereby relieved damage from reactive oxygen species occurred from osmotic stress.

Increase resistance to root pathogens

Many evidences suggested the role of AMF in suppression soil-borne fungal diseases without using synthetic chemicals (Borowicz, 2001). The examples of plant pathogens reduced by AMF are white rot disease in onions caused by *Sclerotium cepivorum*, Fusarium root rot disease in asparagus caused by *Fusarium oxysporum*, and root and stem rot disease in mung bean caused by *Rhizoctonia solani* (Dar et al., 1997; Kjøller and Rosendahl, 1998; Torres-Barragán et al., 1996). The effect of AMF on disease reduction was mainly on decrease severity more than microbial inhibition. To maximize the efficiency of AMF in protecting crop plant, the colonization of AMF needed to be taken place before pathogen attack. The colonization of AMF could induce plant resistance according to the defense mechanisms were up-regulated by the infection of AMF prior pathogenic fungi infection (Whipps, 2004).

Other organisms which are causing agents of diseases and plant damage such as pathogenic nematodes, above ground fungal diseases, and herbivores were also suppressed by AMF colonization (Gosling et al., 2006). The mechanisms are remained unclear but it probably involves with defensive chemical changing in leaf due to the nutritional altered by AMF. The level of disease control is found to be varied among AMF species probably resulting

from the specificity between plant, AMF, and disease which so far is still unpredictable (Vicari et al., 2002).

Increasing soil fertility

Soil fertility usually refers to soil quality and active microbial. AMF can turn small soil particle into larger particle through binding them with AMF hyphae (Tisdall et al., 1997). AMF have been reported in many researches for their secretion of extracellular glycoprotein called glomalin which act as a glue for soil compact (Bethlenfalvay et al., 1999). Besides, there was evidence suggested that exudation from hyphae provided carbon source to soil microorganisms which indirectly increased soil aggregation stability (Jastrow et al., 1998). However, Piotrowski et al. (2004) revealed that the positive or negative results of this phenomenon were depending on the combination of host and AMF species.

Not only plant pathogens are affected by AMF, but also other soil microorganisms as well. Once AMF symbiosis occurred, their hyphae have impact the development of bacterial community (Andrade et al., 1997). The alteration of beneficial bacteria such as N-fixing bacteria and plant growth promoting rhizobacteria (PGPR) was reported (Arias et al., 1991). One of the important strongly impacted microbe from occurrence of AMF was rhizobium symbiosis with legume consequently increase nodules of root legumes (Scheublin et al., 2004).

2.1.3 Previous study of AMF on lettuce

Most AMF are known to be able to have symbiosis with lettuce and are used for many agriculture purposes. Eighteen farms cultivated lettuce in different varieties/cultivars and different cultivation method in California revealed that the symbiosis could be found in all studied area (Miller and Jackson, 1998). The results also suggested that soil management including

nutrients improvement, increase number of plant host species, higher soil pH, and finer soil texture presented positive correlation with AMF colonization ability in lettuce root but did not involve with spore number found in soil. The applications of AMF in lettuce crop improvement are studied in several aspects. According to AMF major benefits, nutrients acquisition enhancement are mostly studied due to lettuce cultivation high water and nutrients demand (Jackson, 1995). At low phosphorous level in soil, AMF increased all nutrients uptake including micronutrients such as Cu, Fe, and Zn at all tested nitrogen level. In contrast, increasing phosphorous level together with nitrogen exhibited reverse effect (Azcón et al., 2003). Jackson et al. (2002) found that the presence of *Glomus intraradices* without addition of phosphorous had the highest shoot and root biomass comparing with combination of fungi and fertilizer. The increasing in micronutrients uptake resulting from AMF colonization in lettuce was also reported in (San Martín et al., 2014). Cu, Zn and Fe as well as total soluble solid, proline, and starch were induced in lettuce colonizing with the mixture of *Rhizophagus intraradices* and *Funneliformis mosseae*.

There are many reports presented the use of AMF in alleviating lettuce crop from environmental stresses, both caused from abiotic and biotic factors. Water uptake efficiency of individual six *Glomus* sp. colonizing lettuce were studied and found that all species could increase relative water uptake under drought stress but the level was different (Marulanda et al., 2003). *G. intraradices* and *G. mosseae* showed potentiality in ameliorating lettuce growth ability or damage under drought stress by improving soil aggregation stability which resulted from increase of glomalin-related soil protein and increase total soluble carbohydrate which act as osmoprotectant chemical accumulated in cell for reducing damage from osmotic stress (Kohler et al., 2009). Salt stress was characterized as a negative effect on strigolactones, AMF germination stimulant secreted from plant root, but lettuce colonizing with *G. intraradices* could increase this stimulant five-folds comparing to uninoculation lettuce at high salt concentration (Aroca et al., 2013). This research also

presented effect of *G. intraradices* on photosynthesis performance in both normal and stress conditions. Photosystem II efficiency and stomatal conductance of *G. intraradices* colonized lettuce were higher than non-mycorrhizal treatment under all level of salinity and normal conditions. The increase of these two parameters correlated with lower ABA level in *G. intraradices* colonized lettuce.

AMF were reported to overcome yield reduction of plants in inappropriate growing season as they can affected growth induction particularly in winter by inducing nutrients uptake such as N, P and Fe (Baslam et al., 2013). Change of nutrition, in term of antioxidant substances, in AMF inoculating lettuce resulted in induction of total carotenoid content in both inner and outer leaves of all studied local cultivar in Spain as well as total phenolic, ascorbic acid, and anthocyanin content (Baslam et al., 2011). The induction of antioxidant enzymes, ascorbate peroxidase and guaiacol peroxidase, in both root and shoot were reported in rescue lettuce from Zn contamination (Farshian et al., 2007). Moreover, growth of *G. mosseae* inoculation lettuce was greater than non-inoculation under soil contaminating with phytotoxin from olives mill residues (Martın et al., 2002).

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant materials

3.1.1.2 Sorghum

Sorghum (*Sorghum biocolor* L.) seeds were kindly provided by National Corn and Sorghum Research Center, Kasetsart University. Seeds were kept at 4°C in the refrigerator for long time storage.

3.1.1.3 Butterhead

Butterhead (*Lactuca sativa* L.) seeds used in this study were purchased from ACK Hydrofarm Co.Ltd. Bangkok, Thailand. The seeds was used within 3 months after purchased.

3.1.2 Equipment

3.1.2.1 Equipment for working with mycorrhiza

Soil collection

Shovel

Polyethylene bag

Spore separation

Balance

75, 150, 180, and 850 μm Sieve

Plastic beaker

Wash bottle

50 ml centrifuge tube

Whatman filter paper no. 4

90 mm petri dish

Suction flask

Buchner funnel

Vacuum machine

Refrigerated centrifuge: Universal 2R (Hettich, Germany)

Spore propagation

Stereo microscope

Black 8 inches plastic pot

Autoclave: TC-459 (Taichung, Taiwan)

Commercial soil (Sida)

Erlenmeyer flask

Cylinder

Root staining

50 ml centrifuge tube

6-wells plate

5 ml Pipette (Gilson, France) and 5 ml pipette tip

Morphological identification

Needle

Forcep

Glass slide and cover slip

Olympus BH-2 microscope (Olympus America Inc., USA)

Olympus DP70 camera (Olympus America Inc., USA)

3.1.2.2 Equipment for plant cultivation

Pot experiment

Balance

Black 8 inches plastic pot

Shovel

Commercial soil (Sida)

Coconut fiber

Manure

Watering can

Field experiement

Balance

Shovel

Hoe

Coconut fiber

Watering can

3.1.2.3 Equipment for sample collection

Growth parameter measurement

Balance (2-3 digits)

Balance (4 digits)

Shovel

Scissor

Measuring tape

Ruler

Newspaper

Tissue paper

Soil sampling

Shovel

Zip bag

Deep freezer

Plant tissue sampling

Balance

Aluminum foil

Scissor

-20^{o}C deep freezer

3.1.2.4 Equipment for photosynthetic performance study

Chlorophyll content determination

Mortars and pestles

Spatula

15 ml centrifuge tube

5 ml Pipette (Gilson, France) and 5 ml pipette tip

Spectrophotometer: 8453E UV-Vis (Agilent Technologies Inc., Germany) and cuvette

Photosynthesis measurement

Portable Photosynthesis System: LI-6400XT (LI-COR, USA)

3.1.2.5 Equipment for antioxidant substances analysis

Enzymatic and Non-enzymatic antioxidant assay

Mortars and pestles

Spatula

Micro pipettes; Pipetman (Gilson, France)

1.5 ml microcentrifuge tube

Ice bucket

pH meter (Denver Instrument Company, USA)

Refrigerated centrifuge: Universal 2R (Hettich, Germany)

Spectrophotometer: 8453E UV-Vis (Agilent Technologies Inc., Germany) and cuvette

50 ml centrifuge tube

Water bath

Vortex mixer

3.1.2.6 Equipment for rhizosphere microbes changes analysis

Balance

PowerSoil DNA isolation kit (MoBio, USA)

Micro pipettes; Pipetman (Gilson, France)

Bead beater machine

1.5 ml microcentrifuge tube

Refrigerated centrifuge: Universal 2R (Hettich, Germany)

Vortex mixer

Water bath

Refrigerator

Spectrophotometer: 8453E UV-Vis (Agilent Technologies Inc., Germany) and cuvette

Gel electrophoresis system

Gel Doc™ 2000 and UV transilluminator (Bio-Rad, USA)

3.1.3 Chemicals

3.1.3.1 Chemical for working with mycorrhiza

Spore separation

50% Sucrose

Spore propagation

5% Chlorox

70% Ethanol

Autoclaved distilled water

Root staining

Staining solution (Preparation please see Appendix A)

5% Hydrochloric acid

10% Potassium hydroxide

Morphological identification

PVLG (Preparation please see Appendix A)

Melzer's reagent (Preparation please see Appendix A)

3.1.3.2 Chemical for sample collection

Liquid nitrogen

3.1.3.3 Chemical for photosynthetic performance study

CO_2 cylinders (LI-COR, USA)

Anhydrous calcium sulfate or Drierite desiccant (W.A. Hammond Drierite Co., Ltd., USA)

80% acetone

3.1.3.4 Chemical for antioxidant substances analysis

Enzymatic antioxidant assay

Extraction buffer (Preparation please see Appendix A)

0.1 M potassium phosphate buffer pH 7.0 (Preparation please see Appendix A)

100 mM hydrogen peroxide in 50 mM potassium phosphate buffer pH 7.0

500 mM Ethylenediaminetetraacetic acid (EDTA)

10 mM ascorbate

2 mg/ml bovine serum albumin

Autoclaved distilled water

BioRad protein assay reagent B (BioRad, CA)

Non-enzymatic antioxidant determination

6% metaphosphoric acid (Preparation please see Appendix A)

2M acetic acid

2% 2,6-dichlorophenolindolphenol (DCIP)

2% thiourea (Preparation please see Appendix A)

2% dinitrophenylhydrazine (DNPH) (Preparation please see Appendix A)

90% sulfuric acid

1 mg/ml ascorbate

80% methanol

0.2 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH)

Autoclaved distilled water

Folin Ciocalteu reagent

2% di-sodium carbonate

2 mg/ml gallic acid

80% acetone

3.1.3.5 Chemical for rhizosphere microbes changes analysis

Agarose

5x tris borate EDTA (TBE) buffer (Preparation please see Appendix A)

Ethidium bromide (Gibco BRI, USA)

DNA loading dye (Preparation please see Appendix A)

1 kB DNA ladder (Fermentus, xx)

3.2 Methods

3.2.1 AMF spore inoculum preparation

3.2.1.1 Soil collection and AMF spore separation

Approximately 30 cm depth of rhizosphere soil were collected from 3 organically cultivated lettuce (*Lactuca sativa* L.) farms. The collected soil samples were kept in plastic bags at room temperature until analysis (Brundrett et al., 1996). A hundred gram of collected soil, three sets from each location, was suspended in 1L tap water for at least 30 minutes. Then, soil suspension was decanted through a series of sieves (750, 180, 150, and 75 µm, respectively). The fractions from 150 and 75 µm sieves were collected. Soil fractions were centrifuged and the obtained pellet was resuspended with 50% (w/v) sucrose solution. Then, soil mix was centrifuged again and supernatant was collected. The supernatant was poured on 75 µm sieve then washed for removing sucrose and filtrated through whatman paper in a Buchner funnel before vacuum infiltration. Spores were kept on whatman paper in pertridish at 4°C until used.

3.2.1.2 AMF spore propagation

Obtained spores were separated into groups according to shape, size, and color and the representative healthy spores were selected for propagation. Individual spore was placed at the point sorghum seeds were germinated. Seeds of sorghum (*Sorghum bicolor* L.) were

cleaned with 5% Sodium hypochlorite followed by 70% ethanol before used. They were soaked with autoclaved water for a night before germinating in pot containing autoclaved soil. Sorghum was watered once a day and cultivated for 3 months. Pure inoculum was continuing used for mixing with autoclaved soil cultivated sorghum to produce large amount of spores for using in the downstream experiments.

3.2.1.3 AMF spore identification

The spores were identified to family using a stereomicroscope based on color, size, ornament, spore shape, shape of subtending hypha, and spore wall thickness. Spore identification to family was performed following the International Culture Collection of Arbuscular Mycorrhiza and Vesicular-Arbuscular Endomycorrhizal fungi (INVAM, www.invam.caf.wvu.edu). The significant characters for identifying to family were described in table 3.

For identifying selected AMF spore isolate to species, spores of that isolate were picked from filtered paper and put on glass slide. Then, they were stained with PVLG solution for intact spore observation and Melzer's reagent for crushing spore morphology.

Table 3 Main characters of arbuscular mycorrhizal fungi for classifying into family

Family	Spore characters
1. Glomeraceae	Spore wall arranges simply with thick wall. Inner wall do not react with Melzer's reagent. At least one layer of spore wall is continuous with wall of subtending hypha. Arbuscule and vesicle can be found in colonized root.
2. Acaulosporaceae	Wall structure was more complex. Inner wall presents many layers and may react with Melzer's

Family	Spore characters
	reagent. Outer wall appears ornament. Spore contents are partitioned from subtending hypha. Subtending hypha presents sporiferous saccule. Germination shield may be observed.
3. Gigasporaceae	Spore size is large (usually more than 200 µm). Subtending hypha is bulbous shape. Spore contents are partitioned from subtending hypha. Outer wall layer permanently enclose inner layers. No vesicle formed in colonized root.

3.2.2 To study the effect of isolates and amounts of AMF spores on growth of butterhead

3.2.2.1 To study effects of AMF isolates on growth of butterhead

Experimental design

The experiment was conducted outdoor from August to October 2012 in Bangkok, Thailand. Average temperature and rainfall were 28.3-29.0°C and 401 mm·month^{-1}, respectively. Seeds of butterhead were individually germinated in 8 inches diameter plastic pots containing a commercial soil mixture with 20 spores of each obtained AMF isolate comparing to control treatment which was uninoculated plants. They were cultivated for 60 days and watered twice daily. A completely randomized design (CRD) was used with 6 replications per treatment.

Growth parameters measurement

At harvesting time, plant diameter was measured and leaf number was counted. Leaves and roots were separated for further analysis. Fresh and dry weights of leaves were determined. Width and length of the largest leaf, 3rd or 4th from outside, were recorded. Percent colonization was determined according to the method of

Phillip and Hayman (1970) (Appendix B). Roots were cut and cleared by autoclaving with 10% KOH. Cleared roots were washed with tap water, neutralized with 5% HCl, and stained in 0.05% tryphan blue. Stained roots were counted for percentage of root colonization under stereoscopic microscope by gridline-intersect method (Giovannetti and Mosse, 1980) (Appendix B).

Statistical analysis

Data were analyzed by ANOVA using SPSS (ver. 20.0, Chicago, IL) at 95% confidence. Means were compared with Least Significance Difference test. Five of the isolates which have significantly potential to increase butterhead yield were chosen for varying spore number in the following experiment.

3.2.2.2 To study effects of numbers of AMF spores on growth of butterhead

Experimental design

The experiment was conducted outdoor in March to May 2013 in Bangkok, Thailand. Average temperature and rainfall were 31.0-31.3°C and 96.26 mm·month^{-1}, respectively. Butterhead seed were germinated in individual pots amended with cattle manure at the rate 10:1 (soil:manure) by weight. Spore from selected isolates were counted and spore inoculum at 50, 100, or 200 spores for a plant were used as treatments. Uninoculated pots were amended with cattle manure at the same ratio and were used as the control. A completely randomized design (CRD) was conducted with 12 replications per treatment. Plants were watered twice daily and cultivated for 60 days.

Growth parameters measurement

At harvesting time, growth parameters were measured followed 3.2.2.1. Percent colonization was also determined by the same method.

Chlorophyll content determination

Photosynthetic pigment, chlorophyll a and b, were determined using the biggest leaf (3rd or 4th leaf from outside). A gram of fresh leaf was extracted with 80% acetone and kept in dark place for 24 hours. Then, the absorbances of supernatant were measured using spectrophotometer at 470, 646.8, and 663.2 nm and calculated followed the method of Porra (2002). The equations for calculating chlorophyll a and b were shown as follow

Chlorophyll a (µg/ml) = 12.25 $A^{663.2}$ - 2.55 $A^{646.8}$

Chlorophyll b (µg/ml) = 20.31 $A^{646.8}$ – 4.91 $A^{663.2}$

Statistical analysis

SPSS (ver. 20.0, Chicago. IL) was used for data analysis. Raw data were analyzed by ANOVA at 95% confidence. Means were compared with Least Significance Difference test. The interaction between spore isolate and number of spore was analyzed by Univariate General Linear Model. The correlations of colonization percentage and each growth parameters were calculated with Pearson's correlation coefficient. The best isolate and amount of AMF was chosen for studying in the field experiment.

3.2.3 To study the application of selected AMF spore amount and isolate on yield and quality of butterhead in natural field cultivated under organic farming system

3.2.3.1 Field preparation and Experimental design

Field preparation

Land used in this study was located at Center of Learning Network for the Region of Chulalongkorn University, Keang Koi District, Saraburi Province, Thailand. The land was abandoned for more than 10 years with no agricultural performance. Plowing was carried out before starting the experiment. The plots were set up 0.3 m from ground with 1 m in width and 8 m in length. There was 0.5 m space between each

plot. After setting up the plots, coconut fiber at the rate 5 kg/plot was mixed throughout each plot and the plots were left they stand for 2 days before transplantation.

Experimental design

There were total 8 plots in this experiment. The experiment consisted of two treatments which were uninoculated butterhead as a control (no-AMF) and butterhead inoculated with the best treatment from 3.2.2.2. A completely randomized design (CRD) was performed. There were 4 plots in each treatment with randomly ordered. Butterhead seeds were germinated in seeding tray for two weeks before transplanting into each plot containing two rows of plant with 0.35 m spacing between each plant. At transplantation time, soil inoculum containing the best amount and isolate of AMF from 3.2.2.2 was placed and mixed roughly with soil at the point seedling were transplanted. There was no any fertilizer amended in soil for the entire experiment. Plants were watered twice daily and continued cultivated for 60 days.

3.2.3.2 Growth parameter measurement

Growth at each sampling time

Four butterhead lettuces were randomly collected from each plot and growth parameters were observed. Growth parameters on day 15, 30, 45 and 60 were measured for number of leaf, leaf fresh weight, root fresh weight, leaf width, and leaf length. Percentage ofcolonization was analyzed followed the method in 3.2.2.1.

Growth at harvesting time

At harvesting time, six plants of another butterhead set were randomly collected from each plot then leaf and root were examined separately. Growth parameters and percent colonization were measured according to 3.2.2.1.

Statistical analysis

The difference between treatment and timing were tested by t-test and one-way ANOVA, respectively, using SPSS (ver.20.0, Chicago, IL) at 95% confidence. The correlations of colonization percentage and each growth parameter were calculated with Pearson's correlation coefficient.

3.2.3.3 Photosynthesis measurement

Chlorophyll content determination

Chlorophyll contents were analyzed according to the method in 3.2.2.2.

Photosynthesis performance

Seven days before harvesting time, net photosynthesis, transpiration rate, intracellular CO_2, and stomatal conductance were determined by a portable photosynthesis system (Li-cor 6400, Lincoln, NE, USA) on the biggest leaf (3^{rd} or 4^{th} leaf from outside). The photosynthetic photon flux density (PPFD) and and CO_2 concentration obtained from light response curve were 800 µmol $m^{-2}s^{-1}$ and 400 µmol/mol, respectively.

3.2.3.4 Soil properties and plant nutrients analysis

Soil and plant sampling

Three sets of rhizosphere soil were collected for analysis. Those sets consisted of soil before transplanting, control soil at harvesting time, and AMF inoculated soil at harvesting time. They were collected in zip bag and kept at room temperature until analysis. For plant nutrient analysis, leaf and root at harvesting time were harvested and dried in 60°C hot air oven for 3 days. Then, they were grounded to powder for further analysis. Soil and plant samples were subjected to and analyzed by Soil Fertilizer Environment Scientific Development Project, Department of Soil Science, Faculty of Agriculture, Kasetsart University.

Soil properties

Physiochemical properties including pH, organic matter (OM), available phosphorous, and exchangeable potassium, magnesium, and calcium were investigated. Organic matter and available phosphorous were determined using Walkley and Black method and Bray II method, respectively (Bray and Kurtz, 1945; Nelson and Sommers, 1996). Exchangeable potassium, calcium, and magnesium were extracted with 1N NH_4OAc (pH 7.0) before determining with atomic absorption (Jackson, 1958).

Plant nutrients analysis

Dried leaf and root were investigated for total nitrogen, phosphorous and potassium. Total nitrogen in plant was quantified by semi microkjeldahl method followed by the distillation (Motsara and Roy, 2008). Total phosphorous in plant was analyzed using Vanado molybdophosphoric acid yellow method and total potassium in plant was digested with concentrated nitric acid followed by determining with atomic absorption (Jackson, 1958).

3.2.3.5 Determination of antioxidant enzyme activities and contents

Plant tissue sampling

At harvesting time, six plants were randomly collected from each plot. They were individually cut into small pieces. A gram of cut leaf for each antioxidant substance assay was randomly collected in aluminum foil and submerged in liquid nitrogen. Plant tissues were kept in -20^{O}C until analysis.

Catalase and ascorbate peroxidase

Activities of catalase (CAT) and ascorbate peroxidase (APX) were assayed. One gram of butterhead leaf was grounded to powder with liquid N_2 and extracted with extraction buffer (containing 50 mM phosphate buffer pH 7.0, 1% (w/v) PVPP, 1 mg/ml DTT, and 100mM PMSF). The extraction was centrifuged at 9,000 rpm for 10 min at 4^{O}C,

then supernatant were collected and determined for activity of CAT and APX followed the method described by Aebi (1984) and Nakano and Asada (1981), respectively (Appendix B).

Ascorbic acid

Ascorbic acid was extracted with 6% metaphosphoric acid in 2M acetic acid then the amount was quantified as described in Shin et al. (2007). The absorbance of ascorbic acid assay at wavelength 540 nm were observed and compared with standard curve of ascorbate (Appendix B).

Phenolic content

Plant tissues were grounded with liquid nitrogen and extracted with 80% methanol. The extraction was centrifuged for 5 min at 9,000 rpm at 25°C. Phenolic content were determined using Folin-Ciocalteu method and the absorbance at 750 nm was calculated and expressed as mg gallic acid equivalents (Choi et al., 2006) (Appendix B).

Carotenoid

Carotemoid was extracted from plant tissue by the same method as chlorophylls content (3.2.2.2). Then, the absorbance was calculated according to equation of Devesa et al. (2007) as followed

$$\text{Carotenoid} = 1000\ A^{470} - 3.27\ C_a - 104\ C_b$$

C_a: Chlorophyll a content

C_b: Chlorophyll b content

DPPH scavenging assay

Plant tissues were grounded and extracted with the same method of phenolic content determination. The amount of free radical $DPPH^{\bullet}$ was determined by measuring the decrease in absorbance at 515 nm and expressed as percentage of inhibition (Yu et al., 2003) (Appendix B). The absorbance was calculated to $DPPH^{\bullet}$ inhibition percentage according to the equation as followed

% DPPH$^{\bullet}$ inhibition = [(1 – A^{sample})/ $A^{control}$] x 100

A^{sample}: Absorbance of reaction with extraction added

$A^{control}$: Absorbance of reaction without extraction added

3.2.4 To study bacterial and fungal community of arbuscular mycorrhizal fungus (*Glomus mosseae*) inoculated soil planting butterhead lettuce

3.2.4.1 Field preparation and Experimental design

Field preparation, experimental design, and plant cultivation were the same as in 3.2.3.1. There were total 6 plots consisting of 2 treatments which were the best treatment from 3.2.2.2 as an AMF treatment and uninoculation as a control. They were randomly ordered between the two treatments.

3.2.4.2 Soil sampling

Three sets of rhizosphere soil collected from field consisted of soil before transplantation (after field preparation), rhizosphere soil of control and AMF treatment at harvesting time. Soil before transplantation was randomly collected from 3 spots in the field. Meanwhile, rhizosphere soil in this study was defined as approximately 15 cm in depth and 10 cm in diameter of soil around root. Soil samples were collected from 4 plants in each plot and they were mixed as representative of soil sample in a plot. Totally 9 samples were collected for studying microbial community and kept in zip bag in deep freezer at -20°C for DNA extraction.

3.2.4.3 Soil DNA extraction, quantification and qualification

Microbial DNA were extracted from 0.5 g of representative soil using commercial DNA isolation kit, PowerSoil DNA Isolation Kit (Mo Bio, Carlsbad, CA), according to manufacturer procedure (Appendix B). DNA

extraction was performed from 4 difference replications of each representative soil and then mixed. Pooled DNA were quantified and qualified by Qubit fluorometric quantification and Agarose gel electrophoresis technique, respectively. Qualified samples were kept in -20°C freezer until downstream process.

3.2.4.4 Next-Gen Sequencing

V4 region of 16s rDNA and ITS1 region of ITS were used for constructing bacterial and fungal libraries, respectively. Primers for each libraries were shown in Table 4. Genomic DNA were used as template for amplification of each libraries and the amplicons were purified through gel-electrophoresis. Then, they were converted to blunt end using T4 DNA polymerase, Klenow polymerase and T4 Polynucleotide kinase followed by adapters ligation at the end of each amplicon. The sequencing was performed using 250 pair-end on the Miseq Illumina platform.

Table 4 Primers for 16s rDNA and ITS libraries construction

Primer	Sequence
16s rDNA	
515F	5’ GTGCCAGCMGCCGCGGTAA 3’
806R	5’ GGACTACHVGGGTWTCTAAT 3’
ITS	
ITS1-F	5’ CTTGGTCATTTAGAGGAAGTAA 3’
ITS2	5’ GCTGCGTTCTTCATCGATGC 3’

3.2.4.5 Bioinformatic analysis

Data filtering

Raw data was filtered to get clean data according to these parameters: truncation of sequence reads have average quality not less than 20 of 25 windows frame according to Phred algorithm and

trimmed read length not less than 75% of original length, no contamination with 15 bps of adaptor with maximum 3 bps mismatch allowed, no contamination with ambiguous base (N), read must not contain 10 consecutive same bases. If there are any read not matched to those parameters, both read and its pair are filtered out. Then, clean reads were pair-end using FLASH (V.1.2.11) with minimum overlapping length at 15 bps and not more than 10% mismatch and unpaired reads were removed.

OTU analysis

USEARCH (V.7.0.1090) was used for clustering paired-reads to operational taxonomic unit (OTU) at 97% threshold and obtained the representative of each OTU by UPARSE. OTU of 16s rDNA and ITS were mapped with gold database and UNITE, respectively, for chimera screening and removed by UCHIME (V.4.2.40). The abundance of each OTU was carried out by mapping all paired reads with OTU using USEARCH GLOBAL.

Diversity analysis

The OTUs were classified by RDP classifier in QIIME using Greengenes and UNITE database for 16s rDNA and ITS, respectively. Species diversity (alpha diversity) was calculated and presented in term of chao1 and Shannon indices using Mothur. The difference between groups was tested by Kruskal Wallis test and Metastat test was performed for comparing diversity of microbial community between control and AMF inoculation. Complexity comparison was analyzed by Jackknife diversity script in QIIME and presented as Bray Curtis dissimilarity index with weight Unifrac distance.

Remark: DNA qualification and quantification, Libraries construction, sequencing were carried out by BGI Tech Co.Ltd., China.

CHAPTER IV

RESULTS

4.1 Effect of isolates and amounts of AMF spore on growth of butterhead lettuce

4.1.1 The identification of spore collected from organic farm cultivated lettuce

AMF spores were isolated from lettuce cultivated soil and 21 spores from Nakhonratchasima, 20 spores from Ratchaburi, and 6 spores from Pathumthani were selected as representatives from each location. However, only 7 spores from Nakhonratchasima (N), 12 spores from Ratchaburi (R), and 4 spores from Pathumthani (P) could be propagated by inoculating into sorghum root. Totally 23 AMF isolates were named as N1-N7, R1-R12, and P1-P4. These AMF isolates were identified into 3 families which were Glomeraceae, Acaulosporaceae and Gigasporaceae. The pictures of AMF spores were shown in Figure 3. Most of the obtained AMF spores, 11 out of 23, were in the Glomeraceae followed by Acaulosporaceae and Gigasporaceae (Table 5). The number of AMF spores that could be propagated from single spore inoculated sorghum planted soil was high in Gigasporaceae followed by Glomeraceae and Acaulosporaceae (Figure 4). The statistical analysis showed significant difference between numbers of propagated spores from single spore inoculation by Gigasporaceae and Acaulosporaceae while numbers of propagated spores of Glomeraceae were not significantly different from the other 2 families.

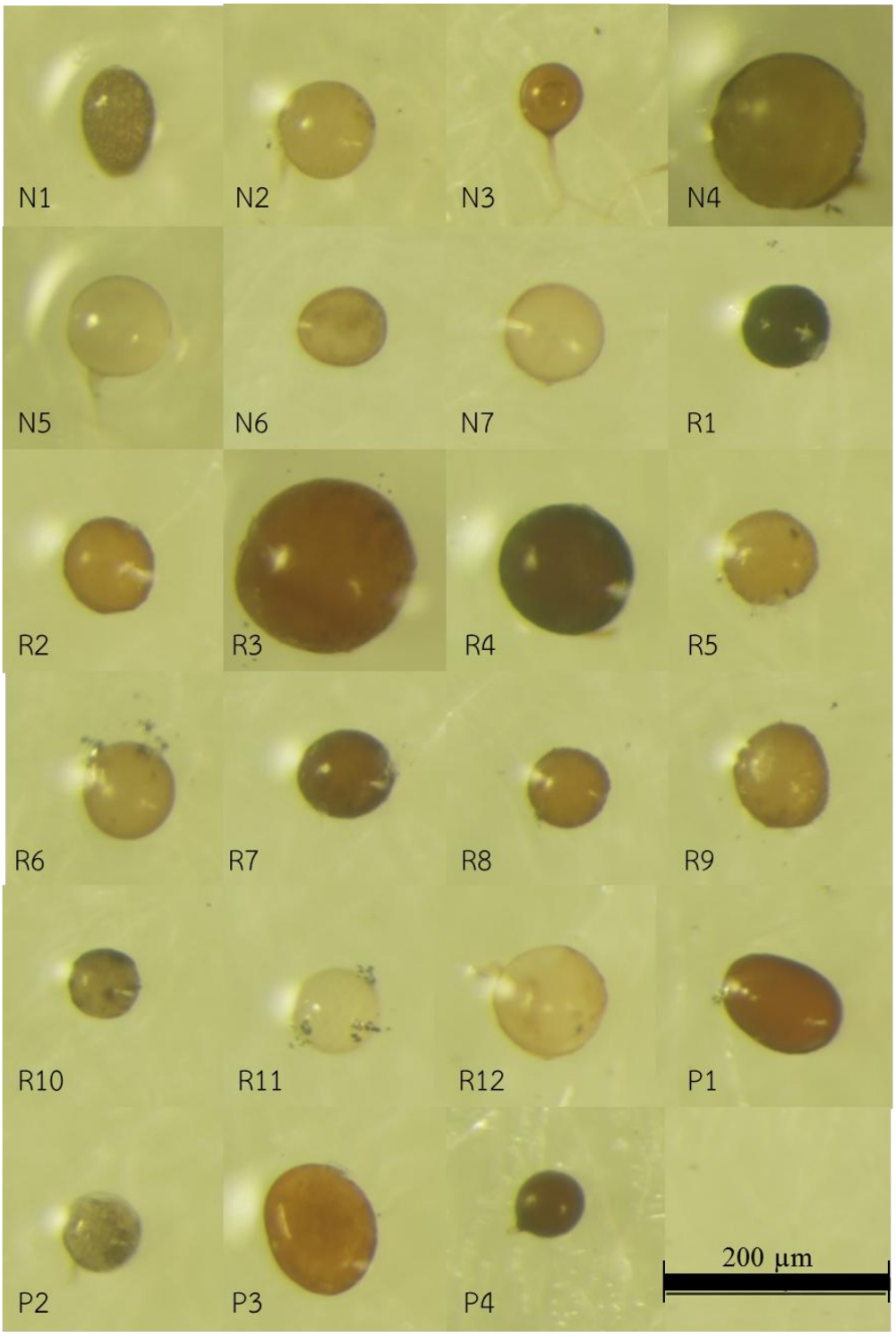

Figure 3 Propagated AMF spore isolates collected from 3 organic lettuce farms in Nakhonratchasima (N), Ratchaburi (R), and Pathumthani (P) provinces.

Table 5 Identification of AMF isolates collected from 3 organic lettuce farms in Nakhonratchasima (N), Ratchaburi (R), and Pathumthani (P) provinces.

Location	Isolate	Family
Nakhonratchasima (N)	N1	Acaulosporaceae
	N2	Glomeraceae
	N3	Glomeraceae
	N4	Glomeraceae
	N5	Glomeraceae
	N6	Acaulosporaceae
	N7	Glomeraceae
Ratchaburi (R)	R1	Glomeraceae
	R2	Acaulosporaceae
	R3	Gigasporaceae
	R4	Gigasporaceae
	R5	Acaulosporaceae
	R6	Glomeraceae
	R7	Acaulosporaceae
	R8	Glomeraceae
	R9	Glomeraceae
	R10	Acaulosporaceae
	R11	Glomeraceae
	R12	Gigasporaceae
Pathumthani (P)	P1	Gigasporaceae
	P2	Acaulosporaceae
	P3	Glomeraceae
	P4	Gigasporaceae

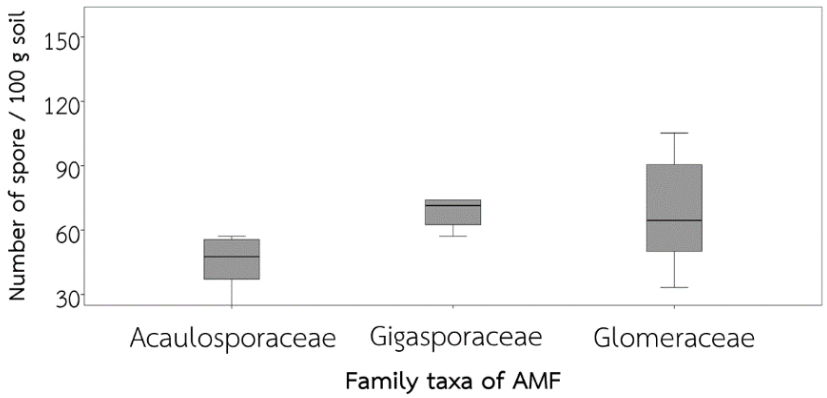

Figure 4 Number of propagated spores from single spore inoculation of 3 months inoculated Sorghum planted soil.

4.1.2 Effect of AMF isolates on growth of butterhead

Butterhead inoculated with AMF isolates exhibited differences in plant growth and development as shown in Figure 5. For each growth parameter, the greatest head diameter, and leaf numbers, the highest leaf fresh and dry weights, the widest leaves and the longest leaves were shown in plants inoculated with isolate N3. Diameters of butterhead head in this crop varied from 2.67-10.33 cm (Figure 6). All the butterheads inoculated by AMF isolates from Nakhonratchasima, Ratchaburi, and Pathumthani except R7, R9, P1, P2, and P3 had significantly wider head than uninoculated butterhead. Leaf number of butterhead was enhanced in all AMF isolates inoculation except R7 (Figure 7). The average leaf number of uninoculation butterhead was 5.83 leaves/plant while the highest leaf number of AMF isolates inoculated butterhead were 17.50 leaves/plant which was approximately three folds of uninoculated plant. Most of AMF isolates increased fresh weight of butterhead except R7, R9 and all isolates from Pathumthani (Figure 8). The highest fresh weight was in butterhead inoculated with isolate N3 followed by isolate N2 (5.58 and 3.77 g, respectively) which was significant difference from uninoculated butterhead (0.2 g). Even though most of AMF isolate inoculated butterhead had higher fresh weight, but there were only isolate N2, N3, and N5 significantly showed an increase in dry weight (Figure 9).

Leaf area represented as leaf width and leaf length presented the same trend. Butterhead inoculated with all AMF isolates except R7, R9, P2, and P3 had significantly wider leaf compared to unioculated butterhead. The great leaf width induction isolates except isolates except R2, R10, and P1 also showed significantly longer leaf than uninoculated treatment (Figure 10 and 11). In summary, all the AMF isolates from Nakhonratchasima could enhance growth of butterhead in terms of head diameter, leaf number, leaf fresh and leaf area. Isolate R5 from Ratchaburi had the highest leaf fresh weight among R isolates. Only R7 and R9 from Ratchaburi did not have any effect on butterhead growth. All the 4 AMF isolates from Pathumthani could increase only leaf number significantly from control but did not affect other growth parameters. Moreover, growth of butterhead inoculated with spores identified to be in the same family differed. Most of AMF isolates in Glomeraceae had greater efficiency on growth induction than those in Acaulosporaceae and Gigasporaceae. However, spores from these 2 families also exhibited potential to improve growth significantly better than in uninoculated butterhead plants.

Determination of percentage of root colonization in butterhead inoculated with AMF isolates showed that isolate P4 had the highest root colonization (73.03%) followed by isolate R3, R1, R4, and R9 (66.06-68.04%) as shown in Figure 12 but plant growth with these isolates were not among the best. Three AMF isolates that potentially increased overall growth of butterhead, N2 N3 and N4, had colonization percentage at only 21.50, 48.74 and 58.56, respectively. AMF spore isolate identified in Glomeraceae showed various colonization levels from nearly lowest (N2) to highest (R1) percentage, as well as in Gigasporaceae. However, all of AMF isolates in Glomeraceae except isolate N2, R8, and P3 had higher level of colonization percentage than uninoculated root. AMF isolates in Acaulosporaceae had low to medium level colonization percentage.

According to the growth enhancement results, isolates N2, N3, N4, N5, and R5 were in top five AMF isolates that could increase all growth parameters of buttherhead, so they were chosen to study effects of spore concentration on Butterhead growth induction.

Figure 5 Sixty days old butterhead lettuce inoculated with 20 spores of propagated AMF isolates.

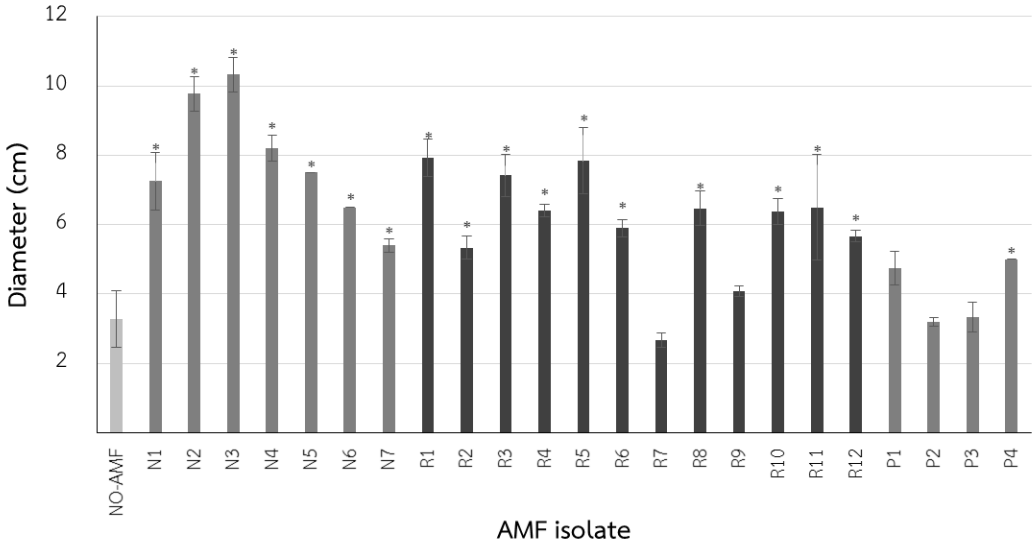

Figure 6 Diameter of 60 days old butterhead lettuce inoculated with 20 spores of propagated AMF isolates (Asterick indicated mean significantly higher than control, no-AMF).

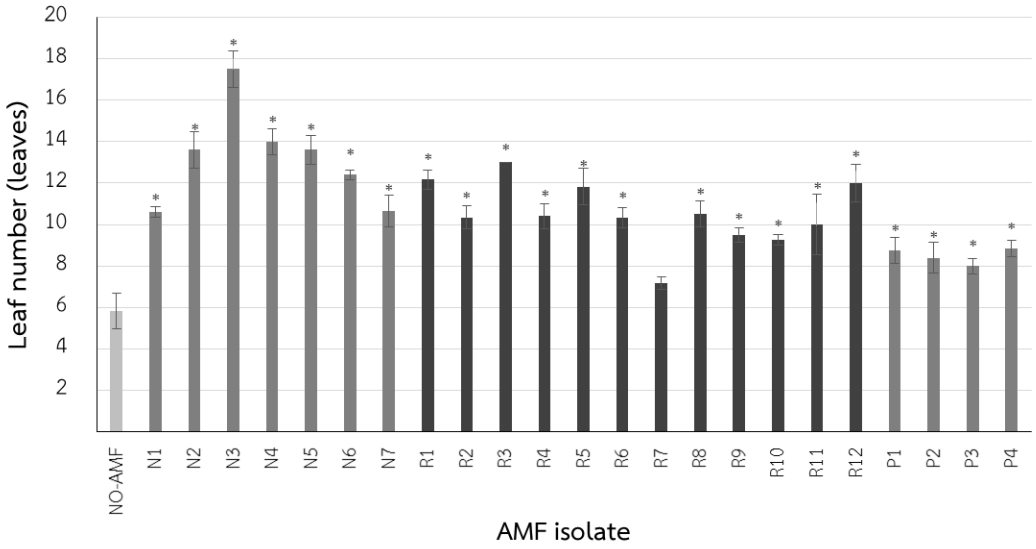

Figure 7 Leaf number of 60 days old butterhead lettuce inoculated with 20 spores of propagated AMF isolates (Asterick indicated mean significantly higher than control, no-AMF).

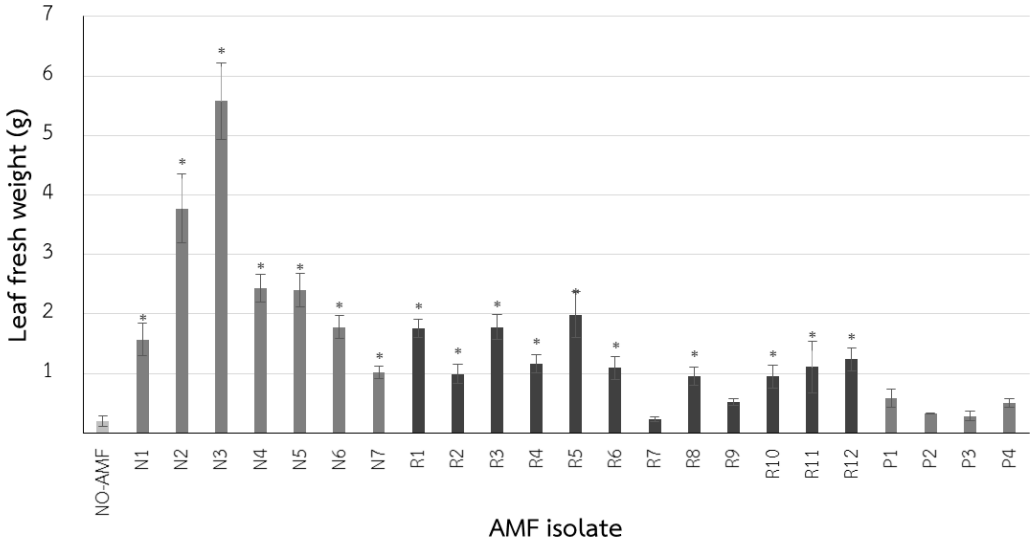

Figure 8 Leaf fresh weight of 60 days old butterhead lettuce inoculated with 20 spores of propagated AMF isolates (Asterick indicated mean significantly higher than control, no-AMF).

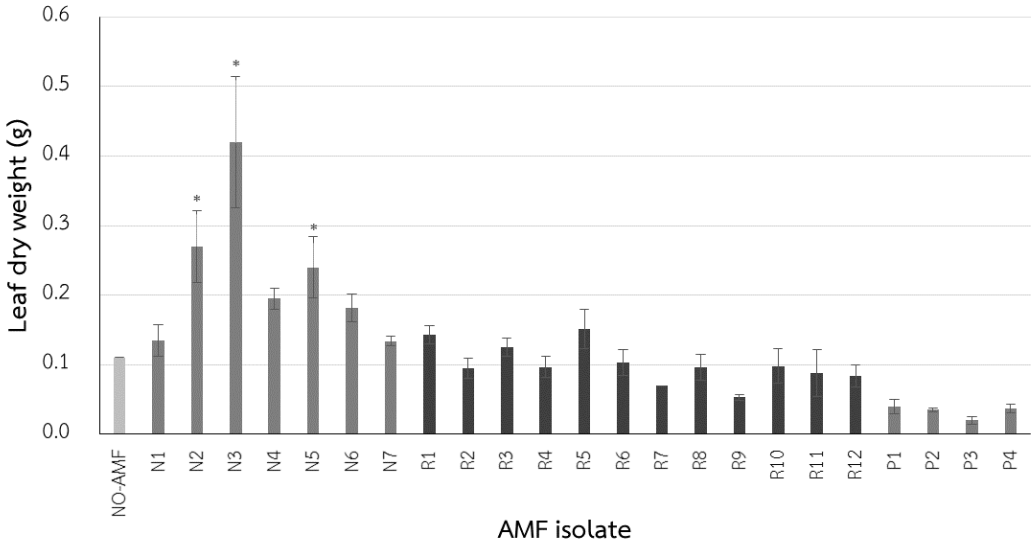

Figure 9 Leaf dry weight of 60 days old butterhead lettuce inoculated with 20 spores of propagated AMF isolates (Asterick indicated mean significantly higher than control, no-AMF).

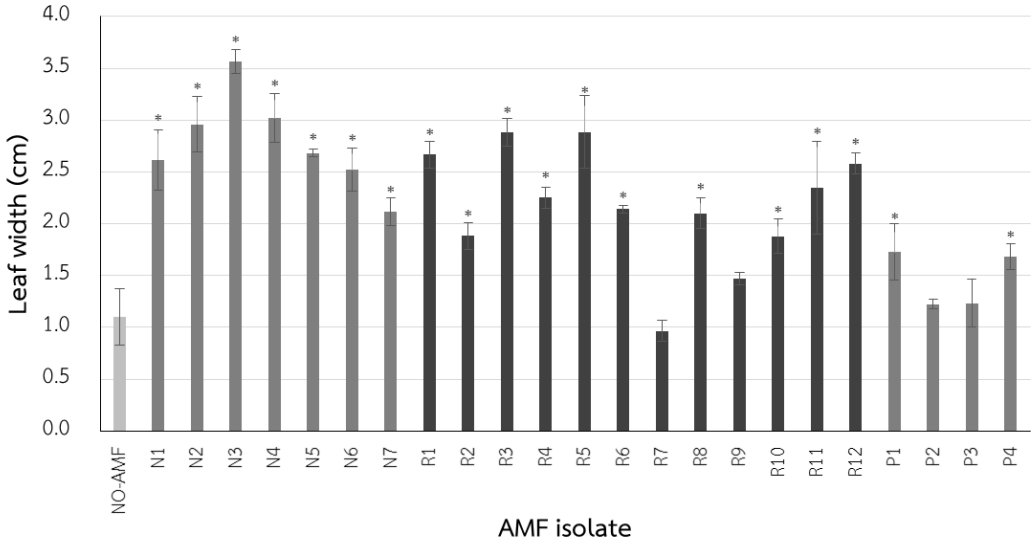

Figure 10 Leaf width of 60 days old butterhead lettuce inoculated with 20 spores of propagated AMF isolates (Asterick indicated mean significantly higher than control, no-AMF).

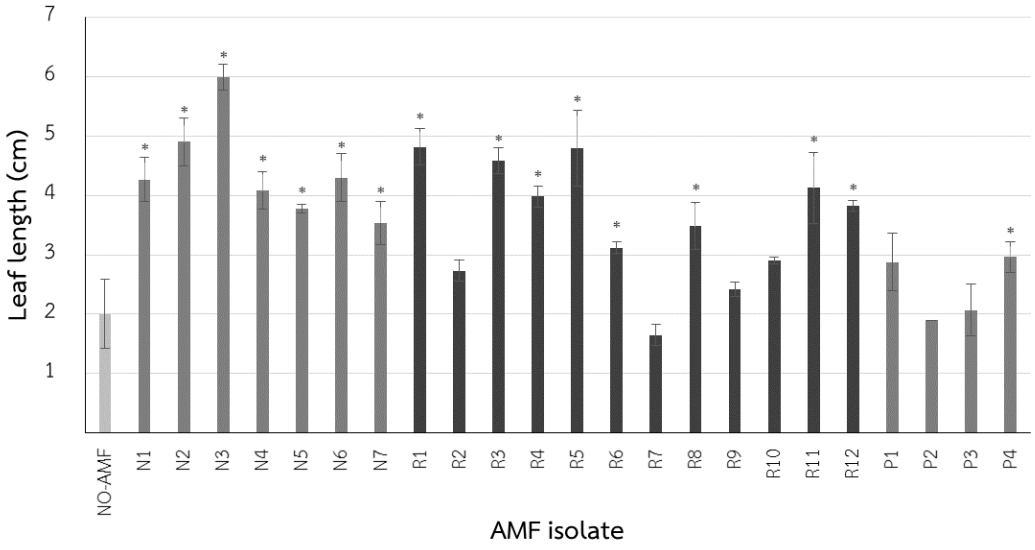

Figure 11 Leaf length of 60 days old butterhead lettuce inoculated with 20 spores of propagated AMF isolates (Asterick indicated mean significantly higher than control, no-AMF).

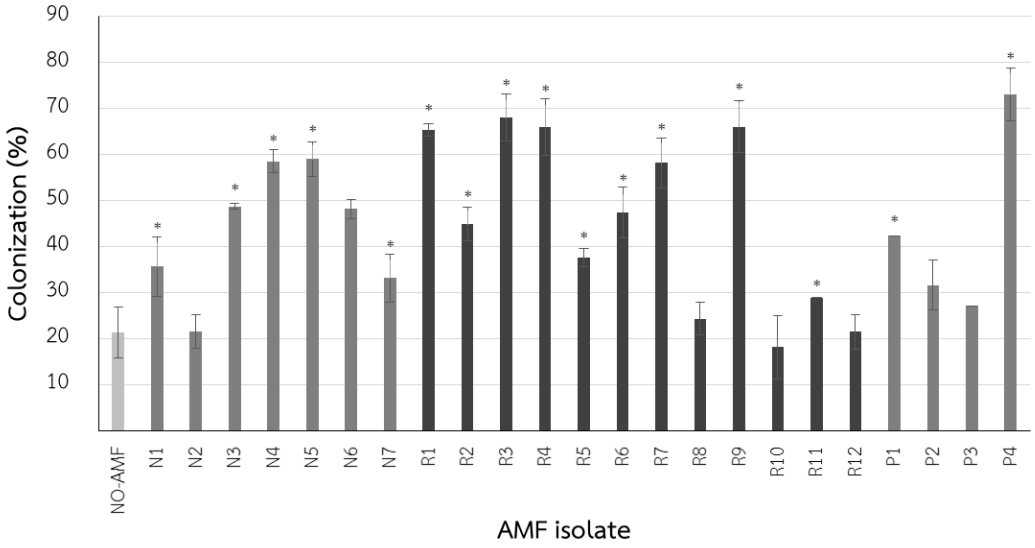

Figure 12 Colonization percentage of 60 days old butterhead lettuce inoculated with 20 spores of propagated AMF isolates (Asterick indicated mean significantly higher than control, no-AMF).

4.1.3 Effect of spore number on growth of butterhead

From 4.1.2, the best five AMF isolates (N2, N3, N4, N5, and R5) were chosen to evaluate an appropriated spore amount used to improve growth of butterhead. Four of the isolates (N2-N5) were in Glomeraceae and another isolate was in Acaulosporaceae. Manure at the rate 10:1 (soil:manure) by weight was applied due to size of butterhead in the previous experiment was commercially unacceptable. It was clearly seen that manure amendment increased all growth parameters of butterhead. However, adding manure affected efficiency of AMF application in this experiment. Average head diameter of butterhead in this experiment ranged from 15.77-18.94 cm which was acceptable in the market. The treatment having largest head diameter was 100 spores of isolate N4 followed by 50 spores of isolate N5 which both were significant difference from uninoculated treatment.

Increased spore amounts of isolate N4 and R5 improved growth of butterhead by enhancing number of leaf and leaf fresh weight while increased

spore amounts of isolate N3 and N5 decreased both parameters (Figure 14 and 15). However, only butterhead inoculated with 50 spores of isolate N5 and 200 spores of isolate R5 produced more leaves than uninoculated treatment and the greatest leaf fresh weight was obtained from 200 spores of isolate R5 treatment (Figure 15). No significant differences in leaf dry weight, leaf width and leaf length were presented in all treatments varied spores numbers and AMF isolates comparing to uninoculated treatment (Figure 16, 17 and 18).

All AMF treatments had greater colonization percentage than the control (Figure 19). The colonization percentage of roots inoculated with isolates N2, N5 and R5 increased when AMF spore numbers increased. Colonization percentage of isolates N3 and N4 were not positively related to spore numbers. Increased numbers of N3 spores resulted in decreased colonization percentage and 100 spores of N4 appeared to exhibit a higher colonization percentage than did 50 and 200 spores. There were no correlations between colonization percentage and plant growth. Treatments potentially increased yield were 200 spores of R5 and 50 spores of N5, but they had low colonization percentage.

The study of interaction effect between spore isolates and spore numbers suggested that they had significant interaction (Table 6). Leaf width was the only parameter that was not resulted from spore isolates, spore numbers, and interaction of them. When considering each factor, the statistical analysis revealed that spore isolates affected all growth parameters while spore numbers affected only leaf dry weight. The result can be implied that increasing in number of AMF spore did not correlated with growth of butterhead but isolate of AMF had influence ability of butterhead growth improvement. Moreover, the interaction of the two factors were need to be studied in order to obtain the best condition for using in each plant species. The interaction between spore isolate and spore number affected colonization percentage while single factor did not have influence on the colonization (Table 6).

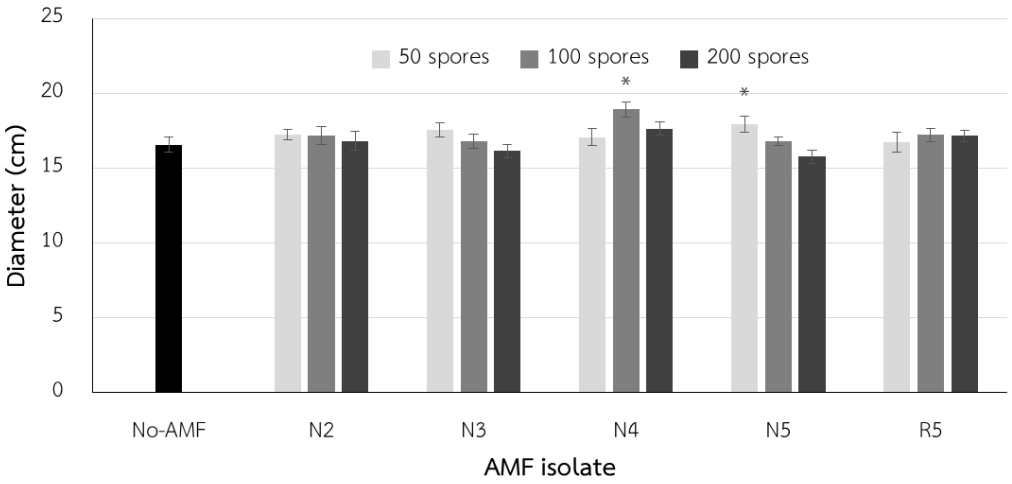

Figure 13 Diameter of 60 days old butterhead lettuce inoculated with 50, 100, and 200 spores of selected AMF isolates (Asterick indicated mean significantly higher than control, no-AMF).

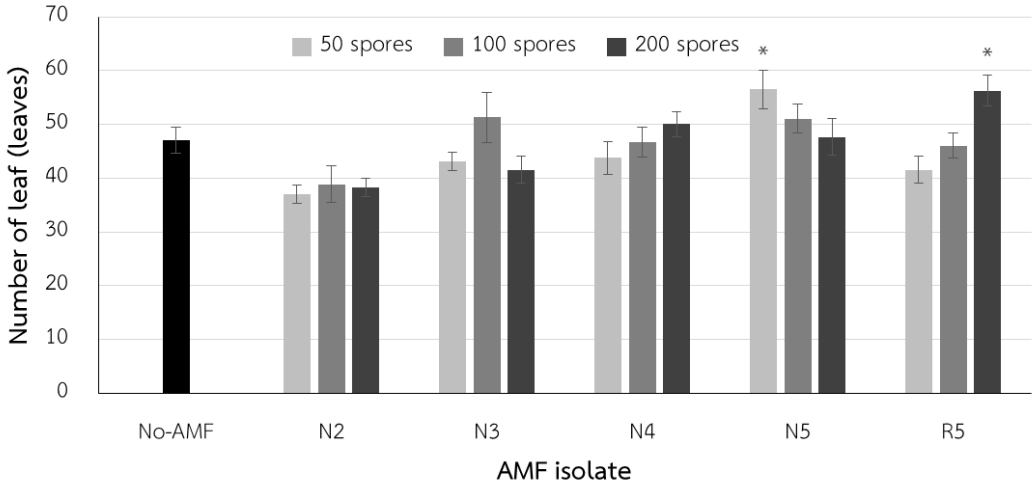

Figure 14 Leaf number of 60 days old butterhead lettuce inoculated with 50, 100, and 200 spores of selected AMF isolates (Asterick indicated mean significantly higher than control, no-AMF).

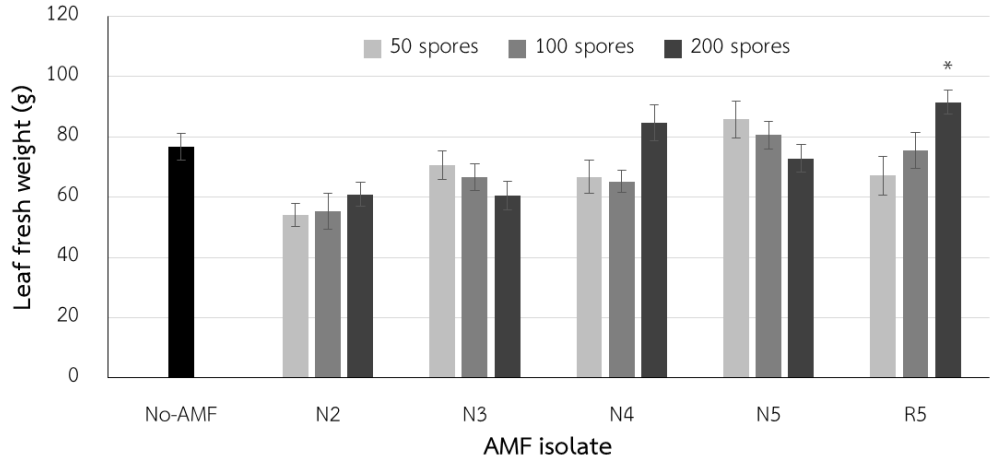

Figure 15 Leaf fresh weight of 60 days old butterhead lettuce inoculated with 50, 100, and 200 spores of selected AMF isolates (Asterick indicated mean significantly higher than control, no-AMF).

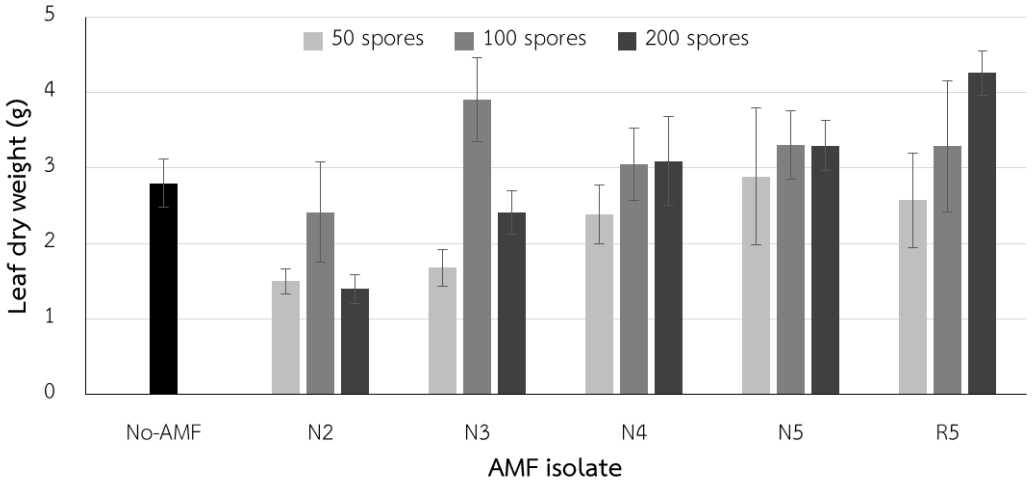

Figure 16 Leaf dry weight of 60 days old butterhead lettuce inoculated with 50, 100, and 200 spores of selected AMF isolates (Asterick indicated mean significantly higher than control, no-AMF).

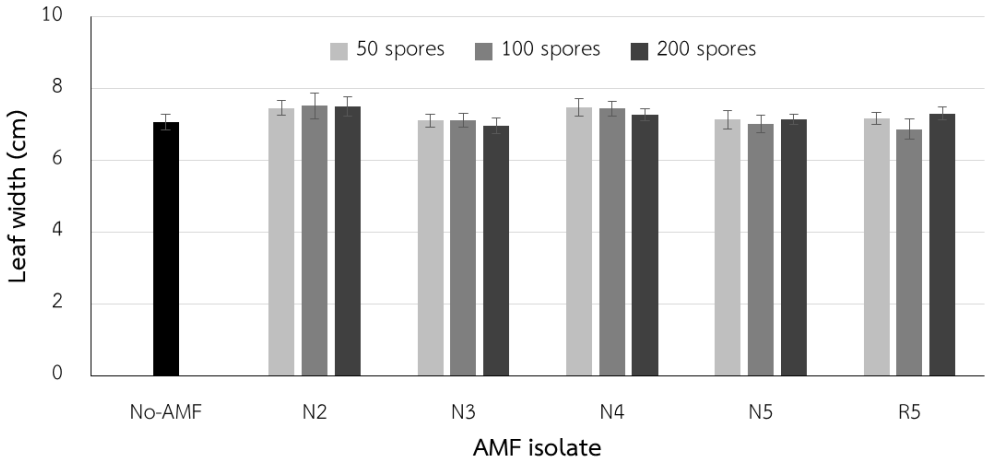

Figure 17 Leaf width of 60 days old butterhead lettuce inoculated with 50, 100, and 200 spores of selected AMF isolates (Asterick indicated mean significantly higher than control, no-AMF).

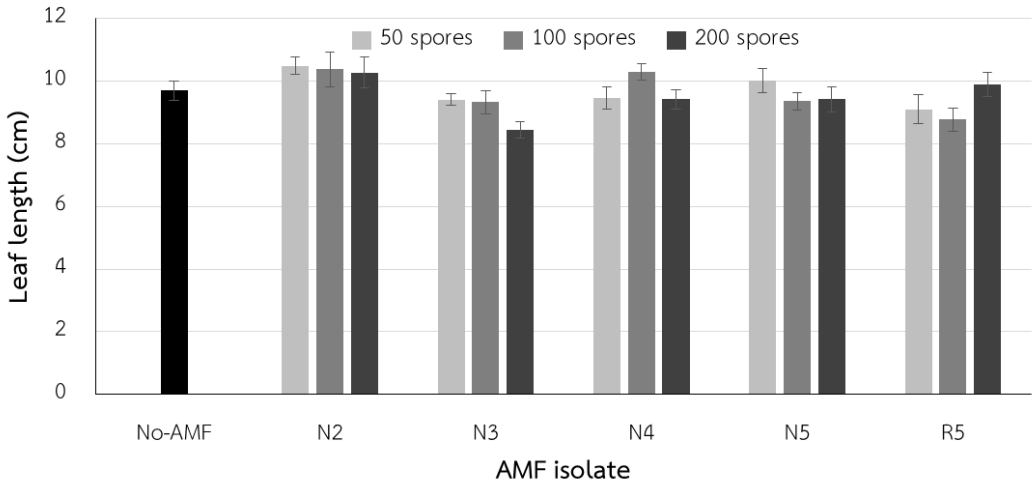

Figure 18 Leaf length of 60 days old butterhead lettuce inoculated with 50, 100, and 200 spores of selected AMF isolates (Asterick indicated mean significantly higher than control, no-AMF).

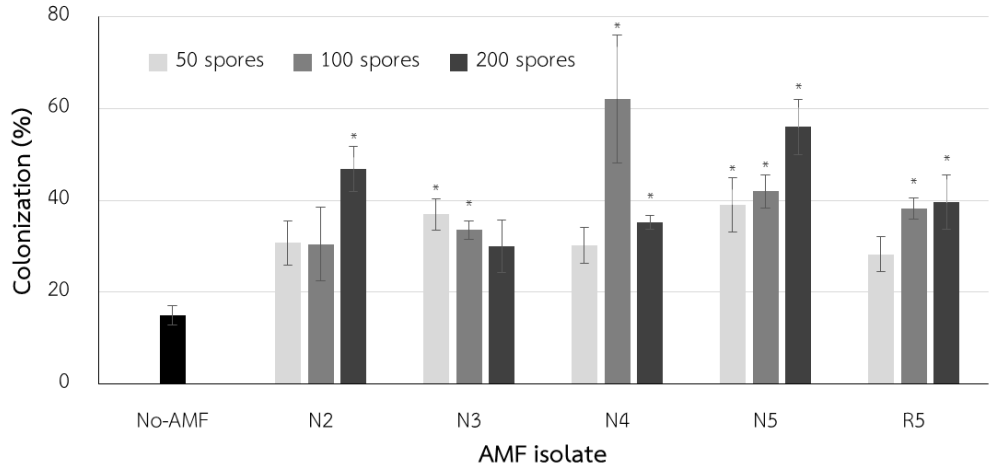

Figure 19 Colonization percentage of 60 days old butterhead lettuce inoculated with 50, 100, and 200 spores of selected AMF isolates (Asterick indicated mean significantly higher than control, no-AMF).

Table 6 Effect of spore isolates (I) and spore numbers (N) of AMF on growth parameters of 60 days old butterhead

Treatment	Probability					
	Diameter	Leaf number	Leaf fresh weight	Leaf dry weight	Leaf width	Leaf length
I	$p<0.05$	$p<0.01$	$p<0.01$	$p<0.01$	NS	$p<0.01$
N	NS	NS	NS	$p<0.01$	NS	NS
I x N	$p<0.05$	$p<0.01$	$p<0.01$	$p<0.01$	NS	$p<0.05$

Interaction effect was tested at 95% confidence and NS represents non-significant at $p<0.05$

Photosynthetic pigments of butterhead treated with AMF in different spore amounts and AMF isolates were varied. Effect of AMF on chlorophyll a and b were similar (Figure 20 and 21). The increase chlorophyll contents when number of AMF spore used increased was obtained in treatment inoculated with isolate N5 and R5. Meanwhile, the higher number of isolate N2 inoculation resulted in lower contents of both chlorophyll. No certain effect of isolate N3 and N4 treatment on chlorophyll contents of butterhead was observed. The highest chlorophyll a and b contents were found in treatment inoculated with 50 spores of isolate N2 (Figure 20). However, there was no significant difference between treatments in chlorophyll b content (Figure 21).

The statistical analysis of interaction effect between spore isolates and spore numbers of chlorophyll contents presented similarly with growth parameters (Table 7). Spore isolates had an effect on both chlorophyll a and b while spore numbers did not. Spore isolates and spore numbers had significantly interaction effect on chlorophyll a and b.

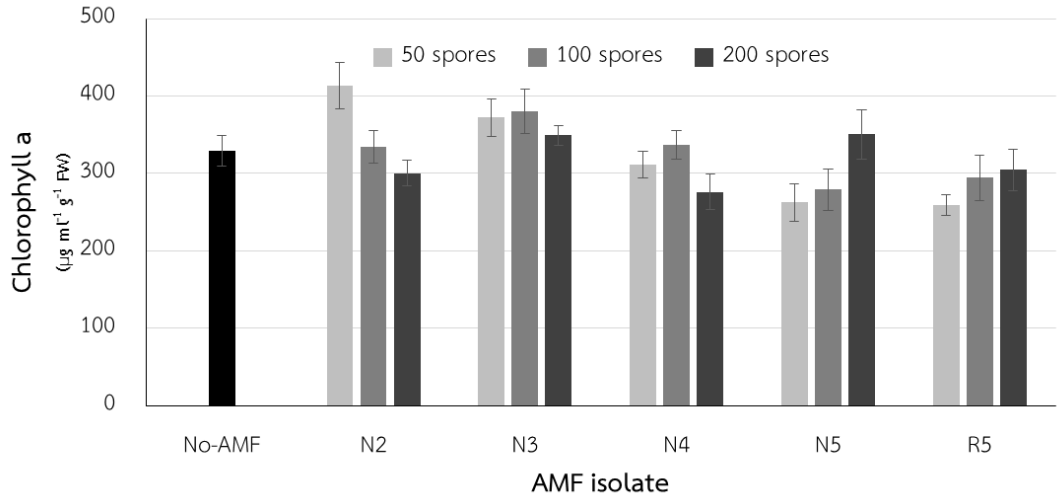

Figure 20 Chlorophyll a content of 60 days old butterhead lettuce inoculated with 50, 100, and 200 spores of selected AMF isolates (Asterick indicated mean significantly higher than control, no-AMF).

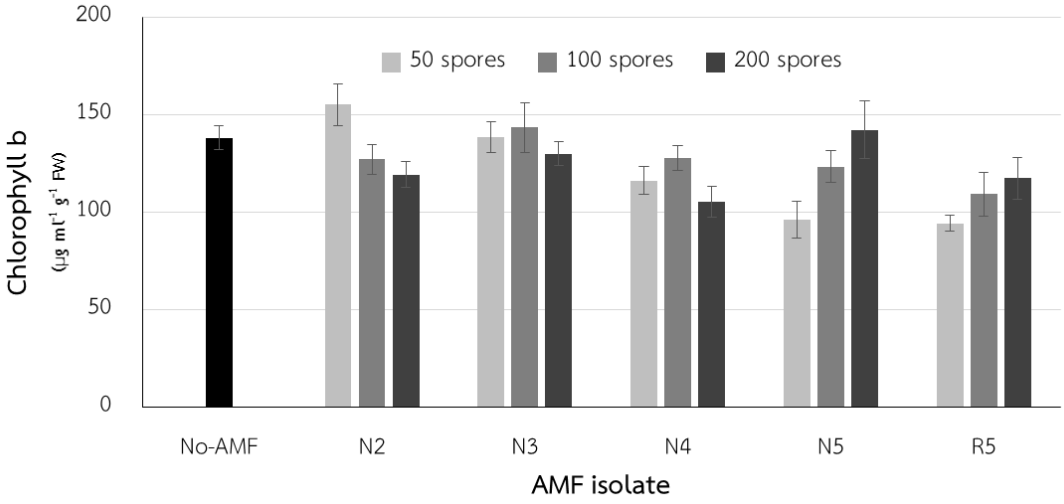

Figure 21 Chlorophyll b content of 60 days old butterhead lettuce inoculated with 50, 100, and 200 spores of selected AMF isolates (Asterick indicated mean significantly higher than control, no-AMF).

Table 7 Effect of spore isolates (I) and spore numbers (N) of AMF on colonization percentage and photosynthetic pigments of 60 days old butterhead

Treatment	Probability		
	Colonization	Chlorophyll a	Chlorophyll b
I	NS	$p<0.01$	$p<0.01$
N	NS	NS	NS
I x N	$p<0.05$	$p<0.01$	$p<0.01$

Interaction effect was tested at 95% confidence and NS represents non-significant at $p<0.05$

Statistical analysis for correlation between colonization percentage and growth parameters as well as photosynthetic pigments are shown in Table 8. All correlations were not significance with colonization percentage. So, the result of correlation can be used to confirm that growth enhancement ability cannot be determined from colonization efficiency but the specificity between plant and AMF isolate was needed to be concerned when applying in plant cultivation.

According to the result of yield of butterhead in this experiment which considered from head diameter, number of leaf, and leaf fresh weight, treatments with 50 spores inoculation of isolate N5 and 200 spores inoculation of isolate R5 were the best for improving growth of plant. So, 50 spores of isolate N5 was chosen for studying in natural field due to their ability in improving plant growth and their practicality in term of cost-effective which was better than using 200 spores of another species.

Table 11 Growth parameters of 60 days old butterhead inoculated with *Glomus mosseae* and cultivated in organic farming system.

Treatments	Diameter (cm)	Number of leaf (leaves)	Fresh weight (g)	Dry weight (g)	Leaf width (cm)	Leaf length (cm)	Colonization (%)
No-AMF	17.55	15.77	14.50	0.95	5.41	9.38	40.15
AMF	16.93	19.41	30.37	1.02	5.36	8.88	58.59
p value	NS	$p<0.01$	$p<0.01$	NS	NS	NS	$p<0.01$

NS represents mean did not have significant difference at $p<0.05$, AMF-soil treated with *Glomus mosseae*

Morphological structures of N5 isolate (suggested to be the best isolate) were observed for fungal identification. The presence of spores inside root tissue was observed (Figure 22A). Root staining presented dark blue color of fungal structure (Figure 22B and 22C). Also, N5 spore showed thick wall and funnel shape of subtending hyphae (Figure 22D). Therefore, together with 120-150 µm in size, yellow to brown color, and sphere shape, this isolate was identified as *Glomus mosseae*.

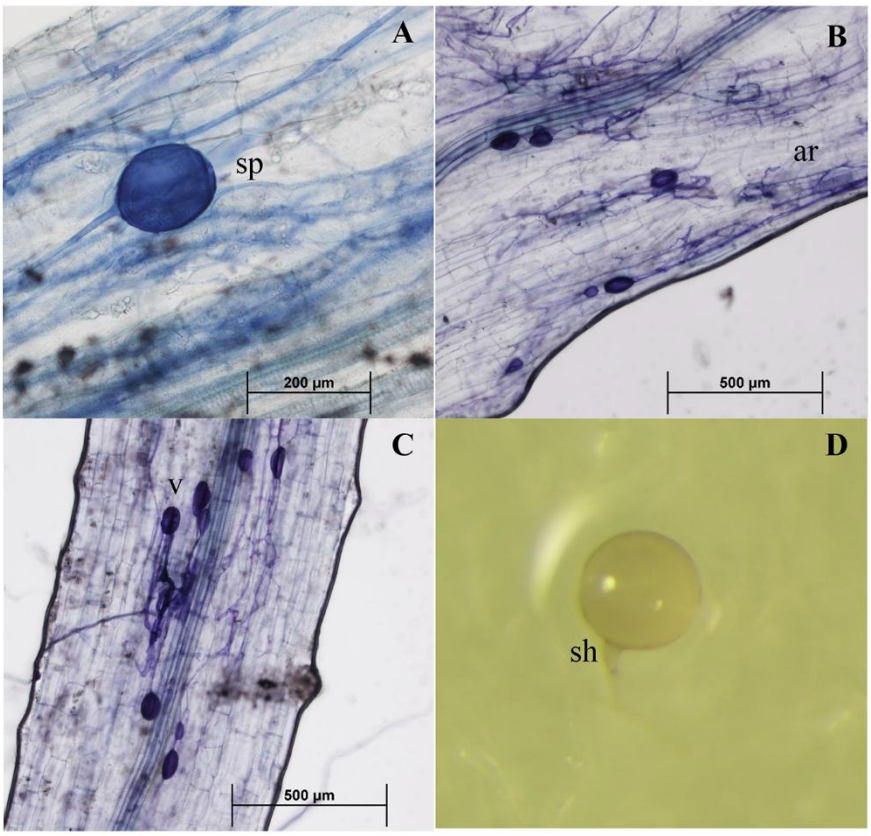

Figure 22 Morphological characters of isolate N5 identified as *Glomus mosseae*. A, B, and C are lettuce root stained with 0.05% tryphan blue (sp-spore, ar-arbuscule, and v-vesicle). D shows funnel shape of subtending hypha (sh) of *Glomus mosseae*.

4.2 The application of selected AMF spore amount and isolate on yield and quality of butterhead in trial field cultivated under organic farming system

4.2.1 Plant growth measurement

Fifty spores of *Glomus mosseae* inoculated butterhead were compared with uninoculated butterhead in trial field. The study in plant growth measurement was divied into 2 parts, growth observation by timing and at harvesting time. For timing butterhead growth, number of leaf, leaf and root fresh weight, leaf width and length, and colonization percentage were observed. The result intended to present difference in changes of growth pattern of the two treatments. Number of leaf in both uninoculated and *G. mosseae* inoculated butterhead continuously increased along the cultivation period (Figure 23). There was no difference between treatments on day 15, and then the significant higher leaf number was presented on day 30 and day 45 in *G. mosseae* inoculated treatment. Even though number of leaf on day 60 was not significant different but *G. mosseae* inoculated treatment had greater number of leaf approximately 0.5 fold than uninoculated butterhead. Leaf and root fresh weight of butterhead slightly increased after transplantation and the significant higher in *G. mosseae* inoculation could be observed on day 30 (Figure 24 and 25). Then, leaf and root weight dramatically increased until day 60. Leaf fresh weight was higher in *G. mosseae* treatment along the cultivation time. Leaf fresh weight on day 60 of *G. mosseae* inoculation was significantly higher approximately 2-folds than control. The higher of root weight in *G. mosseae* inoculation treatment appeared 2 folds more than unioculated treatment but not significance (Figure 25). Root fresh weight on day 45 and 60 of *G. mosseae* inoculation also presented approximately 0.5 fold higher than uninoculation but no significant difference. *G. mosseae* inoculation compared with uninoculation did not affect leaf width and leaf length of butterhead in all sampling times (Figure 26 and 27).

Colonization percentage of *G. mosseae* inoculated butterhead dramatically rose up to nearly 3-folds higher than uninoculated root on day 15 after transplantation (Figure 28). Then, colonization percentage of *G. mosseae*

inoculation increased until last day of observation while colonization percentage of uninoculation slightly increased until day 30 and then it was constant until last day of observation.

Growth parameter statistical analysis comparing between each sampling time presented similar scheme between treatments (Table 9). The analysis was done by comparing means of sampling times within treatment in each growth parameter. The result showed that *G. mosseae* inoculation obviously increased leaf number 15 days earlier than unioculation. Leaf and root fresh weight did not have difference in changing pattern. Also, leaf width and leaf length showed similar pattern between each other and between treatments. Colonization changing pattern appeared difference in *G. mosseae* inoculation on day 45 by significantly increase comparing to day 30. Even though, colonization in root of uninoculated butterhead was presented but no significant difference was observed between sampling times.

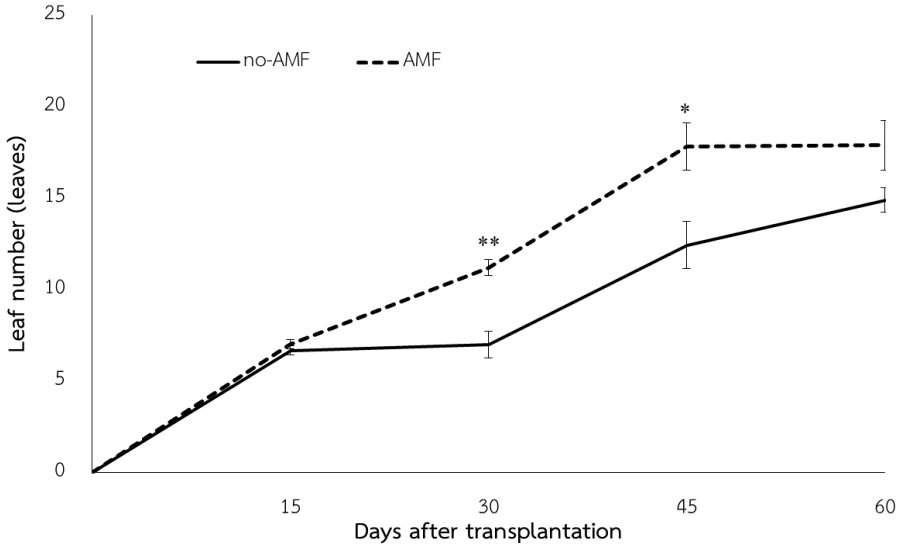

Figure 23 Leaf number of 60 days old butterhead lettuce inoculated with 50 spores of *G. mosseae in trial field (* indicated mean significant difference between treatments at p<0.05 and ** indicated at p<0.01).*

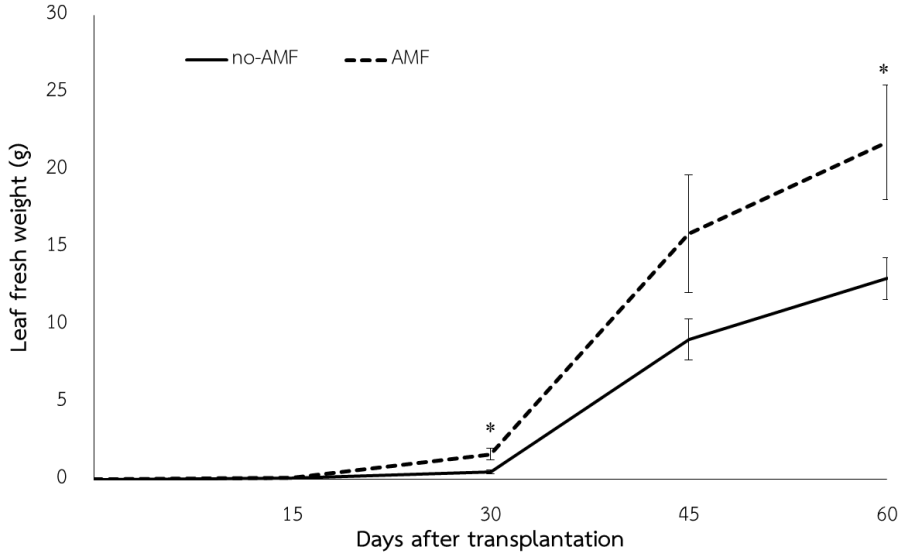

Figure 24 Leaf fresh weight of 60 days old butterhead lettuce inoculated with 50 spores of *G. mosseae* in trial field (* indicated mean significant difference between treatments at p<0.05).

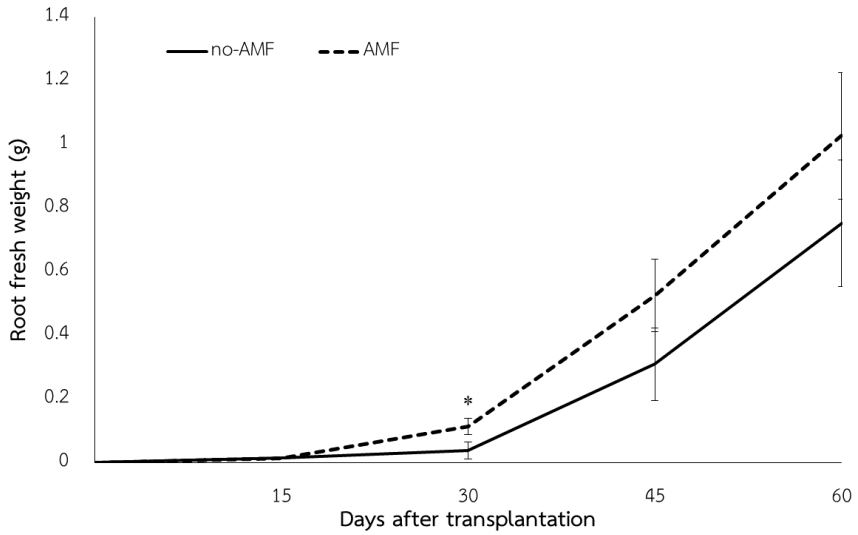

Figure 25 Root fresh weight of 60 days old butterhead lettuce inoculated with 50 spores of *G. mosseae* in trial field (* indicated mean significant difference between treatments at $p<0.05$).

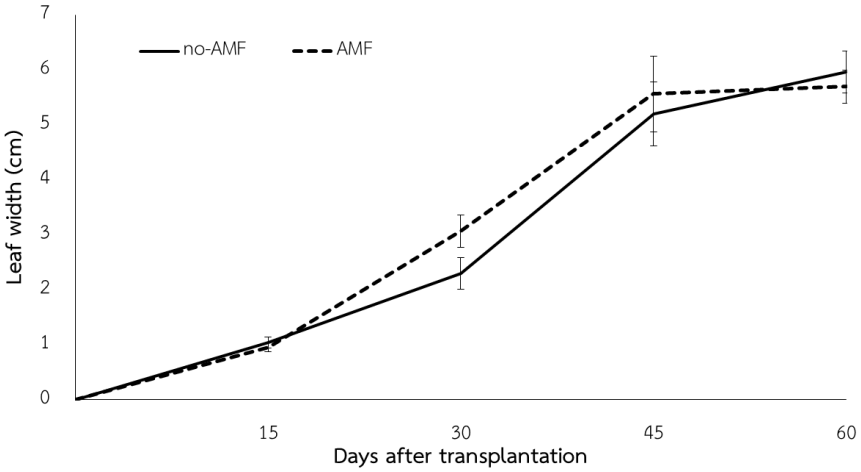

Figure 26 Leaf width of 60 days old butterhead lettuce inoculated with 50 spores of *G. mosseae in trial field.*

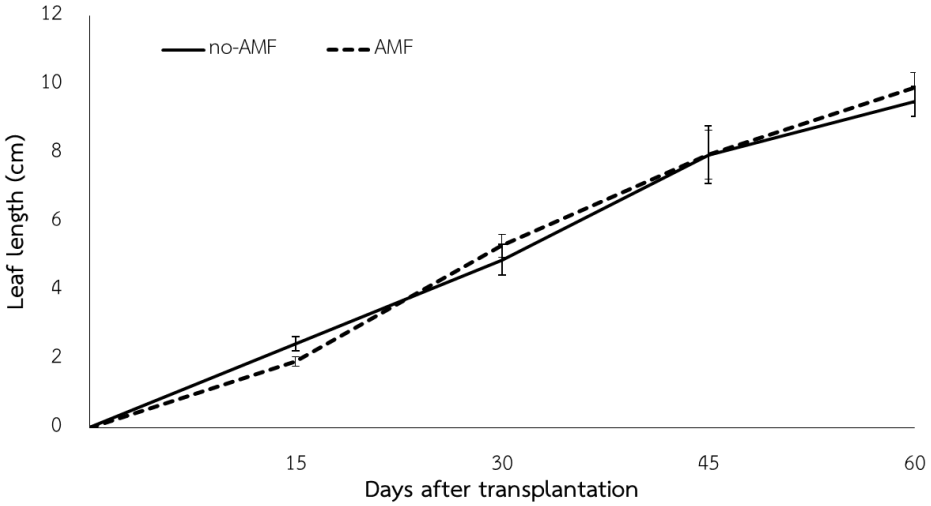

Figure 27 Leaf length of 60 days old butterhead lettuce inoculated with 50 spores of *G. mosseae in trial field.*

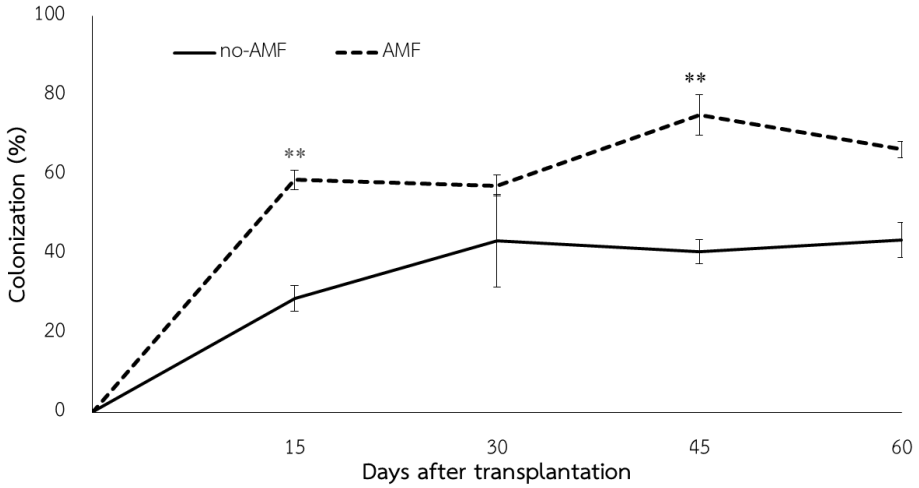

Figure 28 Colonization percentage of 60 days old butterhead lettuce inoculated with 50 spores of *G. mosseae* in trial field (** indicated mean significant difference between treatments at $p<0.01$).

Table 9 Butterhead growth statistical analysis comparing between sampling times within each treatment

	Day 15	Day 30	Day 45	Day 60
AMF Leaf number (leaves)				
Unioculation	6.67[a]	7.00[a]	12.43[b]	14.89[c]
G. mosseae	7.00[a]	11.20[b]	17.83[c]	17.89[c]
Leaf fresh weight (g)	0.11[a]	0.49[a]	9.06[b]	12.98[c]
Unioculation	0.10[a]	1.62[a]	15.86[b]	21.79[c]
G. mosseae				
Root fresh weight (g)	0.01[a]	0.04[a]	0.31[b]	0.75[c]
Unioculation	0.01[a]	0.11[a]	0.53[b]	1.03[c]
G. mosseae	1.04[a]	2.30[b]	5.20[c]	5.96[c]
Leaf width (cm)	0.95[a]	3.07[b]	5.56[c]	5.70[c]
Unioculation				
G. mosseae	2.45[a]	4.89[b]	7.95[c]	9.50[d]
Leaf length (cm)	1.92[a]	5.30[b]	7.96[c]	9.90[d]
Unioculation				
G. mosseae	28.77[a]	43.34[a]	40.57[a]	43.42[a]
Colonization (%)	58.66[a]	57.26[a]	75.14[b]	66.33[ab]
Unioculation				
G. mosseae				

* Significant difference were tested by LSD and different letters within a row represent significant difference at $p<0.05$. Means were compared within treatment.

The correlation of *G. mosseae* inoculation and growth of butterhead was shown in Table 10. In uninoculated treatment, colonization of no-AMF treatment did not appear to correlate with any growth parameter. The significant correlation of colonization was observed in *G. mosseae* inoculated treatment with leaf number, leaf fresh weight, leaf width, and leaf length. Pearson correlation value suggested positive change of growth parameters when colonization percentage increased. Only root fresh weight in *G. mosseae* inoculated treatment did not have correlation with colonization percentage.

Table 10 Correlation of colonization and growth parameters of butterhead lettuce inoculated with AMF.

	Leaf number	Leaf fresh weight	Root fresh weight	Leaf width	Leaf length
Correlation with colonization					
no-AMF					
Pearson correlation	0.296	0.369	0.363	0.338	0.345
p-value	NS	NS	NS	NS	NS
AMF					
Pearson correlation	0.639	0.552	0.371	0.652	0.614
p-value	*p<0.05*	*p<0.05*	NS	*p<0.05*	*p<0.05*

Correlation coefficients analyzed with Pearson test. NS represents non-significant at $p<0.05$.

Growth parameters of Butterhead at harvesting time were shown in table 11. AMF inoculated treatment had significantly greater numbers of leaf and leaf fresh weight than uninoculated treatment. The *G. mosseae* inoculated Butterhead had fresh weight approximately 2 folds higher than uninoculation. Looser head formation was observed on Butterhead with no-AMF inoculation (Figure 29). However, leaf dry weight, leaf width, and leaf length did not show significant differences between treatments. Colonization percentage in root of Butterhead inoculated with *G. mossea* was 0.5 fold and was significantly higher than uninoculation.

Figure 29 Sixty days old butterhead lettuce inoculated with 50 spores of *G. mosseae* (left) comparing to uninoculation (right) in trial field.

Table 11 Growth parameters of 60 days old butterhead inoculated with *Glomus mosseae* and cultivated in organic farming system.

Treatments	Diameter (cm)	Number of leaf (leaves)	Fresh weight (g)	Dry weight (g)	Leaf width (cm)	Leaf length (cm)	Colonization (%)
No-AMF	17.55	15.77	14.50	0.95	5.41	9.38	40.15
AMF	16.93	19.41	30.37	1.02	5.36	8.88	58.59
p value	NS	$p<0.01$	$p<0.01$	NS	NS	NS	$p<0.01$

NS represents mean did not have significant difference at $p<0.05$, AMF-soil treated with *Glomus mosseae*

4.2.2 Photosynthesis measurement

Net photosynthesis and transpiration rate of AMF inoculated butterhead were significantly higher than those of control (Table 12). Net photosynthesis of *G. mosseae* inoculation was 0.5-fold higher than uninoculation. Intercellular CO_2 tended to be lower in AMF inoculated butterhead but the statistical analysis did not show significant difference between treatments. Stomatal conductance of both no-AMF and AMF treatments were equivalent which the value was 0.4 mol H_2O $m^{-2}s^{-1}$.

Increased amount of total chlorophyll content was significantly higher in *G. mosseae* inoculated butterhead than uninoculation (Table 12). When analyzed separately, only chlorophyll a content in *G. mosseae* inoculation was significantly greater than uninoculation while chlorophyll b did not have any significant difference. However, chlorophyll b content slightly increased in *G. mosseae* inoculation.

Table 12 Photosynthesis performances and photosynthetic pigments (Ch; chlorophyll) of 60 days old butterhead inoculated with *Glomus mosseae* and cultivated in organic farming system.

	Photosynthesis performances				Pigments (μg ml^{-1} g^{-1} FW)		
Treatments	Photo	C_i	Trmmol	Cond.	Total Ch	Ch a	Ch b
no-AMF	4.27	208.18	0.71	0.04	520.67	374.17	146.49
AMF	6.12	174.43	0.85	0.04	602.58	437.05	165.53
p value	$p<0.01$	NS	$p<0.05$	NS	$p<0.05$	$p<0.05$	NS

NS represents mean did not have significant difference at $p<0.05$, AMF-soil treated with *Glomus mosseae*. Photo, Net photosynthesis (μmol CO_2 $m^{-2}s^{-1}$); C_i, Intercellular CO_2 (μmol CO_2 mol^{-1}); Trmmol, Transpiration rate (mmol H_2O $m^{-2}s^{-1}$); Cond., Stomatal conductance (mol H_2O $m^{-2}s^{-1}$)

4.2.3 Soil properties and plant nutrients analysis

Soil properties were displayed as pH, organic matter (OM), available phosphorous, and exchangeable potassium, magnesium, and calcium (Table 13). Soil in planting area before transplanting was low acidity. Rhizosphere soil pH of both uninoculated and *G. mosseae* inoculated treatments increased at harvesting time but only *G. mosseae* inoculation showed significant difference from before transplantation. OM percentage showed similar changing pattern of pH. OM percentage of *G. mosseae* inoculation was about 1.5 fold higher than before transplantation and 0.5 fold-higher than control on harvesting day. Available phosphorous in *G. mosseae* inoculation at harvesting time significantly and extremely high compared to before transplantation from 2 mg/kg soil up to 12.33 mg/kg soil while available phosphorous of uninoculation was only 3.67 mg/kg soil which was not significant difference from before transplanting. Exchangeable potassium exhibited a same trend with phosphorous but there was no significant difference between treatments at collecting time. Exchangeable magnesium and calcium presented similar changing pattern in which the amount of these nutrients in both uninoculated and *G. mosseae* inoculated treatments significantly decreased after cultivation. However, only exchangeable calcium in *G. mosseae* inoculation was significantly higher than control.

Nitrogen, phosphorous and potassium in both leaf and root of butterhead at harvesting time was analyzed (Figure 30). The concentration of nutrients in leaf was significantly more than in root. All leaf and root nutrients in *G. mosseae* inoculated treatment were higher than uninoculated treatment. Nutrients in leaf of *G. mosseae* inoculated treatment increased only 17%, 6%, and 5% for nitrogen, phosphorous, and potassium, respectively. Meanwhile, the nutrients in root were enhanced up to 38%, 26%, and 20% for nitrogen, phosphorous, and potassium, respectively compared to uninoculation.

Table 13 Properties of rhizosphere soil before and 60 days after transplanting butterhead cultivated in organic farming system.

Treatments	pH	OM (%)	Avail. P (mg/kg)	Exch. K (mg/kg)	Exch. Ca (mg/kg)	Exch. Mg (mg/kg)
Before transplanting	5.9[a]	1.85[a]	2.00[a]	181.33[a]	1,469.00[c]	240.00[b]
At harvesting time						
no-AMF	6.2[ab]	2.40[ab]	3.67[a]	223.00[a]	1,202.67[a]	212.67[a]
AMF	6.4[b]	3.07[b]	12.33[b]	238.67[a]	1,362.67[b]	224.00[a]

Vertically different letters represent mean significantly difference at $p<0.05$, AMF-soil treated with *Glomus mosseae*

OM, Organic matter; Avail. P, Available phosphorous; Exch. K, Exchangeable potassium; Exch. Ca, Exchangeable calcium; Exch. Mg, Exchangeable magnesium

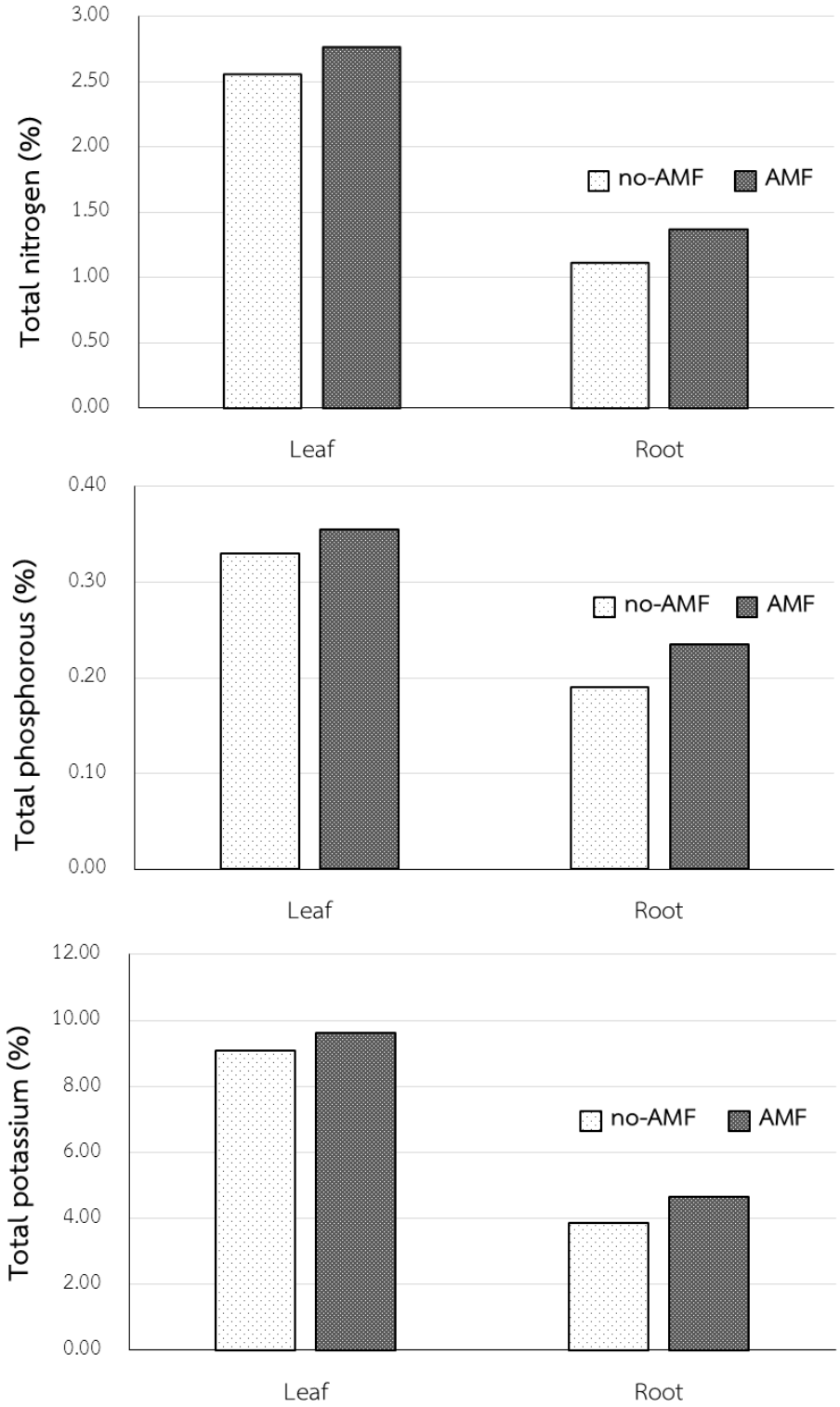

Figure 30 Total nitrogen, phosphorous and potassium of leaf and root of 60 days old butterhead inoculated with *G. mosseae* in trial field

4.2.4 Determination of antioxidant enzyme activities and contents

Both CAT and APX activities of butterhead inoculated with *G. mosseae* were increased (Table 14). However, both of the enzymes were not significant difference in statistical analysis. Non-enzymatic antioxidants, ascorbic acid and carotenoid, showed slightly higher in *G. mosseae* inoculated butterhead with no significant difference comparing to uninoculation. Phenolic content was not affected from *G. mosseae* inoculation. Total free radical scavenging was presented as $DPPH^{\bullet}$ inhibition percentage. Interestingly, percentage of $DPPH^{\bullet}$ inhibition in *G. mosseae* inoculated butterhead was approximately 2 fold-higher than control.

Table 14 Enzymatic and non-enzymatic antioxidant substances of 60 days old butterhead inoculated with *Glomus mosseae* and cultivated in organic farming system.

	Enzymatic		Non-Enzymatic			
Treatment	Catalase (Unit)	Ascorbate Peroxidase (Unit)	Ascorbic acid (mg g^{-1} FW)	Phenolic content (mg g^{-1} FW)	Carotenoid (μg ml^{-1} g^{-1} FW)	$DPPH^{\bullet}$ scavenging (%)
No-AMF	35.23	87.37	0.31	0.50	58.49	24.12
AMF	39.30	95.05	0.33	0.47	64.37	53.85
p value	NS	NS	NS	NS	NS	$p<0.01$

NS represents mean did not have significant difference at $p<0.05$, AMF-soil treated with *Glomus mosseae*. One unit of enzyme defines as an amount of enzyme that can convert 1 mol of substrate.

4.3 Bacterial and fungal community of arbuscular mycorrhizal fungus (*Glomus mosseae*) inoculated soil planting butterhead lettuce

4.3.1 Bacterial community in rhizosphere soil of butterhead inoculated with *G. mosseae*

Rhizosphere bacterial communities were compared between before transplantation and at harvesting time with 2 treatments, uninoculated and *G. mosseae* inoculated treatments. V4 region of 16s rDNA was used to be target for libraries construction. Obtained operational taxonomic unit (OTU) was significant difference between groups of soil sample ($p<0.05$). The average OTU found in before transplantation, uninoculation, and *G. mosseae* inoculation were 4660.67±249.52, 5678.67±228.42, and 6463.00±225.96, respectively (Figure 31). OTU was appeared higher after plant cultivation and *G. mosseae* inoculation was found to have more OTU than uninoculation.

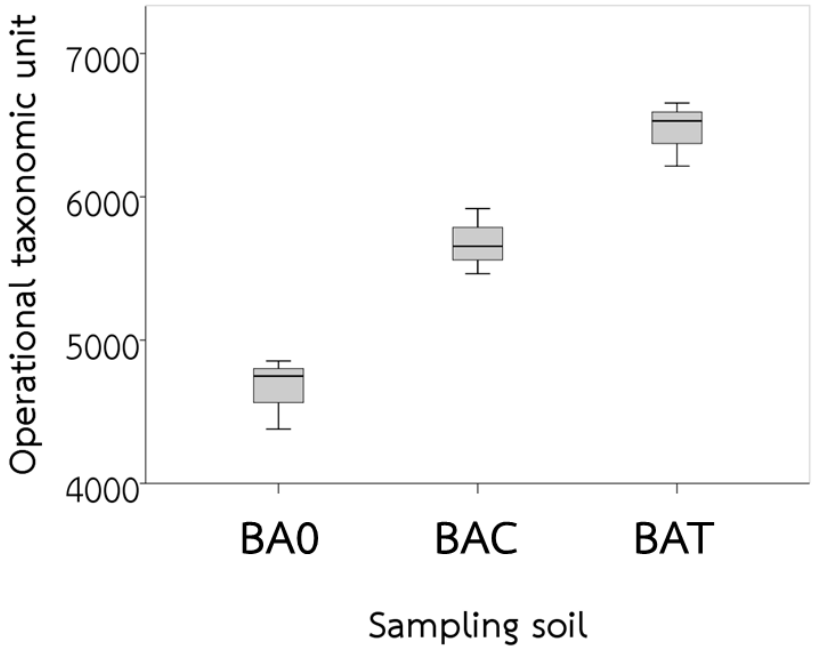

Figure 31 Operational taxonomic unit number of bacteria in 3 groups of soil samples, before transplantation (BA0) and at harvesting time of uninoculated (BAC) and *G. mosseae* inoculated (BAT) treatments.

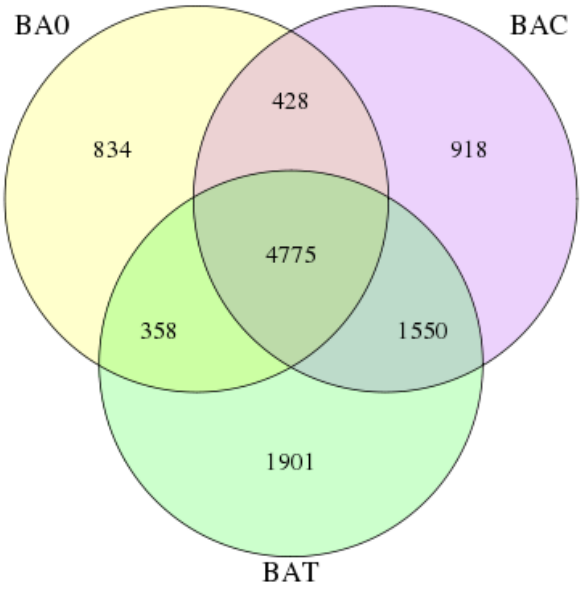

Figure 32 Venn diagram of shared operational taxonomic unit number of bacteria between 3 groups of soil samples, before transplantation (BA0) and at harvesting time of uninoculation (BAC) and *G. mosseae* inoculation (BAT) treatments.

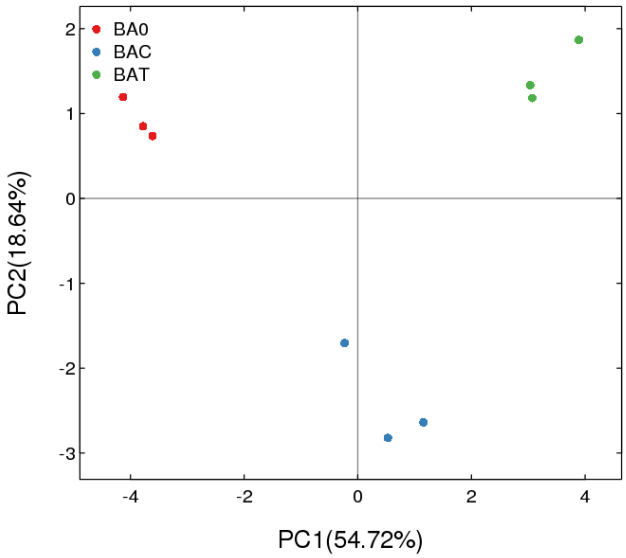

Figure 33 Principal components analysis based on OUT abundance of bacterial community. The number in brackets represented contributions principal components to differences among samples.

There were total 10,764 OTUs identified as bacteria found in soil of this experiment (Figure 32). Approximately 44% of them could be found in all soil groups. OTUs found only in *G. mosseae* inoculation soil were 17% and approximately 2 folds higher than those merely found in another 2 groups of soil. Forty percent of OTUs appeared after performing butterhead cultivation which 35% of them were shared between uninculation and *G. mosseae* inoculation treatments. The OTUs principle component analysis (PCA) of individual sample based on OTU abundance information suggested soil samples could be divided into 3 clusters followed their treatments (Figure 33).

The identification of each OTU was performed using Ribosomal Database Project Classifier. The number of OTUs and their count were calculated for diversity indices. Chao and Shannon-weiner diversity indices were expressed in Figure 34. Both Chao and Shannon's indices were highest in *G. mosseae* inoculation treatment followed by uninoculated treatment and soil before transplantation. Besides, both diversity indices also presented significant difference from each other.

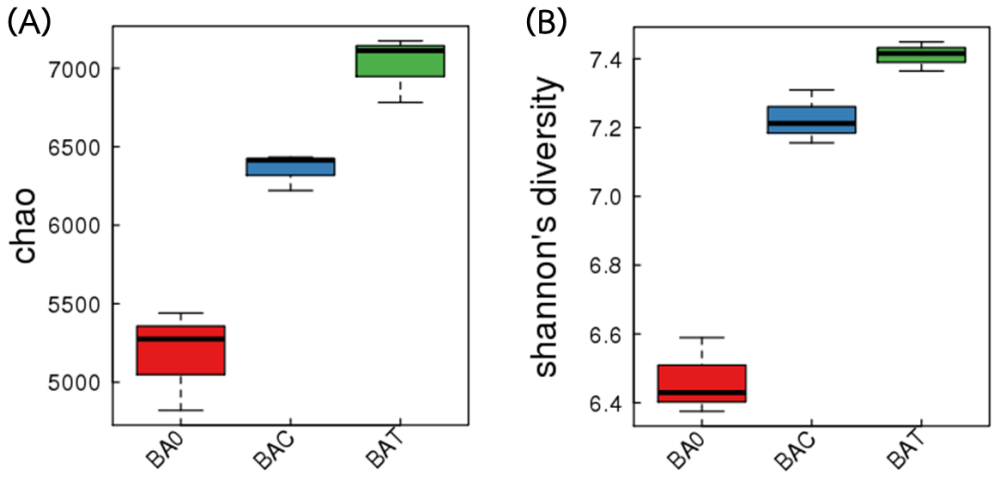

Figure 34 Diversity indices of bacteria based on OTUs number comparing between 3 groups of soil samples, before transplantation (BA0), at harvesting time of uninoculated (BAC) and *G. mosseae* inoculated (BAT) treatments.

Relative abundance of OTUs in each soil group at phylum taxa was presented in Figure 35. Total 48 bacterial phyla could be identified. Soil before transplantation group contained 40 phyla while uninocualated and *G. mosseae* inoculated soil contained 44 and 47 phyla, respectively. Means of relative abundance and statistical analysis of all obtained phyla are shown in Appendix C. The highest phylum abundance in soil before transplantation was phylum Proteobacteria followed by Acidobacteria and Verrucomicrobacteria. There was significant increase of Proteobacteria occurred in *G. mosseae* inoculation soil when comparing with soil before transplantation but uninoculation soil did not have any significant difference other treatments. The abundance of Acidobacteria after butterhead cultivation significantly decreased and G. mosseae inoculated soil presented lowest abundance among soil groups which nearly 2 folds lower than soil before transplantation. Even though, there was no significant difference of relative abundance of Verrucomicrobia among soil groups but this phylum exhibited lower after cultivation and had lowest in G. mosseae inoculation soil. Bacteroidetes was higher after plant cultivation which the abundance of this phylum in G. mosseae inoculated soil was obviously higher than uninoculation but it was not significant.

Moreover, there were many phyla were affected from plant cultivation and *G. mosseae* inoculation (see Table 16 in Appendix C). Lentisphaerae, PAUC34f, and ZB3 phyla were not detected in soil before transplantation and uninoculation group, but they could be found in very little amount in G. mosseae inoculation group. Relative abundance of Cyanobacteria and TM6 significantly increased at harvesting time comparing with before transplantation and the abundances of these phyla in *G. mosseae* inoculation were also significantly higher than uninocualtion. Actinobacteria was significanty decreased in *G. mosseae* inoculation soil after butterhead cultivation and also significantly lower than uninoculation group. This pattern of change was also found in phylum Chlamydie, GN02, and NKB19. Phylum Chlorobi and SRB1093 relative abundance after butterhead cultivation were significantly higher but it was only in *G. mosseae* inoculation group.

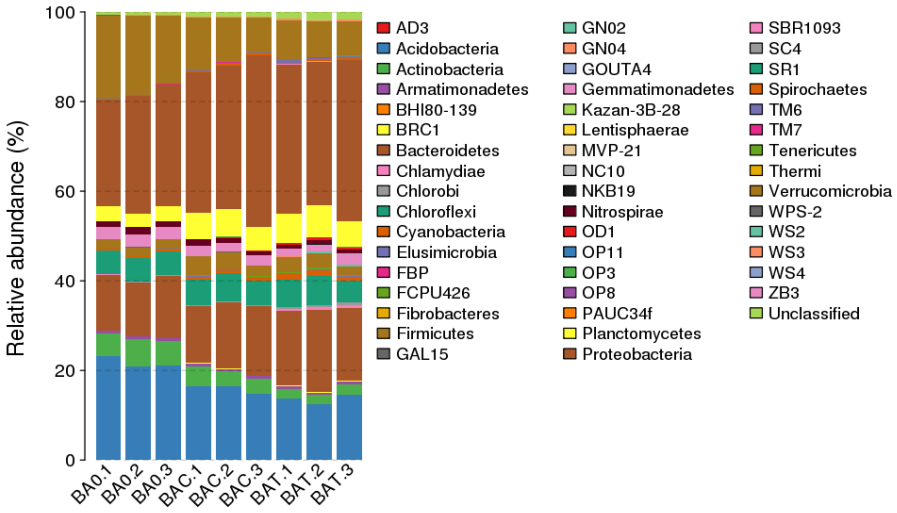

Figure 35 Relative abundance of bacteria at phylum taxa comparing between 3 groups of soil samples, before transplantation (BA0) and at harvesting time of uninoculation (BAC) and *G. mosseae* inoculation (BAT) treatments.

The highest abundance of bacteria at genus taxa was found in genus *DA101* followed by *Flavisolibacter* and *Kaistobacter*. *DA101* of soil before transplantation group was significantly higher than both groups of soil after butterhead cultivation which *G. mosseae* inoculation presented the lowest abundance among the other. *Flavisolibacter* and *Kaistobacter* was also significant decreased after cultivation. Genera which their relative abundance was higher after cultivation and highest in *G. mosseae* inoculation were *Anaeromyxobacter*, *Alicyclobacullus*, *Planctomyces*, *Flavobacterium*, *Aquicella*, and *Rhodoplanes* but only *Planctomyces*, *Aquicella*, and *Rhodoplanes* were significant (Figure 36). However, there were more genera which were affected from *G. mosseae* inoculation (see Table 17 in Appendix C). Genera *Hyphomicrobium*, *Pedomicrobium*, and *Psuedomonas* were higher after plant cultivation and highest among other soil groups while *Amycolatopsis*, *Candidatus_Koribacter*, *Streptomyces*, and *Novosphingobium* were decrease after plant cultivation and lowest among other soil samples. All the genera exhibited significant difference between uninoculation and *G. mosseae* inoculation group.

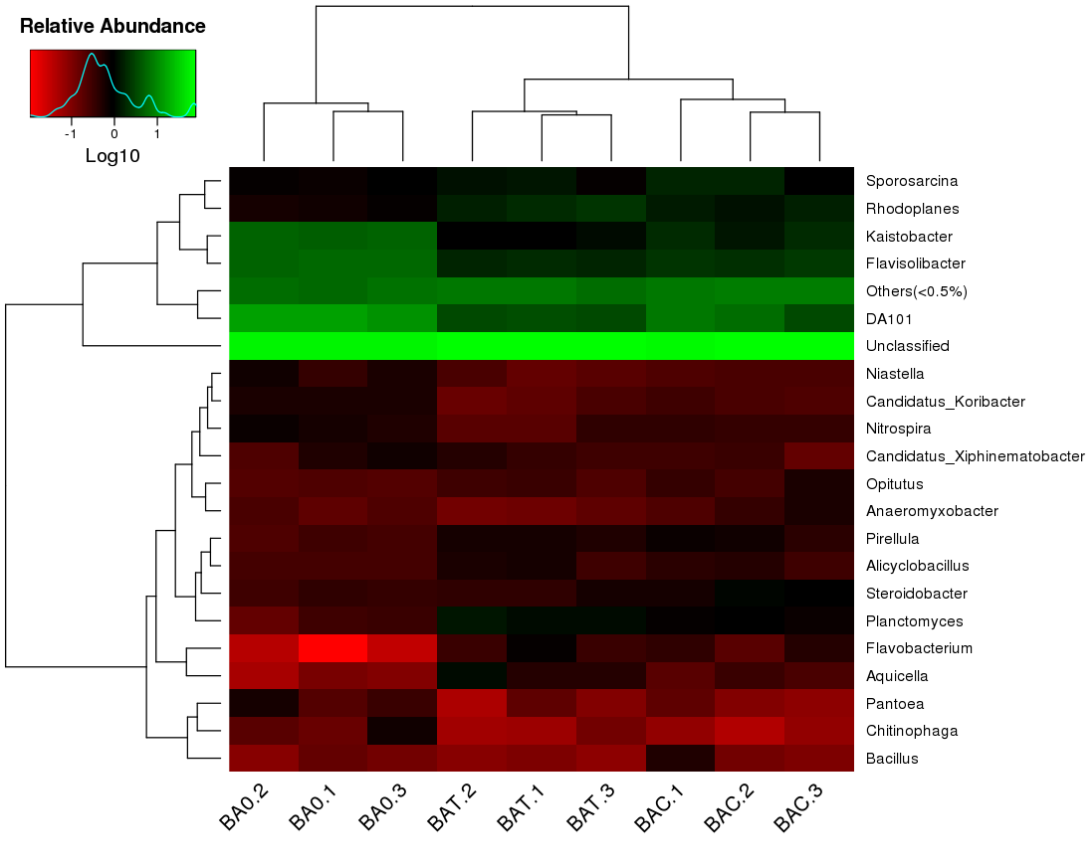

Figure 36 Heat map of Relative abundance at genus taxa of bacteria comparing between 3 groups of soil samples, before transplantation (BA0) and at harvesting time of uninoculation (BAC) and G. mosseae inoculation (BAT) treatments.

There were only 74 species totally found in all soil samples. Most of the top abundance species of bacteria were not affected from *G. mosseae* inoculation. *Psuedomonas alcaligenes* and *Lacibacter cauensis* were two species significant increased after plant cultivation and found to be the highest in *G. mosseae* inoculation group (Table 15). *Methylotenera mobilis* was very low and significantly difference in soil before transplantation. *G. mosseae* inoculation soil appeared lower abundance of this species than uninoculation group. See Table 16-18 in Appendix C for more significant difference in each taxa. Beyond those significant, there were many more interesting species affected plant growth. For some examples, *Burkholderia bryophila* was 3 folds lower after plant cultivation in uninoculation soil but *G. mosseae* could maintain abundance of this species at the same amount with before transplantation.

Table 15 Significantly different species from statistical analysis of relative abundance (mean) between each soil sample.

Species	BA0	BAC	BAT	*P value*
Methylotenera mobilis	0.0003	0.0420	0.0146	0.0265
Pseudomonas alcaligenes	0.0001	0.0238	0.0641	0.0265
Clostridium cellulovorans	0.0003	0	0.0003	0.0347
Siphonobacter aquaeclarae	0.0154	0	0.0006	0.0347
Pseudoxanthomonas kaohsiungensis	0.0016	0.0321	0.0087	0.0390
Legionella nagasakiensis	0.0004	0.0007	0.0003	0.0459
Lacibacter cauensis	0.0010	0.0022	0.0053	0.0486

4.3.2 Fungal community in rhizosphere soil of butterhead inoculated with *G. mosseae*

Rhizosphere fungal communities were compared as well as in bacterial community study. ITS1F-2 region of 16s rDNA was used to be target for libraries construction. Contrasting with bacteria, Obtained OTU did not show significant difference between groups of soil sample. The average OTU found in before transplantation, uninoculation, and *G. mosseae* inoculation were 428.67±3.28, 533.33±39.98, and 498.33±32.77, respectively (Figure 37). OTU of fungal community at harvesting time in both treatments seemed to be higher than community before transplantation. However, there was no significant difference in statistical analysis.

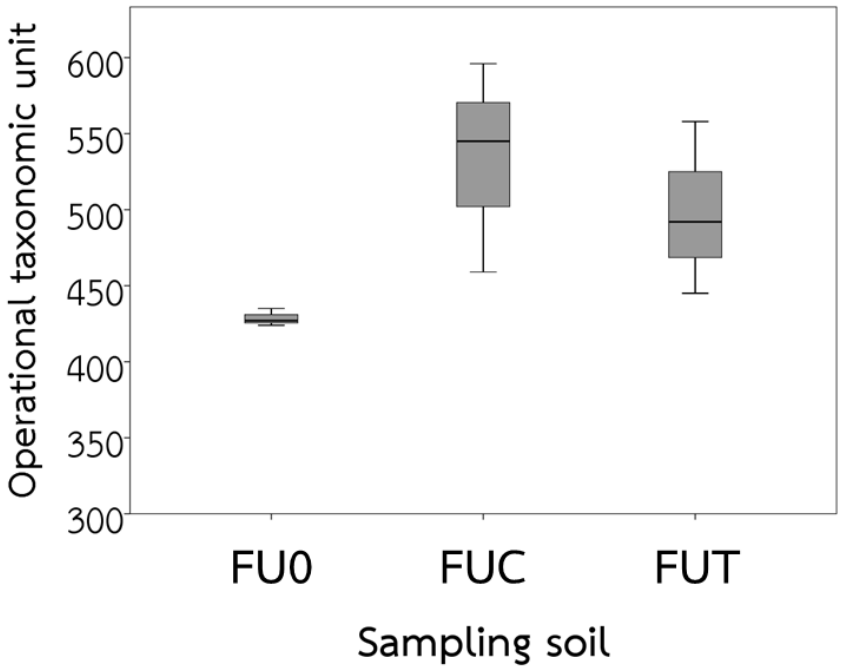

Figure 37 Operational taxonomic unit number of bacteria in 3 groups of soil samples, before transplantation and at harvesting time of uninoculation and *G. mosseae* inoculation treatments.

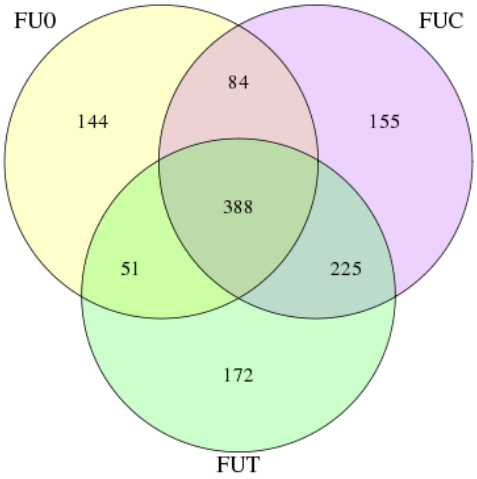

Figure 38 Venn diagram of shared operational taxonomic unit number of fungal between 3 groups of soil samples, before transplantation (FU0) and at harvesting time of uninoculation (FUC) and *G. mosseae* inoculation (FUT) treatments.

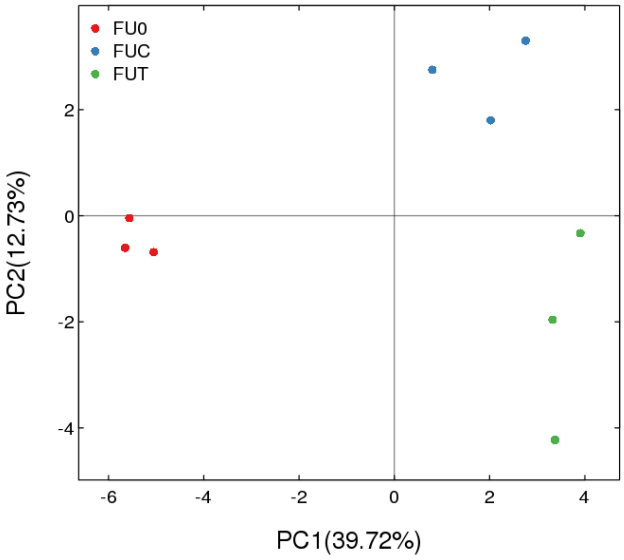

Figure 39 Principal components analysis based on OUT abundance of fungal community. The number in brackets represented contributions principal components to differences among samples.

For fungal clustering, there were total 1,219 OTUs identified as fungi found in soil of this experiment (Figure 38). Approximately 32% of them were shared OTUs among all soil groups. Approximately 45% of OTUs appeared after performing butterhead cultivation which 41% of them were shared between uninculation and *G. mosseae* inoculation treatments. *G. mosseae* inoculation had number of OTUs found only in this group higher than those found only in before transplantation and uninoculation groups. The PCA analysis based on OTU abundance information suggested the individual sample could be separate into clusters according to their groups of sampling (Figure 39).

The identification of each OTU was performed using UNITE database. The number of OTUs and their count were calculated for the same diversity indices with bacteria diversity, Chao and Shannon-weiner diversity indices. The statistical analysis suggested the significance differences of Chao index between before transplantation group and both groups after transplantation. The highest Chao index was found in uninoculation soil (Figure 40A). Shannon's index did not present differences between groups of soil (Figure 40B).

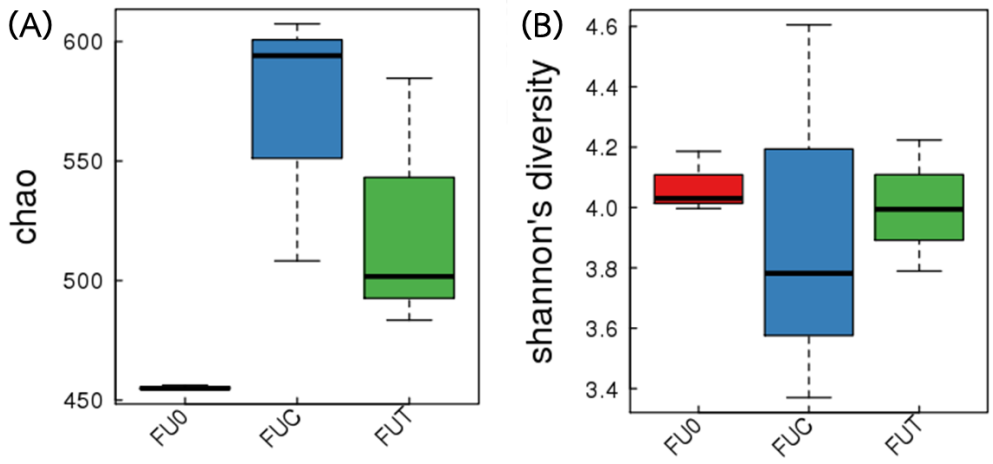

Figure 40 Diversity indices of fungi based on OTUs number comparing between 3 groups of soil samples, before transplantation (FU0) and at harvesting time of uninoculation (FUC) and *G. mosseae* inoculation (FUT) treatments.

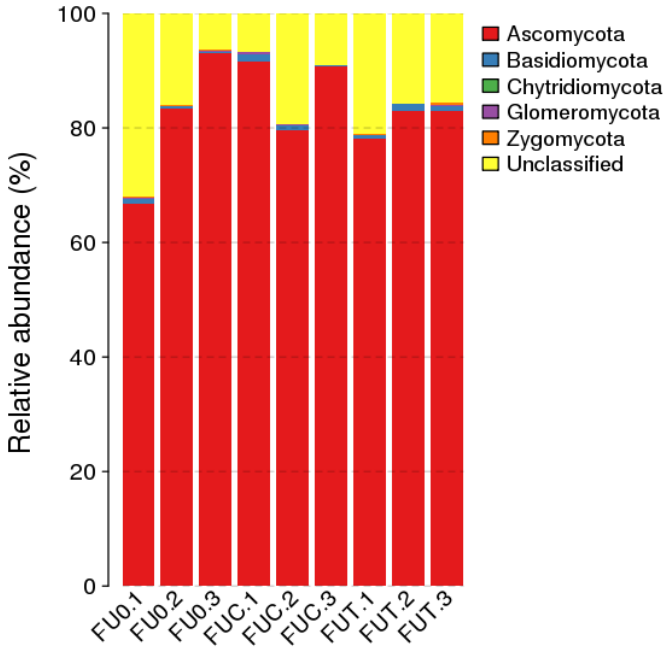

Figure 41 Relative abundance of fungi at phylum taxa comparing between 3 groups of soil samples, before transplantation (FU0) and at harvesting time of uninoculation (FUC) and *G. mosseae* inoculation (FUT) treatments.

The OTUs which could be identified presented 5 phylums, 80 families, 136 genera, and 193 species of fungi. Phylum of fungi most found in the community was in Ascomycota (Figure 41). The percentage of this phylum presenting in soil before transplantation, uninocualtion and *G. mosseae* inoculation treatment at harvesting time were 81.05, 87.28, and 81.30%, respectively which was not significant difference between each group. The other phyla found in community were Basidiomycota, Zygomycota, Glomeromycota, and Chytridiomycota which range from high to low abundance, respectively. At genus taxa, several genera were affected by *G. mosseae* inoculation. The highest abundance found in soil before transplantation was *Thielavia* followed by *Cochliobolus* and *Chaetomium* (Figure 42). The abundance of *Thielavia* and *Cochliobolus* in soil after plant cultivation dramatically decreased approximately 6 and 8 folds, respectively, and *G. mosseae* inoculation soil of both genera appeared lower than uninoculation. *Chaetomium*

also presented constant decrease after plant cultivation and was the lowest in *G. mosseae* inoculation soil but the statistical analysis was not significant.

Other groups of fungi with high abundance in fungal community were *Periconia*, *Aspergillus*, and *Furarium*. Even though these genera were not significant difference in statistical analysis, but they were affected by *G. mosseae* inoculation (see Table 20 in Appendix C). *Periconia* and *Aspergillus* were lower after plant cultivation and the lowest abundance of these 2 genera was found in *G. mosseae* inoculation soil. *Fusarium* abundance in soil after plant cultivation was decrease. However, the abundance of this genus was not difference between treatments. There were many more genera that were significantly affected by *G. mosseae* inoculation. The abundance of *Emericella* was extremely higher in both groups of soil after plant cultivation and *G. mosseae* inoculation soil exhibited 2 fold lower than uninoculation. The abundance of *Curvularia* after plant cultivation in both treatments was also extremely decreased and *G. mosseae* inoculation showed the lowest abundance by having approximately 27 folds and 6 folds lower than before transplantation and uninoculation soil at harvesting time, respectively. Significant increase of abundance after plant cultivation and the highest presented in *G. mosseae* inoculation was found in *Phaeosphaeriopsis*, *Dokmaia*, and *Pyrenochaetopsis*. *Candida* was another interesting genus which decreased after plant cultivation in both treatment. Meanwhile, the abundance of this genus was higher in *G. mosseae* inoculation when compared to uninoculation.

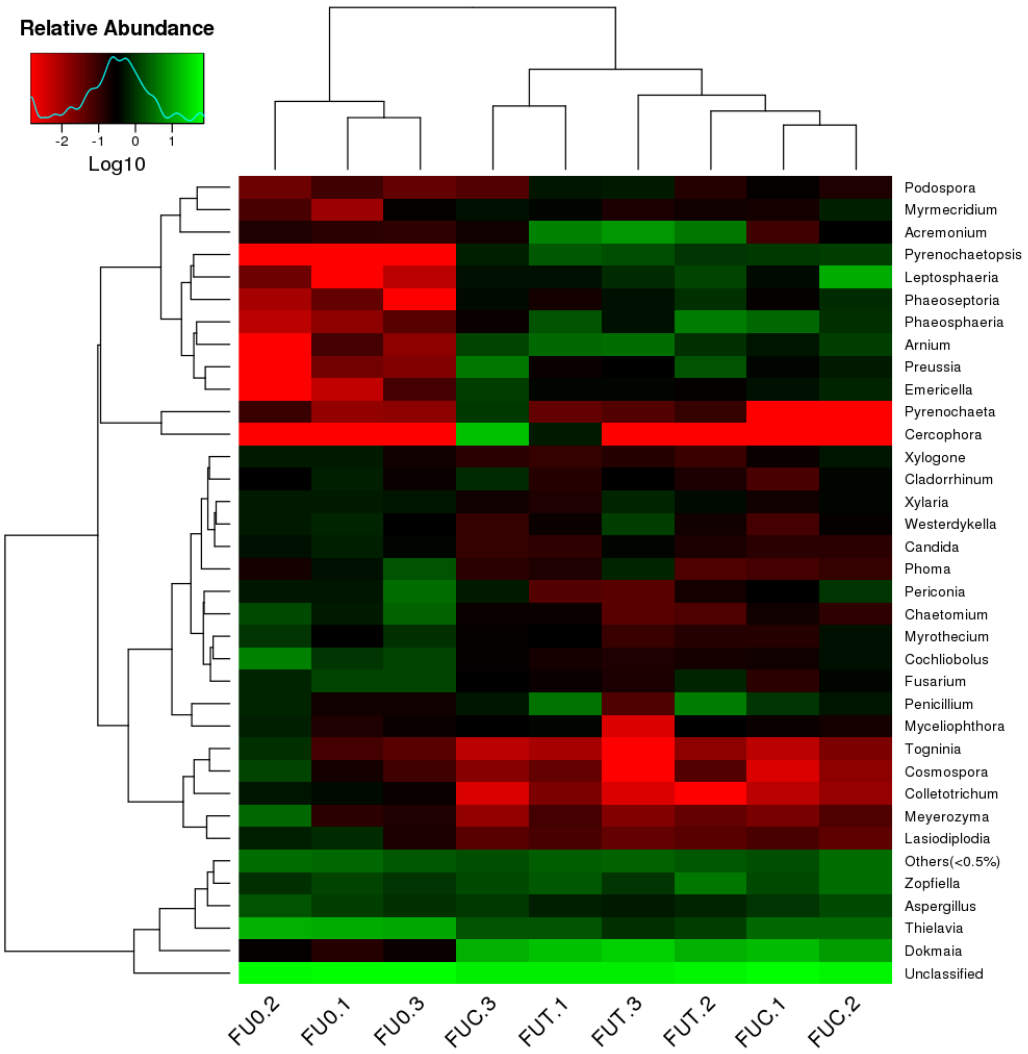

Figure 42 Heat map of Relative abundance at genus taxa of fungi comparing between 3 groups of soil samples, before transplantation (FU0) and at harvesting time of uninoculation (FUC) and *G. mosseae* inoculation (FUT) treatments.

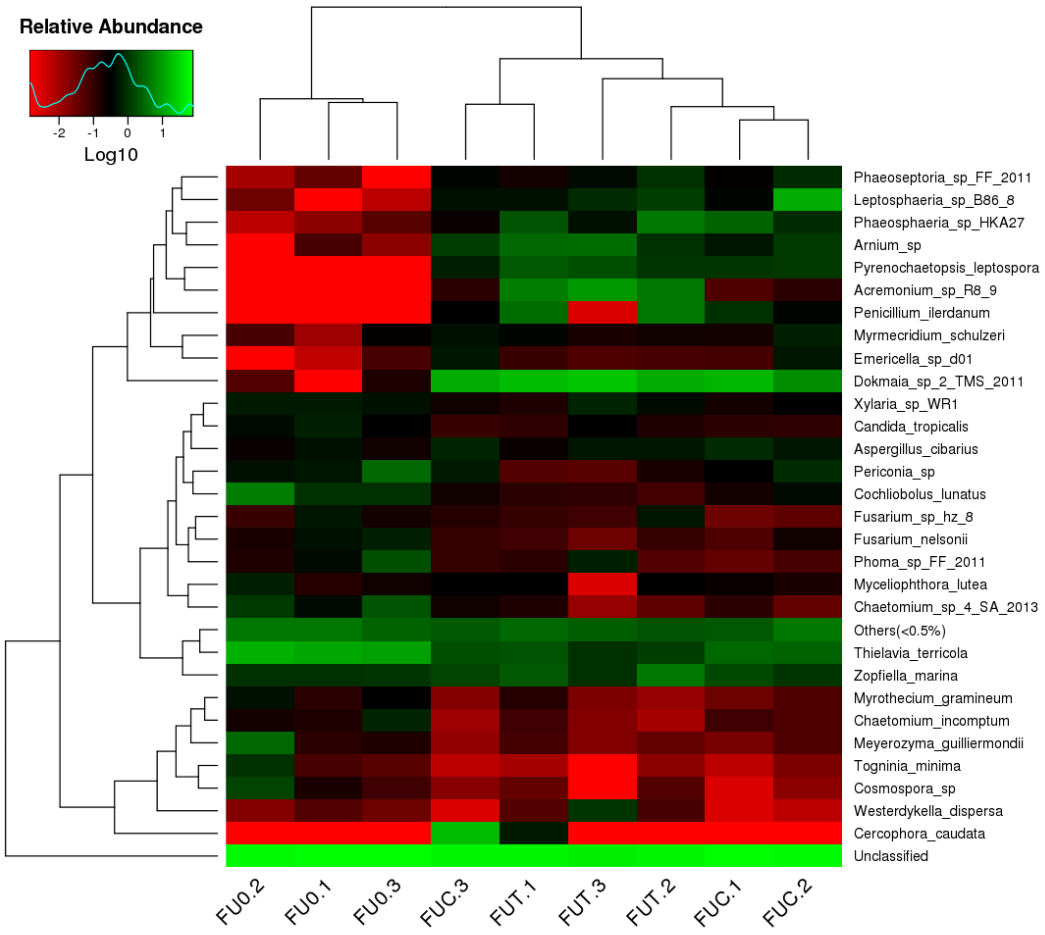

Figure 43 Heat map of Relative abundance at species taxa of fungi comparing between 3 groups of soil samples, before transplantation (FU0) and at harvesting time of uninoculation (FUC) and *G. mosseae* inoculation (FUT) treatments.

There were total 193 species that could be identified from all soil samples which was only 15.8% from all OTUs (see Table 21 in Appendix C). Figure 43 presented dynamic of fungi in species level. Means and statistical analysis was in Table 21 in Appendix C. Many species were found to have significantly effect from *G. mosseae* inoculation. *Thielavia terricola* and *Cochliobolus lunatus* were 2 of the highest abundance species. In soil before transplantation, their abundances exhibited extreme reducing after plant cultivation in both treatments with number of this species in *G. mosseae* inoculation soil was about 2 fold lower than

uninoculation. *Pseudallescheria angusta* was another species that its abundance dramatically decreased after plant cultivation but there was no significant difference observed between 2 treatments at harvesting time. There were many species presented the change similar to *Pseudallescheria angusta* such as *Cosmospora* sp., *Chaetomium incomptum*, *Myrothecium gramineum*, *Phaeoacremonium fuscum*, and *Alternaria alternata*. Fungal species which the abundance in *G. mosseae* inoculation was less than uninoculation and were showed the absence before plant cultivation then later found in community after plant cultivation were *Schizothecium carpinicola*, *Stachybotrys microspore*, and *Thermomyces lanuginosus*.

On the other hand, *Acromonium* sp. R8_9, *Phaeosphaeriopsis* sp. C652, *Dokmaia* sp.2 TMS 2011, and *Pyrenochaetopsis leptospora* dramatically increased after plant cultivation. *G. mosseae* inoculation exhibited significant enhancement the abundance of these species higher than uninoculation. Moreover, others important species were also affected from *G. mosseae* inoculation but they did not show significant difference. For examples, *Aspergillus cibarius* in uninoculation soil increased approximately 2 fold higher than before transplantation while this species in *G. mosseae* inoculation group presented similar abundance number with soil before transplantation (see Table 21 in Appendix C). *Penicillium ilerdanum* was absence in soil before transplantation. At harvesting time, the abundance of this species in *G. mosseae* inoculation was 5 folds higher than uninoculation soil. The abundance of *Togninia minima* and *Fusarium nelsonii* dramatically reduced after plant cultivation and *G. mosseae* inoculation soil exhibited slightly lower than uninoculation soil. *Phoma medicagnis* abundance in *G. mosseae* inoculation soil was 12 folds lower than before transplantation and 4 folds lower than uninoculation soil. *Candida tropicalis* abundance was higher in *G. mosseae* inoculation comparing to uninocualtion which both treatments were also lower than before transplantation.

CHAPTER V

DISCUSSION

5.1 Effect of isolate and amount of AMF spores on growth of butterhead

All the single AMF spores selected as representative from each location were healthy. However, only approximately 50% of them could infect host plant or propagate to get enough amount of spores. Even though sorghum, the host for large scale production of AMF spores in this study, was reported as wide range for AMF infection (Carrenho et al., 2002) but the result showed that not all species of AMF can be propagated using this host. Carrenho and colleagues also presented the proportion of cultureable AMF species in that 85% of them were in Glomeraceae followed by Acaulosporaceae and Gigasporaceae. This proportion was similar to a report by Schalamuk and Cabello (2010) indicated that Glomeraceae was found to be the highest abundance in natural soil followed by Acaulosporaceae and Gigasporaceae. In this study, 50% of obtained AMF spores were Glomeraceae that could confirm those finding. There were more than a family could be observed from a location which may contain more than 1 species in each family. However, the diversity of AMF in each location depends on many factors which the most important one is soil management (Brundrett et al., 1999).

From the result, AMF in different families presented different ability to promote plant growth. A major factor impacting plant growth in limited condition environments is the compatibility and specificity between AMF isolate and plant host (Estrada et al., 2013). To maximize the beneficial role of AMF, the isolate needs to be chosen to benefit each plant species. This conclusion was confirmed by comparison of growth within AMF families in the study. Family Glomeraceae in especially *Glomus* spp. is the most AMF to be studied and reported as the best that had advantages on plant growth (Quilambo, 2003; Sebuliba et al., 2012).

Lettuce growth between the 2 pot experiments appeared to be different. Yield obtained from all treatments in the second experiment was greater than in the first

experiment which the purpose was to select potential AMF isolates. In order to evaluate the exact effects of AMF isolates, no fertilizer was used in the first experiment and difference in growth induction occurred. However, yield in the first experiment was not satisfactory due to small heads. In the second experiment, manure was added to improve quality and inoculation with AMF improved yield, head size and appearance. The positive effect of AMF combined with manure on growth has been reported in other crops (Nwangburuka, 2010).

Lettuce is a short-lived plant which may not be affected by AMF as much as annual or perennial plants due to duration of colonization (Azcón-Aguilar and Barea, 1997a). The hypothesis of supplementing lettuce with higher numbers of AMF spores was to improve colonization in plant roots. However, increasing numbers of spores may not always lead to increased plant growth. There is evidence that increased spore density (varying between 265-3180 spores) did not always improve plant growth (Mala et al., 2010). Study of effect of *G. intraradices* on sorghum biomass also presented best efficiency of using the fungus at optimum concentration (Dabiré et al., 2007). The research showed sorghum biomass had no significant difference between 10 and 100 spores of the fungus inoculation in non-disinfected soil. Use of 50 spores of *G. mosseae* per plant was the most appropriate inoculum concentration for this species for improving yield. The interaction effect of AMF isolate and the number of spores also confirmed the efficiency of AMF application on plant growth improvement. So, it was necessary to find the suitable condition of AMF application on each plant species or even varieties. Moreover, there was specificity of using each AMF isolate in each cultivation condition as well (Orłowska et al., 2012).

The mechanism of AMF *G. mosseae* in improving plant growth is not only due to increase nutrient uptake, but also to increase chlorophyll a and b contents (Latef and Chaoxing, 2011). A greater photosynthetic rate was reported in *G. intraradices* inoculated plants (Demir, 2004). However, the result of chlorophyll contents, both chlorophyll a and b, in pot experiment was different. The treatments contain high amount of chlorophyll comparing to other treatments were not in the high ranking of butterhead growth. Besides, the AMF can alleviate osmotic stress, affect PGPR induction and phytoremediation (Quilambo, 2003). This study aimed to determine

proper AMF species and concentration of spores to use to improve growth of butterhead. So, fifty spores of *G. mosseae* were chosen due to the best and suitable amount to enhancement of plant growth in simulated organic production.

The colonization of plants by AMF depends on plant species and external factors such as temperature, other soil-microorganisms and plant growth matrix (Hayman, 1983). The first pot experiment in this study was done during the rainy season and the second during the hot, dry season, with differences in temperature and rainfall. Temperature can affect length of extraradical mycelium but may not affect colonization capability in roots of host plants (Heinemeyer and Fitter, 2004; Vierheilig and Ocampo, 1991), and variation in colonization ability occurred between experiments in this study. Colonization of AMF in uninoculated roots occurred due to the commercial soil used not being sterile. It may also be that the manure contained some mycorrhizal inoculum which could account for exhibiting infection and colonization in control treatment. Colonization percentage of all AMF isolates, except isolate N2, tended to decrease in the second experiment. It has been noted that AMF do better in colonizing plant roots under low nutrient conditions, especially phosphorous (Ijdo et al., 2010). Use of manure in the second pot experiment may have led to increased availability of nutrients in soil resulting in a lower colonization percent compared to the first experiment.

The relationship between butterhead growth and colonization percent was similar to previous research with other varieties of lettuce (*L. sativa* L. var. *capitata* and var. *longifolia*) under different growing conditions (Baslam et al., 2011). It was determined that colonization percentage differed due to AMF species and growth induction was not related to colonization level. Most isolates of family Glomeraceae in this study presented great colonization percentage, however, only some isolates had ability to induce butterhead growth. Growth of inoculated plants in the second pot experiment did not differ from the control as much as in the first experiment. It was possible that the lower colonization percent led to lower growth.

The variation of growth enhancement level in selecting appropriated isolate and amount of AMF for enhancing growth of butterhead lettuce was shown in this study. The chosen isolate, *G. mosseae*, was suggested as one of the popular species

to study according to the report of their several specific advantages on plant growth. This fungal species can increase nitrogen fixation of rhizobium and induce nodulation (Ganry et al., 1982). It was found that the species can secrete chemical substance which contain plant growth regulator such as auxin, cytokinin and gibberellin (Barea and Azcón-Aguilar, 1982).

5.2 The application of selected AMF spore amount and isolate on yield and quality of butterhead in natural field cultivated under organic farming system

The usage of *G. mosseae* inoculation in butterhead cultivation enhanced growth of plant 15 days earlier than uninoculation. Similar to application of *G. mosseae* in rice production, rice growth inoculated with this AMF species was enhanced 11 days earlier than uninoculated plant (Li et al., 2012). The results of this study exhibited potential application of AMF in yield improvement of butterhead cultivated in more than 10 years abandon area. Morphological study of butterhead root in uninoculation treatment showed root colonization which could result from existing AMF spores in natural soil. However, the correlation analysis between colonization percentage and plant growth not only confirmed that *G. mosseae* inoculation affected on growth of butterhead lettuce but could also confirm that colonization occurred in uninocualted treatment didn't involve with growth of butterhead lettuce. The result of correlation between colonization percentage and growth parameters in uninoculated treatment also revealed that plant growth induction by AMF application needs specificity between plant and fungal species.

Even though, advantages of AMF on plant growth in difference aspects have been introduced since 1980s, most studies have agreed that the effects of AMF on plant growth and development are complex by reasons of the interaction between host plant genotype and AMF isolate (Baum et al., 2015). The above pot experiments indicated this specificity in term of growth promotion ability. The effect of growth improvement of AMF was fundamentally by increasing in nutrients uptake, particularly phosphorous, from soil to plant root via their hyphae (Smith and Read, 1997b). This effect was confirmed by high fungal colonization that led to high leaf number and

fresh weight. Benefit of AMF in plant production improvement obviously showed on fresh weight and number of leaf that reflected yield of butterhead crop, however, plant biomass obtained from dry weight presented only a slight increase. Colonized plants with AMF that had no effect on change of plant dry weight were previously reported in soybean, lettuce, maize and etc. (Köhler et al., 2008; Porcel and Ruiz-Lozano, 2004; Zhu et al., 2012). The strongly fresh weight induction of AMF inoculated butterhead may be due to upper level of water content in leaves affected from AM colonization (Augé, 1987). Allen (1982) presented that AMF could directly absorb and transfer soil water to inoculated root. Previous researches also suggested the attribute of AMF on soil moisture extraction and transportation to plant root via their hyphae that may cause higher leaf water content (Hardie and Leyton, 1981).

Photosynthesis activity and chlorophyll contents were investigated to illustrate the mechanism of growth induction by AMF. The response pattern of plant performance in increasing photosynthesis level in this study was corresponding to the study of *G. intraradice* in maize (Zhu et al., 2012). However, the effect of AMF isolate on photosynthesis was reported to be diverse in plant species and AMF isolates (Wu and Xia, 2006; Zhu et al., 2014). Review by Augé (2001) indicated that colonizing with different species of AMF could produce different results of photosynthesis parameters under abiotic stresses. For example, *G. fasiculatum* colonization tended to increase stomatal conductance in many plants while *G. mosseae* colonization presented less often in simulating this parameter, similarly with no difference observed in stomatal conductance in this study. Slightly lower of intercellular CO_2 under similar value of stomatal conductance in *G. mosseae* inoculation may result from high CO_2 usage for high photosynthesis rate. However, photosynthesis rate often increased in most of the AMF isolates inoculation (Augé, 2001; Wu and Xia, 2006; Zhu et al., 2014). Net photosynthesis of lettuce was varied from 2.76-12.02 μmol CO_2 $m^{-2}s^{-1}$ according to many environment conditions (Caporn et al., 1994) while net photosynthesis observed in this study measured early in the morning was 4.27-6.12 μmol CO_2 $m^{-2}s^{-1}$. Not only environment, but also time of measurement affected photosynthesis performance due to the evidence presenting change of the parameter along day and night time (Ogle et al., 2012). The study also suggested time showing the highest photosynthesis

performance differed according to plant species. Transpiration rate was directly influenced by soil moisture which was increase by effect of AMF inoculation (Kozlowski, 1987).

Many studies have agreed that AMF inoculation had positive effect on chlorophyll contents in most of the plant species (Hazzoumi et al., 2015; Rahmaty and Khara, 2011; Riveros et al., 2013) including local varieties of lettuce in Spain (Baslam et al., 2011). The increase in chlorophyll contents was suggested as resulting from major ability of AMF in higher nutrients uptake especially magnesium which is a main component of chlorophyll structure (Stobart et al., 1985). This chlorophyll induction was suggested to induce some core proteins involving with photosystem I and II and light-harvesting complex II in Indica rice and consequently increased photosynthesis capacity of plant (Davies et al., 1993; Kang et al., 2012). The higher of available phosphorous in AMF inoculated soil was also suggested as another important factor in photosynthesis induction of lettuce by enhancing energy in form of ATP for CO_2 assimilation in Calvin cycle (Luo et al., 2009).

Rhizosphere pH in AMF inoculated soil appeared to be more neutral than no-AMF treatment when compared to before transplanting soil pH. AMF were reported to be able to neutralize soil pH by production or consumption of H^+ or by secretion of some organic substances (Jolicoeur et al., 1998). The effectiveness of AMF in plant growth induction also involved with their species. *G. mosseae* was stated for capability to absorb nutrients from soil in wide range of pH, both alkaline and calcareous soil (Clark et al., 1999). This AMF species was also reported to associate with some plant growth promoting rhizobacteria (PGPRs), such as *Psuedomonas* sp. and *Bacillus* sp., living on or inside AMF spore wall which benefits plant (Bharadwaj et al., 2008). These PGPRs are responsible for decomposition of both inactive and active soil organic matter (Vijayabharathi et al., 2015). Thus, the increase in organic matter in AMF inoculated soil can be implied that it may result from induction numbers of these PGPRs. These organisms have ability in releasing several nutrients into available form that plant root and fungi can uptake and finally transport to root cells.

Available phosphorous of rhizosphere soil before transplanting was classified into low level. Supplementing butterhead cultivation with AMF strongly enhanced

level of phosphorous in usable form. Increase of phosphorous is significant and unique characteristic of AMF due to their ability to produce and secrete phosphatase to convert phosphorous in inactive form into phosphate (Koide and Mosse, 2004). In accordance with soil properties improvement, major nutrients in both leaf and root were induced. The inductions were apparently observed in root more than leaf when comparing between treatments. It could be because lettuce is short-lived plant which have inoculation period less than 60 days and nutrients in leaf are influenced by AMF less than longer-lived plant (Azcón-Aguilar and Barea, 1997a).

Unlike many researches, antioxidant enzyme activities, CAT and APX, in our study were not induced by AMF inoculation. Blilou et al. (2000) demonstrated that *G. mosseae* could induce activities of CAT and APX in root of tobacco, yet it was transient. They also suggested that the highest activity of both enzymes related with the first appressoria entering of AMF into plant root, after that the activity of both enzymes decreased over time. The increasing of these antioxidant enzymes occurred via defense mechanism of plant responding to infection by fungi including AMF (Gianinazzi-Pearson, 1996). The overall activities of antioxidant were commonly expressed as percentage inhibition of DPPH free radical, representing radical quenching kinetics (Hogg et al., 1961; Xie and Schaich, 2014). AMF were recently reported the ability in inducing DPPH scavenging activity of many plant species such as cyclamen and onions (Maya and Matsubara, 2013; Mollavali et al., 2015). Study in onions also indicated that the capability of DPPH scavenging was affected by interaction between plant and AMF species. Amount of chosen non-enzymatic antioxidants, AA, and CAR, in both control and inoculated treatment were similar. According to increasing of water content in butterhead leaves, the dilution of these contents may occur and lead to no difference between treatments. Moreover, there may be other induced antioxidants substances affected by AMF in which consequently resulting in greater DPPH scavenging activity. Nevertheless, the mechanism of AMF in antioxidant induction is still unclear.

5.3 Bacterial and fungal communities of arbuscular mycorrhizal fungus (*Glomus mosseae*) inoculated soil planting butterhead lettuce

AMF were reported to have impact on bacterial and fungal communities in many ways such as increasing plant growth promoting rhizobacteria (PGPR) or decreasing some plant pathogens (Hodge and Fitter, 2010). The examples of PGPR genera are *Bacillus*, *Burkholderia*, *Pseudomonas, Rhizobium (Bhattacharyya and Jha, 2012).* The study of *G. mosseae* inoculation in this study was shown significant impact on soil properties. However, the mechanism was still unclear. Observation on changes of bacterial and fungal communities could partially explain the effects occurred. The results in this experiment suggested that many organisms in both bacterial and fungal communities were altered by *G. mosseae* inoculation. Similar to the previous suggestion, the number of total rhizosphere bacteria in the present study was increased by AMF inoculation (John, 2001). Meanwhile, abundance of several fungi was lower in *G. mosseae* inoculated treatment in agreement with Waschkies et al. (1994). However, *G. mosseae* inoculation did not change dominant bacteria and fungi of the community in all taxa. The diversity indices, Chao1 index was used to represent species richness and Shannon-weiner's index was used to represent species richness and evenness. Species richness and evenness of bacterial community were found to increase after plant cultivation in both uninoculation and *G. mosseae* inoculation. The result was difference in fungal community that the fungal species richness increase after plant cultivation but *G. mosseae* inoculation treatment presented significantly lower than uninoculation. The changes of rhizosphere microbes in both bacterial and fungi can be occurred via direct and indirect effects from specific AMF inoculation. The direct effect was the secretion of substance which can induce or suppress susceptible microbes in the community (Mar Vázquez et al., 2000). For an example, mycelium of *G. intraradices* can secrete exudate which reduce the germination of *Fusarium oxysporum* in carrot root *in vitro* culture (Filion et al., 1999). Other direct effects such as changing soil pH and competing nutrients acquisition (Johansson et al., 2004). Some substances secreted from AMF can induce plant defense response to parasitic

nematodes such as *Radopholus similis* and *Pratylencus coffeae* which is an indirect effect (Elsen et al., 2008).

Proteobacteria and acidobacteria were reported as the highest abundance in many soil bacterial communities which can be implied that bacterial phyla distribution may be similar in many geographic region (Griffiths et al., 2006; Rösch et al., 2002; Sun et al., 2004). These 2 phyla are vary in functions due to their diverse group of organisms. However, their high abundance in soil throughout the world indicate that they may have an indeed impact on soil functioning. Proteobacteria was found to increase in all class (Alpha-, Beta-, Gamma-, and delta-) after performing plant cultivation in both treatments with no difference between them. The availability of carbon increase in planted soil was reported to promote the bacterial population (Fierer et al., 2007). Soil organic matter of both treatments in the field experiment increased which may lead to the increasing in carbon and consequently number of the bacteria. Besides, Smit et al. (2001) hypothesized that the ratio between Proteobacteria and Acidobacteria may involve with nutrient status in soil that the higher ratio represents higher nutrient. Ratio of Proteobacteria to Acidobacteria in this study were 1.19, 2.13, and 2.49 in soil before transplantation, uninoculation and *G. mosseae* inoculation at harvesting time, respectively, which was similar to the change of soil organic matter as well. The decrease of Acidobacteria was resulting from the increase of soil pH according to some of them were acidophilic (Lauber et al., 2009). Moreover, many genera in Proteobacteria were reported as 'mycorrhiza helper bacteria', which is a group of bacteria promoting mycorrhizal symbiosis, such as *Pseudomonas*, *Burkholderia*, *Bradyrhizobium*, *Paenibacillus* and *Streptomyces* (Frey-Klett and Garbaye, 2005).

Previous study reported Verrucomicrobia was less found in soil community (approximately 7%) (Janssen, 2006). The observation of Verrucomicrobia abundance in 181 soil communities from 11 types of area was done and the dissimilarity of abundance was presented (Bergmann et al., 2011). The report found that abundance of this phylum was high in grassland and lower in agriculture performed land which was similar with this study. Verrucomicrobia were also reported for survival ability in low nutrient level (Janssen, 2006). Verrucomicrobia in soil after plant cultivation in

both treatments were lower than before cultivation which may relate to higher in soil nutrients. Lentisphaerae and Chlamydiae were closed to Verrucomicrobia but they were affected by *G. mosseae* in contrasting with Verrucomicrobia. Lentisphaerae was found only in *G. mosseae* inoculation in very small amount. It was possible that these phyla contaminated in the inoculum as well as PAUC34f and ZB3. However, there is no report on the effect of these phyla on plant cultivation.

Glomus hoi was reported to be able to alter community of bacteria in decomposer group (Nuccio et al., 2013). The result of Nuccio et al. (2013) exhibited decreases in both relative abundance of Actinobacteria and Bacteroidetes but the result of the present study was different that Bacteroidetes was induced in *G. mosseae* inoculated soil. It could be implied that there was different in ability of each AMF species on changes of bacterial population. The increase of Cyanobacteria is benefit on plant production. *G. mosseae* was suggested to replace the use of fertilizer especially nitrogen (Karthikeyan et al., 2007). *G. mosseae* was previous known to have an ability to increase nitrogen fixation in soil (Ganry et al., 1982). It could be indicated that *G. mosseae* can increase nitrogen fixation in soil via increasing of Cyanobacteria. Interestingly, *Pseudomonas alcaligenes* induced by *G. mosseae* inoculation was reported to be antagonist against *Fusarium oxysporum*, a plant pathogen causes *Fusarium* wilt disease (Widnyana et al., 2013) which is one of the agricultural problem in lettuce production around the world (Gordon and Koike, 2015). Thus, *G. mosseae* can also be applied in the field in aspect of preventing the disease.

The occurrence of several soil fungi was influenced by environment and soil management and it can be used to indicate soil health and sustainability (Doran and Zeiss, 2000). Fungi play many important roles in agriculture such as decomposer, plant pathogen, or nutrient cycler, so the change of their community in agriculture field can be a sensitive indicator of soil quality (Kennedy and Smith, 1995). The result suggested no change of composition in fungal community found in this study but some of fungi were obviously affected by *G. mosseae* inoculation. Ascomycota comprises of more than 64,000 named species, thus the phylum is the highest abundance found in soil (Aislabie and Deslippe, 2013). There was an indigenous arbuscular mycorrhiza fungus found in this area which is in family Acaulosporaceae

correlating with colonization percentage occurred in uninoculation treatment (see Table 19 in Appendix C). *Thielavia terricola* was the highest abundance in fungal community and found dramatically decrease after plant cultivation but it was not affected by *G. mosseae* inoculation. *G. mosseae* inoculation can suppress *Cochliobolus lunatus*, cause of leaf blight in rice, similarly with previous study but the mechanism of suppression is still unknown (Koide and Mosse, 2004). *G. mosseae* inoculation was found to have ability in decreasing many fungal genera which were plant pathogens. Those inhibited plant pathogens were *Curvularia*, *Aspergillus*, and *Periconia* which contain many species causing important plant diseases (Oliveira et al., 2015). *Aspergillus niger* was previously found to decrease when it was co-inoculated with *G. mosseae* (McAllister et al., 1995). Vice versa, the germination and hyphal growth of *G. mosseae* was also inhibited by many species in genus *Aspergillus*. The study of Mcallister et al. (1995) also suggested colonization with AMF prior infected with pathogens could protect plant from disease damages. Plant root exudates modification by effect of *G. mosseae* was proposed as possible mechanism of reducing this genus as well as other pathogenic fungi (Schwab and Lindsay, 1983).

Most of the abundance in genus taxa of fungi tended to be reduced by *G. mosseae* inoculation. However, the inoculation appeared to extremely increase some genera such as *Phaeosphaeriopsis*, *Dokmaia*, and *Pyrenochaetopsis*. Nevertheless, these genera have not been reported their effect on plant, so they are very interesting to be characterized due to the extreme change after plant cultivation and the significant higher in *G. mosseae* inoculation. Even though number of identified species of fungi was nearly 3 folds more than bacteria, but only 10% of them were changed in *G. mosseae* inoculation and the function of them remain unknown.

CHAPTER VI

CONCLUSION

6.1 Conclusions

6.1.1 Effect of isolate and amount of AMF spores on growth on butterhead

AMF had an efficiency to induce growth of lettuce. However, difference AMF isolate showed difference efficiency of lettuce growth induction. Isolate N3 was the best isolate that could significantly raise yield of lettuce by increasing diameter, leaf width, leaf length and leaf fresh weight compared to control treatment, followed by N2, N4, N5 and R5, respectively. Percentage of root colonization may not have any correlation with growth of lettuce. The best 5 isolates of AMF (N2, N3, N4, N5, and R5) were selected to study for their effects on growth parameters of lettuce.

Butterhead inoculated with 200 spores of isolate R5 and 50 spores of isolate N5 presented the highest efficiency to increase number of leaf, leaf fresh and dry weight of butterhead significantly different from control. But there were no significantly difference change observed in diameter, leaf width and length. Chlorophyll a content in 50 spores of isolation N2 inoculated butterhead significantly increased while chlorophyll b presented a slight increase with no significant difference from control. Chlorophylls may not involve with growth induction which affected from AMF in pot experiment. According to the results, AMF could enhance yield of butterhead by increasing diameter, leaf number and fresh weight but not leaf size. Fifty spores of isolate N5 was selected and used for field study. The isolate was identified as *Glomus mosseae*.

6.1.2 The application of selected AMF spore amount and isolate on yield and quality of butterhead in natural field cultivated under organic farming system

Growth improvement of 60 days-old butterhead was presented in AMF treatment by significantly increase in leaf number and leaf fresh weight compared to uninoculation. The results in field experiment related with pot experiment. The percentage of colonization also showed significantly higher in *G. mosseae* inoculated treatment. However, there were no difference in diameter, leaf dry weight and leaf size. Similar with pot experiment, chlorophyll a content of *G. mosseae* inoculated butterhead in field showed significantly higher than uninoculation while chlorophyll b content of two treatments were not difference. Net photosynthesis and intercellular CO_2 in which net photosynthesis of 60 days-old butterhead inoculated with *G. mosseae* was significantly higher than uninoculation while intercellular CO_2 of inoculated butterhead was lower. The results suggested that higher growth rate of butterhead derived from increasing in leaf number. Moreover, the results can also be presumed that AMF could increase chlorophyll a content which leads to elevation of net photosynthesis and in growth induction.

Rhizosphere soil of 60 days old Butterhead inoculated with *G. mosseae* was found to have the highest organic matters, available phosphorous and exchangeable potassium. However, only available phosphorous of *G. mosseae* inoculated soil was significant difference from uninoculated soil. Calcium and magnesium in rhizosphere soil decreased after butterhead cultivation. Although, *G. mosseae* inoculated treatment resulted in significantly higher amount of these nutrients than control, total nitrogen, phosphorous and potassium in leaf and root of butterhead were not difference. For studying plant nutrition in term of antioxidant substances, the result showed that activity of CAT and APX were not significant difference between treatments as well as vitamin C and phenolic content. Interestingly, scavenging of DPPH free radical revealed 2 times higher in *G. mosseae* inoculated plants than uninoculation. This may be caused by the

increasing of other non-enzymatic antioxidant substances besides vitamin C and phenolic content.

6.1.3 Bacterial and fungal communities of arbuscular mycorrhizal fungus (*Glomus mosseae*) inoculated soil planting butterhead lettuce

Bacterial and fungal communities in *G. mosseae* inoculated soil presented higher OTUs compared to uninoculation and before transplantation. The diversity indices suggested changes in both bacterial and fungal communities. Many bacterial phyla abundance were changed according to *G. mosseae* inoculation. Proteobacteria and Acidobacteria were among the highest abundance in phyla taxa. Proteobacteria was found to be increased while Acidobacteria was decreased in *G. mosseae* inoculated soil. All fungal phyla did not have difference between soil communities. In genus taxa, the highest fungal genera were *Thielavia* and *Cochliobolus.* Both of them exhibited dramatically decreases in *G. mosseae* inoculated soil. Some of fungi which are plant pathogens were also suppressed by *G. mosseae* inoculation such as *Cochliobolus lunatus* and *Aspergillus* sp. Nevertheless, there were many more genera and species of bacteria and fungi which were found affecting by *G. mosseae* inoculation and also by plant cultivation, but their functions have not been studied yet.

6.2 Suggestion for further study

6.2.1 According to the propagation ability of AMF colonized plant root, butterhead growth observation of second crop in the same field without adding more AMF spore into the field is very interesting to be evaluated.

6.2.2 Some bacteria or fungi with known function affected from both plant cultivation and AMF inoculation should be isolated and studied for other potential improvement factors in plant cultivation.

6.2.3 The co-inoculation between AMF and pathogenic fungi can be performed for studying potential application in agriculture.

REFERENCES

Aebi, H. 1984. Methods in enzymology. Methods in Enzymology 105: 121-126.

Aislabie, J., and Deslippe, J. 2013. Soil microbes and their contribution to soil services. In Dymond, J. (ed.), Ecosystem Services in New Zealand: Conditions and Trends, pp. pp. 143-161. New Zealand.

Allen, M.F. 1982. Influence of Vesicular-Arbuscular Mycorrhizae on Water Movement Through *Bouteloua gracilis* (H.B.K.) Lag Ex Steud. New Phytologist 91: 191-196.

Andrade, G., Mihara, K., Linderman, R., and Bethlenfalvay, G. 1997. Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. Plant and Soil 192: 71-79.

Andrén, O., and Lagerlöf, J. 1983. Soil Fauna (Microarthropods, Enchytraeids, Nematodes) in Swedish Agricultural Cropping Systems. Acta Agriculturae Scandinavica 33: 33-52.

Arias, I., Koomen, I., Dodd, J.C., White, R.P., and Hayman, D.S. 1991. Growth responses of mycorrhizal and non-mycorrhizal tropical forage species to different levels of soil phosphate. Plant and Soil 132: 253-260.

Aroca, R., et al. 2013. Arbuscular mycorrhizal symbiosis influences strigolactone production under salinity and alleviates salt stress in lettuce plants. Journal of Plant Physiology 170: 47-55.

Augé, R.M. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11: 3-42.

Augé, R.M. 2004. Arbuscular mycorrhizae and soil/plant water relations. Canadian Journal of Soil Science 84: 373-381.

Augé, T. 1987. Chromite deposits in the northern Oman ophiolite: mineralogical constraints. Mineralium Deposita 22: 1-10.

Azcón-Aguilar, C., and Barea, J.M. 1997a. Applying mycorrhiza biotechnology to horticulture: significance and potentials. Scientia Horticulturae 68: 1-24.

Azcón-Aguilar, C., and Barea, J.M. 1997b. Arbuscular mycorrhizas and biological control of soil-borne plant pathogens – an overview of the mechanisms involved. Mycorrhiza 6: 457-464.

Azcón, R., Ambrosano, E., and Charest, C. 2003. Nutrient acquisition in mycorrhizal lettuce plants under different phosphorus and nitrogen concentration. Plant Science 165: 1137-1145.

Barea, J.M., and Azcón-Aguilar, C. 1982. Production of plant growth-regulating substances by the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae.* Applied and Environmental Microbiology 43: 810-813.

Baslam, M., Esteban, R., García-Plazaola, J.I., and Goicoechea, N. 2013. Effectiveness of arbuscular mycorrhizal fungi (AMF) for inducing the accumulation of major carotenoids, chlorophylls and tocopherol in green and red leaf lettuces. Applied Microbiology and Biotechnology 97: 3119-3128.

Baslam, M., Garmendia, I., and Goicoechea, N. 2011. Arbuscular mycorrhizal fungi (AMF) improved growth and nutritional quality of greenhouse-grown lettuce. Journal of Agricultural and Food Chemistry 59: 5504-5515.

Baum, C., El-Tohamy, W., and Gruda, N. 2015. Increasing the productivity and product quality of vegetable crops using arbuscular mycorrhizal fungi: A review. Scientia Horticulturae 187: 131-141.

Bending, G.D., Turner, M.K., Rayns, F., Marx, M.-C., and Wood, M. 2004. Microbial and biochemical soil quality indicators and their potential for differentiating areas under contrasting agricultural management regimes. Soil Biology and Biochemistry 36: 1785-1792.

Bengtsson, J., AhnstrÖM, J., and Weibull, A.-C. 2005. The effects of organic agriculture on biodiversity and abundance: a meta-analysis. Journal of Applied Ecology 42: 261-269.

Bergmann, G.T., et al. 2011. The under-recognized dominance of Verrucomicrobia in soil bacterial communities. Soil Biology and Biochemistry 43: 1450-1455.

Besserer, A., et al. 2006. Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. PLoS Biology 4: e226.

Bethlenfalvay, G., Cantrell, I., Mihara, K., and Schreiner, R.P. 1999. Relationships between soil aggregation and mycorrhizae as influenced by soil biota and nitrogen nutrition. Biology and Fertility of Soils 28: 356-363.

Bharadwaj, D.P., Lundquist, P.-O., and Alström, S. 2008. Arbuscular mycorrhizal fungal spore-associated bacteria affect mycorrhizal colonization, plant growth and potato pathogens. Soil Biology and Biochemistry 40: 2494-2501.

Bhattacharyya, P., and Jha, D. 2012. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World Journal of Microbiology and Biotechnology 28: 1327-1350.

Blilou, I., Bueno, P., Ocampo, J.A., and García-Garrido, J.M. 2000. Induction of catalase and ascorbate peroxidase activities in tobacco roots inoculated with the arbuscular mycorrhizal *Glomus mosseae*. Mycological Research 104: 722-725.

Bonfante, P., and Genre, A. 2010. Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. Nature Communications 1: 48.

Borowicz, V.A. 2001. Do arbuscular mycorrhizal fungi alter plant-pathogen relations? Ecology 82: 3057-3068.

Bray, R.H., and Kurtz, L. 1945. Determination of total, organic, and available forms of phosphorus in soils. Soil Science 59: 39-46.

Brundrett, M., Abbott, L., and Jasper, D. 1999. Glomalean mycorrhizal fungi from tropical Australia. Mycorrhiza 8: 305-314.

Brundrett, M., Bougher, N., Dell, B., Grove, T., and Malajczuk, N. 1996. Working with Mycorrhizas in Forestry and Agriculture. Australian Centre for International Agricultural Research Canberra.

Bucher, M. 2007. Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. New Phytologist 173: 11-26.

Buee, M., Rossignol, M., Jauneau, A., Ranjeva, R., and Bécard, G. 2000. The pre-symbiotic growth of arbuscular mycorrhizal fungi is induced by a branching factor partially purified from plant root exudates. Molecular Plant-Microbe Interactions 13: 693-698.

Caporn, S.J.M., Hand, D.W., Mansfield, T.A., and Wellburn, A.R. 1994. Canopy photosynthesis of CO2-enriched lettuce (*Lactuca sativa* L.). Response to short-

term changes in CO2, temperature and oxides of nitrogen. New Phytologist 126: 45-52.

Carrenho, R., Trufem, S.F., and Bononi, V.L. 2002. Effects of using different host plants on the detected biodiversity of arbuscular mycorrhizal fungi from an agroecosystem. Brazilian Journal of Botany 25: 93-101.

Choi, Y., Lee, S.M., Chun, J., Lee, H.B., and Lee, J. 2006. Influence of heat treatment on the antioxidant activities and polyphenolic compounds of Shiitake (*Lentinus edodes*) mushroom. Food Chemistry 99: 381-387.

Clark, R.B., Zobel, R.W., and Zeto, S.K. 1999. Effects of mycorrhizal fungus isolates on mineral acquisition by *Panicum virgatum* in acidic soil. Mycorrhiza 9: 167-176.

Dabiré, A.P., et al. 2007. Responses of soil microbial catabolic diversity to arbuscular mycorrhizal inoculation and soil disinfection. Mycorrhiza 17: 537-545.

Dar, G.H., Zargar, M., and Beigh, G. 1997. Biocontrol of *Fusarium* root rot in the common bean (*Phaseolus vulgaris* L.) by using symbiotic *Glomus mosseae* and *Rhizobium leguminosarum*. Microbial Ecology 34: 74-80.

Davies, F., Potter, J., and Linuerman, R. 1993. Drought resistance of mycorrhizal pepper plants independent of leaf P concentration-response in gas exchange and water relations. Physiologia Plantarum 87: 45-53.

Demir, S. 2004. Influence of arbuscular mycorrhiza on some physiological growth parameters of pepper. Turkish Journal of Biology 28: 85-90.

Devesa, R., Moldes, A., Diaz-Fierros, F., and Barral, M.T. 2007. Extraction study of algal pigments in river bed sediments by applying factorial designs. Talanta 72: 1546-51.

Dodd, J. 2000. The role of arbuscular mycorrhizal fungi in agro-and natural ecosystems. Outlook on Agriculture 29: 55-55.

Doran, J.W., and Zeiss, M.R. 2000. Soil health and sustainability: managing the biotic component of soil quality. Applied Soil Ecology 15: 3-11.

Elsen, A., Gervacio, D., Swennen, R., and De Waele, D. 2008. AMF-induced biocontrol against plant parasitic nematodes in *Musa* sp.: a systemic effect. Mycorrhiza 18: 251-256.

Estrada, B., Aroca, R., Barea, J.M., and Ruiz-Lozano, J.M. 2013. Native arbuscular mycorrhizal fungi isolated from a saline habitat improved maize antioxidant systems and plant tolerance to salinity. Plant Science 201: 42-51.

Farshian, S., Khara, J., and Malekzadeh, P. 2007. Effect of arbuscular mycorrhizal (*G. etunicatum*) fungus on antioxidant enzymes activity under zinc toxicity in lettuce plants. Pakistan Journal of Biological Sciences 10: 1865-1869.

Fierer, N., Bradford, M.A., and Jackson, R.B. 2007. Toward an ecological classification of soil bacteria. Ecology 88: 1354-1364.

Filion, M., St-Arnaud, M., and Fortin, J. 1999. Direct interaction between the arbuscular mycorrhizal fungus Glomus intraradices and different rhizosphere microorganisms. New Phytologist 141: 525-533.

Fitter, A.H., and Moyersoen, B. 1996. Evolutionary Trends in Root-Microbe Symbioses. Philosophical Transactions of the Royal Society of London B: Biological Sciences 351: 1367-1375.

Frey-Klett, P., and Garbaye, J. 2005. Mycorrhiza helper bacteria: a promising model for the genomic analysis of fungal–bacterial interactions. New Phytologist 168: 4-8.

Galvez, L., Douds Jr, D., Drinkwater, L., and Wagoner, P. 2001. Effect of tillage and farming system upon VAM fungus populations and mycorrhizas and nutrient uptake of maize. Plant and Soil 228: 299-308.

Ganry, F., Diem, H.G., and Dommergues, Y. 1982. Effect of inoculation with *Glomus mosseae* on nitrogen fixation by field grown soybeans. Plant and Soil 68: 321-329.

Genre, A., Chabaud, M., Timmers, T., Bonfante, P., and Barker, D.G. 2005. Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. The Plant Cell 17: 3489-3499.

Gianinazzi-Pearson, V. 1996. Plant cell responses to arbuscular mycorrhizal fungi: getting to the roots of the symbiosis. The Plant Cell 8: 1871.

Gianinazzi, S., Gollotte, A., Binet, M.N., Tuinen, D., Redecker, D., and Wipf, D. 2010. Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza 20: 519-530.

Giovannetti, M., and Mosse, B. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytologist 84: 489-500.

Gordon, T.R., and Koike, S.T. 2015. Management of *Fusarium* wilt of lettuce. Crop Protection 73: 45-49.

Gosling, P., Hodge, A., Goodlass, G., and Bending, G.D. 2006. Arbuscular mycorrhizal fungi and organic farming. Agriculture, Ecosystems and Environment 113: 17-35.

Govindarajulu, M., et al. 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature 435: 819-823.

Griffiths, R.I., Bailey, M.J., McNamara, N.P., and Whiteley, A.S. 2006. The functions and components of the Sourhope soil microbiota. Applied Soil Ecology 33: 114-126.

Hardie, K., and Leyton, L. 1981. The influence of vesicular-arbuscular mycorrhiza on growth and water relations of red clover. New Phytologist 89: 599-608.

Hata, S., Kobae, Y., and Banba, M. 2010. Chapter 1 - Interactions Between Plants and Arbuscular Mycorrhizal Fungi. In Kwang, W.J. (ed.), International Review of Cell and Molecular Biology, pp. 1-48. Academic Press.

Hayman, D. 1983. The physiology of vesicular-arbuscular endomycorrhizal symbiosis. Canadian Journal of Botany 61: 944-963.

Hazzoumi, Z., Moustakime, Y., and Joutei, K.A. 2015. Effect of arbuscular mycorrhizal fungi (AMF) and water stress on growth, phenolic compounds, glandular hairs, and yield of essential oil in basil (*Ocimum gratissimum* L.). Chemical and Biological Technologies in Agriculture 2: 1-11.

Heinemeyer, A., and Fitter, A. 2004. Impact of temperature on the arbuscular mycorrhizal (AM) symbiosis: growth responses of the host plant and its AM fungal partner. Journal of Experimental Botany 55: 525-534.

Hodge, A., and Fitter, A.H. 2010. Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling. Proceedings of the National Academy of Sciences 107: 13754-13759.

Hogg, J., Lohmann, D., and Russell, K. 1961. The kinetics of reaction of 2, 2-diphenyl-1-picrylhydrazyl with phenols. Canadian Journal of Chemistry 39: 1588-1594.

Ijdo, M., Cranenbrouck, S., and Declerck, S. 2011. Methods for large-scale production of AM fungi: past, present, and future. Mycorrhiza 21: 1-16.

Ijdo, M., Schtickzelle, N., Cranenbrouck, S., and Declerck, S. 2010. Do arbuscular mycorrhizal fungi with contrasting life-history strategies differ in their responses to repeated defoliation? FEMS Microbiological Ecology 72: 114-122.

Ismail, A., and Cheah, S.F. 2003. Determination of Vitamin C, b-carotene and Riboflavin Contents in Five Green Vegetables Organically and Conventionally Grown. Malaysian Journal of Nutrition 9: 31-9.

Jackson, L. 1995. Root architecture in cultivated and wild lettuce (*Lactuca* spp.). Plant, Cell and Environment 18: 885-894.

Jackson, L., Miller, D., and Smith, S. 2002. Arbuscular mycorrhizal colonization and growth of wild and cultivated lettuce in response to nitrogen and phosphorus. Scientia Horticulturae 94: 205-218.

Jackson, M. 1958. Nitrogen determinations for soil and plant tissue. Soil Chemical Analysis 183-204.

Janssen, P.H. 2006. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. Applied Environmental Microbiology 72: 1719-28.

Jastrow, J., Miller, R., and Lussenhop, J. 1998. Contributions of interacting biological mechanisms to soil aggregate stabilization in restored prairie. Soil Biology and Biochemistry 30: 905-916.

Johansson, J.F., Paul, L.R., and Finlay, R.D. 2004. Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. FEMS Microbiological Ecology 48: 1-13.

Jolicoeur, M., Germette, S., Gaudette, M., Perrier, M., and Bécard, G. 1998. Intracellular pH in arbuscular mycorrhizal fungi a symbiotic physiological marker. Plant Physiology 116: 1279-1288.

Kahiluoto, H., and Vestberg, M. 1998. The effect of arbuscular mycorrhiza on biomass production and phosphorus uptake from sparingly soluble sources by leek (*Allium porrum* L.) in Finnish field soils. Biological Agriculture and Horticulture 16: 65-85.

Kang, Z., et al. 2012. Photosynthetic and physiological analysis of the rice high-chlorophyll mutant (Gc). Plant Physiology and Biochemistry 60: 81-87.

Karandashov, V., and Bucher, M. 2005. Symbiotic phosphate transport in arbuscular mycorrhizas. Trends in Plant Science 10: 22-29.

Karthikeyan, N., Prasanna, R., Nain, L., and Kaushik, B.D. 2007. Evaluating the potential of plant growth promoting cyanobacteria as inoculants for wheat. European Journal of Soil Biology 43: 23-30.

Kennedy, A., and Smith, K. 1995. Soil microbial diversity and the sustainability of agricultural soils. Plant and Soil 170: 75-86.

Kjøller, R., and Rosendahl, S. 1998. Enzymatic activity of the mycelium compared with oospore development during infection of pea roots by *Aphanomyces euteiches.* Phytopathology 88: 992-996.

Kohler, J., Caravaca, F., Carrasco, L., and Roldán, A. 2007. Interactions between a plant growth-promoting rhizobacterium, an AM fungus and a phosphate-solubilising fungus in the rhizosphere of *Lactuca sativa.* Applied Soil Ecology 35: 480-487.

Kohler, J., Caravaca, F., and Roldán, A. 2010. An AM fungus and a PGPR intensify the adverse effects of salinity on the stability of rhizosphere soil aggregates of *Lactuca sativa.* Soil Biology and Biochemistry 42: 429-434.

Kohler, J., Hernández, J.A., Caravaca, F., and Roldán, A. 2009. Induction of antioxidant enzymes is involved in the greater effectiveness of a PGPR versus AM fungi with respect to increasing the tolerance of lettuce to severe salt stress. Environmental and Experimental Botany 65: 245-252.

Köhler, S., Bauer, S., Horn, D., and Robinson, P.N. 2008. Walking the interactome for prioritization of candidate disease genes. The American Journal of Human Genetics 82: 949-958.

Koide, R.T. 2000. Functional complementarity in the arbuscular mycorrhizal symbiosis. New Phytologist 147: 233-235.

Koide, R.T., and Mosse, B. 2004. A history of research on arbuscular mycorrhiza. Mycorrhiza 14: 145-163.

Kozlowski, T. 1987. Soil mositure and absorption of water by tree roots. Journal of Arboriculture 13: 39-46.

Lages Barbosa, G., et al. 2015. Comparison of land, water, and energy requirements of lettuce grown using hydroponic vs. conventional agricultural methods.

International Journal of Environmental Research and Public Health 12: 6879-6891.

Latef, A.A.H.A., and Chaoxing, H. 2011. Arbuscular mycorrhizal influence on growth, photosynthetic pigments, osmotic adjustment and oxidative stress in tomato plants subjected to low temperature stress. Acta Physiologiae Plantarum 33: 1217-1225.

Lauber, C.L., Hamady, M., Knight, R., and Fierer, N. 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and Environmental Microbiology 75: 5111-5120.

Li, W., Fang, M., Shujuan, Z., and Xue, Z. 2012. Effect of *Glomus Mosseae* Inoculation on Growth and Reproduction of Rice. In Zhu, E. and Sambath, S. (ed.), Information Technology and Agricultural Engineering, pp. 935-942. Springer Berlin Heidelberg.

Liao, J.P., Lin, X.G., Cao, Z.H., Shi, Y.Q., and Wong, M.H. 2003. Interactions between arbuscular mycorrhizae and heavy metals under sand culture experiment. Chemosphere 50: 847-853.

Liu, A., Hamel, C., Elmi, A., Costa, C., Ma, B., and Smith, D. 2002. Concentrations of K, Ca and Mg in maize colonized by arbuscular mycorrhizal fungi under field conditions. Canadian Journal of Soil Science 82: 272-278.

Liu, X., et al. 2007. Total phenolic content and DPPH radical scavenging activity of lettuce (*Lactuca sativa* L.) grown in Colorado. LWT - Food Science and Technology 40: 552-557.

Llorach, R., Martínez-Sánchez, A., Tomás-Barberán, F.A., Gil, M.I., and Ferreres, F. 2008. Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole. Food Chemistry 108: 1028-1038.

Logi, C., Sbrana, C., and Giovannetti, M. 1998. Cellular events involved in survival of individual arbuscular mycorrhizal symbionts growing in the absence of the host. Applied and Environmental Microbiology 64: 3473-9.

Lu, G. 2000. Lettuce cultivation techniques. Beijing: Jindun Publishing.

Luo, H., Lee, S., and He, J. 2009. Integrated effects of root-zone temperatures and phosphorous levels on aeroponically-grown lettuce (*Lactuca sativa* L.) in the tropics. The Open Horticulture Journal 2: 6-12.

Maeder, P., Fliessbach, A., Dubois, D., Gunst, L., Fried, P., and Niggli, U. 2002. Soil fertility and biodiversity in organic farming. Science 296: 1694-1697.

Mala, W., Kumari, I., Sumanasena, H., and Nanayakkara, C. 2010. Effective spore density of *Glomus mosseae*, arbuscular mycorrhiza (AM), for inoculation of rooted cuttings of black pepper (*Piper nigrum* Linn.). Tropical Agricultural Research 21: 189-197.

Mar Vázquez, M., César, S., Azcón, R., and Barea, J.M. 2000. Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum*, *Pseudomonas*, *Trichoderma*) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants. Applied Soil Ecology 15: 261-272.

Martın, J., Sampedro, I., Garcıa-Romera, I., Garcıa-Garrido, J., and Ocampo, J. 2002. Arbuscular mycorrhizal colonization and growth of soybean (*Glycine max*) and lettuce (*Lactuc sativa*) and phytotoxic effects of olive mill residues. Soil Biology and Biochemistry 34: 1769-1775.

Marulanda, A., Azcon, R., and Ruiz-Lozano, J.M. 2003. Contribution of six arbuscular mycorrhizal fungal isolates to water uptake by *Lactuca sativa* plants under drought stress. Physiologia Plantarum 119: 526-533.

Maya, M.A., and Matsubara, Y.-i. 2013. Tolerance to *Fusarium* wilt and anthracnose diseases and changes of antioxidative activity in mycorrhizal cyclamen. Crop Protection 47: 41-48.

McAllister, C.B., Garcia-Romera, I., Martin, J., Godeas, A., and Ocampo, J.A. 1995. Interaction between *Aspergillus niger* van Tiegh. and *Glomus mosseae*. (Nicol. & Gerd.) Gerd. & Trappe. New Phytologist 129: 309-316.

McNear Jr, D. 2013. The rhizosphere-roots, soil and everything in between. Nature Education Knowledge 4: 1.

Medina, J.M., Bosque, L.A.R.d., and Río, M.d.l.A.P.d. 2002. Effect of vesicular-arbuscular mycorrhiza on growth and yield of piquin pepper intercropped with citrus. in

The 16th International Pepper Conference. pp. 10-12 November 2002. Tampico, Tamaulipas, Mexico.

Miller, R., and Jackson, L. 1998. Survey of vesicular–arbuscular mycorrhizae in lettuce production in relation to management and soil factors. The Journal of Agricultural Science 130: 173-182.

Mollavali, M., Bolandnazar, S., Nazemieh, H., Zare, F., and Aliasgharzad, N. 2015. The effect of mycorrhizal fungi on antioxidant activity of various cultivars of onion (*Allium cepa* L). International Journal of Biosciences 6: 66-79.

Motsara, M., and Roy, R.N. 2008. Guide to laboratory establishment for plant nutrient analysis. Food and Agriculture Organization of the United Nations Rome.

Nagahashi, G., and Douds Jr, D.D. 1997. Appressorium formation by AM fungi on isolated cell walls of carrot roots. New Phytologist 299-304.

Nakano, Y., and Asada, K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology 22: 867-880.

Navazio, L., et al. 2007. A diffusible signal from arbuscular mycorrhizal fungi elicits a transient cytosolic calcium elevation in host plant cells. Plant Physiology 144: 673-681.

Nelson, D.W., and Sommers, L.E. 1996. Total carbon, organic carbon, and organic matter. Methods of Soil Analysis 3: 961-1010.

Nuccio, E.E., Hodge, A., Pett-Ridge, J., Herman, D.J., Weber, P.K., and Firestone, M.K. 2013. An arbuscular mycorrhizal fungus significantly modifies the soil bacterial community and nitrogen cycling during litter decomposition. Environmental Microbiology 15: 1870-81.

Nwangburuka, C. 2010. Morphological characterization and genetic studies in okra *Abelmoschus esculentus* (L.) moench. PhD Thesis in the University of Agric. Abeokuta, Nigeria.

Oehl, F., Oberson, A., Probst, M., Fliessbach, A., Roth, H.-R., and Frossard, E. 2001. Kinetics of microbial phosphorus uptake in cultivated soils. Biology and Fertility of Soils 34: 31-41.

Oehl, F., et al. 2004. Impact of long-term conventional and organic farming on the diversity of arbuscular mycorrhizal fungi. Oecologia 138: 574-583.

Ogle, K., et al. 2012. Differential daytime and night-time stomatal behavior in plants from North American deserts. New Phytologist 194: 464-76.

Oliveira, M., Pereira, C., Bessa, C., Araujo, R., and Saraiva, L. 2015. Chronological aging in conidia of pathogenic *Aspergillus*: Comparison between species. Journal of Microbiological Methods 118: 57-63.

Orłowska, E., Godzik, B., and Turnau, K. 2012. Effect of different arbuscular mycorrhizal fungal isolates on growth and arsenic accumulation in *Plantago lanceolata* L. Environmental Pollution 168: 121-130.

Parniske, M. 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nature Reviews Microbiology 6: 763-775.

Phillip, J., and Hayman, D. 1970. Improved procedures for clearing roots and staining parasitic and VAM fungi for rapid assessment of infection. Transactions of the British Mycological Society 55: 158-161.

Piotrowski, J., Denich, T., Klironomos, J., Graham, J., and Rillig, M. 2004. The effects of arbuscular mycorrhizas on soil aggregation depend on the interaction between plant and fungal species. New Phytologist 164: 365-373.

Porcel, R., and Ruiz-Lozano, J.M. 2004. Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. Journal of Experimental Botany 55: 1743-1750.

Porra, R. 2002. The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b. Photosynthesis Research 73: 149-156.

Prohens, J., and Nuez, F. 2008. Vegetables I Asteraceae, Brassicaceae, Chenopodicaceae, and Cucurbitaceae. New York: Springer Science+Business Media.

Quilambo, O.A. 2003. The vesicular-arbuscular mycorrhizal symbiosis. African Journal of Biotechnology 2: 539-546.

Rahmaty, R., and Khara, J. 2011. Effects of vesicular arbuscular mycorrhiza *Glomus intraradices* on photosynthetic pigments, antioxidant enzymes, lipid peroxidation, and chromium accumulation in maize plants treated with chromium. Turkey Journal of Biology 35: 51-58.

Redecker, D., and Raab, P. 2006. Phylogeny of the glomeromycota (arbuscular mycorrhizal fungi): recent developments and new gene markers. Mycologia 98: 885–895.

Reganold, J.P., Elliott, L.F., and Unger, Y.L. 1987. Long-term effects of organic and conventional farming on soil erosion. Nature 330: 370-372.

Rigby, D., and Cáceres, D. 2001. Organic farming and the sustainability of agricultural systems. Agricultural Systems 68: 21-40.

Riveros, C., Dechartres, A., Perrodeau, E., Haneef, R., Boutron, I., and Ravaud, P. 2013. Timing and completeness of trial results posted at ClinicalTrials. gov and published in journals. PLoS Medicine 10: e1001566.

Rösch, C., Mergel, A., and Bothe, H. 2002. Biodiversity of denitrifying and dinitrogen-fixing bacteria in an acid forest soil. Applied and Environmental Microbiology 68: 3818-3829.

Ruiz-Lozano, J. 2003. Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. Mycorrhiza 13: 309-317.

Ruiz-Lozano, J.M., and Azcón, R. 1996. Mycorrhizal colonization and drought stress as factors affecting nitrate reductase activity in lettuce plants. Agriculture, Ecosystems and Environment 60: 175-181.

Ruiz-Sánchez, M., Aroca, R., Muñoz, Y., Polón, R., and Ruiz-Lozano, J.M. 2010. The arbuscular mycorrhizal symbiosis enhances the photosynthetic efficiency and the antioxidative response of rice plants subjected to drought stress. Journal of Plant Physiology 167: 862-869.

Ruiz-Lozano, J., and Azcón, R. 1995. Hyphal contribution to water uptake in mycorrhizal plants as affected by the fungal species and water status. Physiologia Plantarum 95: 472-478.

Safir, G.R., Boyer, J.S., and Gerdemann, J.W. 1972. Nutrient status and mycorrhizal enhancement of water transport in soybean. Plant Physiology 49: 700-703.

San Martín, A., et al. 2014. Imaging mitochondrial flux in single cells with a FRET sensor for pyruvate. PLoS ONE 9: e85780.

Satter, M.A., Hanafi, M.M., Mahmud, T.M.M., and Azizah, H. 2006. Influence of arbuscular mycorrhiza and phosphate rock on uptake of major nutrients by *Acacia mangium* seedlings on degraded soil. Biology and Fertility of Soils 42: 345-349.

Schalamuk, S., and Cabello, M. 2010. Arbuscular mycorrhizal fungal propagules from tillage and no-tillage systems: possible effects on Glomeromycota diversity. Mycologia 102: 261-268.

Scheublin, T.R., Ridgway, K.P., Young, J.P.W., and Van Der Heijden, M.G. 2004. Nonlegumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. Applied and Environmental Microbiology 70: 6240-6246.

Schüβler, A., Schwarzott, D., and Walker, C. 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution*. Mycological Research 105: 1413-1421.

Schwab, A., and Lindsay, W. 1983. The effect of redox on the solubility and availability of manganese in a calcareous soil. Soil Science Society of America Journal 47: 217-220.

Sebuliba, E., Nyeko, P., Majaliwa, M., Eilu, G., Kizza, C.L., and Ekwamu, A. 2012. Enhanced growth of multipurpose calliandra (*Calliandra calothyrsus*) using arbuscular mycorrhiza fungi in Uganda. The Scientific World Journal 2012: 1-6.

Shin, Y., Liu, R.H., Nock, J.F., Holliday, D., and Watkins, C.B. 2007. Temperature and relative humidity effects on quality, total ascorbic acid, phenolics and flavonoid concentrations, and antioxidant activity of strawberry. Postharvest Biology and Technology 45: 349-357.

Smit, E., Leeflang, P., Gommans, S., Van Den Broek, J., Van Mil, S., and Wernars, K. 2001. Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. Applied and Environmental Microbiology 67: 2284-2291.

Smith, S.E., and Read, D.J. 1997a. 2 - Colonization of roots and anatomy of VA mycorrhizas. In Read, S.E.S.J. (ed.), Mycorrhizal Symbiosis (Second Edition), pp. 33-II. London: Academic Press.

Smith, S.E., and Read, D.J. 1997b. Mycorrhizal symbiosis 2nd edn. Academic, San Diego.

Stobart, A.K., Griffiths, W.T., Ameen-Bukhari, I., and Sherwood, R.P. 1985. The effect of Cd2+ on the biosynthesis of chlorophyll in leaves of barley. Physiologia Plantarum 63: 293-298.

Sun, H., Deng, S., and Raun, W. 2004. Bacterial community structure and diversity in a century-old manure-treated agroecosystem. Applied and Environmental Microbiology 70: 5868-5874.

Taylor, A.F.S., and Alexander, I.A.N. 2005. The ectomycorrhizal symbiosis: life in the real world. Mycologist 19: 102-112.

Tian, C., He, X., Zhong, Y., and Chen, J. 2002. Effects of VA mycorrhizae and Frankia dual inoculation on growth and nitrogen fixation of *Hippophae tibetana*. Forest Ecology and Management 170: 307-312.

Tisdall, J.M., Smith, S.E., and Rengasamy, P. 1997. Aggregation of soil by fungal hyphae. Soil Research 35: 55-60.

Torres-Barragán, A., Zavaleta-Mejía, E., González-Chávez, C., and Ferrera-Cerrato, R. 1996. The use of arbuscular mycorrhizae to control onion white rot (*Sclerotium cepivorum* Berk.) under field conditions. Mycorrhiza 6: 253-257.

Vicari, M., Hatcher, P., and Ayres, P. 2002. Combined effect of foliar and mycorrhizal endophytes on an insect herbivore. Ecology 83: 2452-2464.

Vierheilig, H., and Ocampo, J. 1991. Susceptibility and effectiveness of vesicular-arbuscular mycorrhizae in wheat cultivars under different growing conditions. Biology and Fertility of Soils 11: 290-294.

Vijayabharathi, R., Sathya, A., and Gopalakrishnan, S. 2015. Plant growth-promoting microbes from herbal vermicompost. In (ed.), Plant-Growth-Promoting Rhizobacteria (PGPR) and Medicinal Plants, pp. 71-88. Springer.

Waschkies, C., Schropp, A., and Marschner, H. 1994. Relations between grapevine replant disease and root colonization of grapevine (Vitis sp.) by fluorescent pseudomonads and endomycorrhizal fungi. Plant and Soil 162: 219-227.

Whipps, J.M. 2004. Prospects and limitations for mycorrhizas in biocontrol of root pathogens. Canadian Journal of Botany 82: 1198-1227.

Widnyana, I.K., Suprapta, D.N., Sudana, I.M., and Temaja, I.G.R.M. 2013. Pseudomonas alcaligenes, potential antagonist against *Fusarium oxysporum* f. sp.

lycopersicum the cause of *Fusarium* wilt disease on tomato. Journal of Biology, Agriculture and Healthcare 3: 163-169.

Wu, Q.-S., and Xia, R.-X. 2006. Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. Journal of Plant Physiology 163: 417-425.

Xie, J., and Schaich, K. 2014. Re-evaluation of the 2, 2-diphenyl-1-picrylhydrazyl free radical (DPPH) assay for antioxidant activity. Journal of Agricultural and Food Chemistry 62: 4251-4260.

Yu, L., Perret, J., Harris, M., Wilson, J., and Haley, S. 2003. Antioxidant properties of bran extracts from "Akron" wheat grown at different locations. Journal of Agricultural and Food Chemistry 51: 1566-1570.

Zhu, X., Song, F., Liu, S., Liu, T., and Zhou, X. 2012. Arbuscular mycorrhizae improves photosynthesis and water status of *Zea mays* L. under drought stress. Plant Soil and Environment 58: 186-191.

Zhu, X.Q., Wang, C.Y., Chen, H., and Tang, M. 2014. Effects of arbuscular mycorrhizal fungi on photosynthesis, carbon content, and calorific value of black locust seedlings. Photosynthetica 52: 247-252.

Zinnen, T. 1988. Assessment of plant diseases in hydroponic culture. Plant disease 72: 96-99.

Zwart, K.B., et al. 1994. Soil ecology of conventional and integrated arable farming systems population dynamics in the belowground food webs in two different agricultural systems. Agriculture, Ecosystems and Environment 51: 187-198.

APPENDIX

APPENDIX A

Chemicals preparation

1. Chemical for working with mycorrhiza

1.1 Staining solution (for 2 dm^3)

Glycerine	1	dm^3
Distilled water	950	ml
Acetic acid	50	ml
Tryphan blue	0.02	g

1.2 PVLG

Polyvinyl alcohol	8.33	g
Distilled water	50	ml
Lactic acid	50	ml
Glycerine	5	ml

1.3 Metzer's reagent

Chloral hydrate	100	g
Distilled water	100	ml
Iodine	1.5	g
Potassium iodine	5	g

2. Chemical for antioxidant substances analysis

2.1 0.1 mM potassium phosphate (Pi) buffer pH 7.0

1 M K2HPO4	61.5	ml
1 M KH2PO4	38.5	ml

Adjust volume with autoclave distilled water up to 1 dm^3

2.2 Extraction buffer

50 mM	Potassium phosphate buffer pH 7.0
1% (w/v)	Polyvinylpolypyrrolidone (PVPP)
1 mg/ml	Dithiothreitol (DTT)
100 mM	Phenylmethylsulfonyl (PMSF) dissolve in isopropanol

2.3 6% metaphosphoric acid

Acetic acid	121	ml
Metaphosphoric acid	60	g

Adjust volume with autoclave distilled water up to 1 dm^3

2.4 2% thiourea

Thiourea	4	g
6% metaphosphoric acid	167	ml

Adjust volume with autoclave distilled water up to 200 ml

2.5 2% 2,4-dinitrophenylhydrazine (DNPH)

2,4-dinitrophenylhydrazine	2	g
Sulfuric acid	45	ml

Dissolve before adjust volume with autoclave distilled water up to 100 ml

Beware: Slowly mix dissolve DNPH with autoclaved distilled water on ice when adjusting volume

3. Chemical for rhizosphere microbes changes analysis

3.1 5x Tris boric EDTA (TBE) buffer

Tris-base	54	g
Boric acid	27.5	g
0.5 EDTA pH 8.0	20	ml

Adjust volume with autoclave distilled water up to 1 dm^3

3.2 6x DNA loading dye

30% glycerol	3	ml
Bromophenol blue	25	g

Adjust volume with autoclave distilled water up to 10 ml

APPENDIX B

Protocols and standard graphs

1. Root staining and colonization

- Place cut root in 50 ml centrifuge tube containing 20 ml of 50% KOH then aultoclaved for clearing root
- Pour off KOH and rinsed cleared root with tap water for 3 times
- Soak cleaned root in 10 ml of 5% HCl for 5 mins and pour off HCl
- Add 10 ml of 10 folds diluted staining solution and left for 2-3 days
- Pour stained root on plate with 1 cm with 2 axis gridlines
- Crossing points between root and gridline were counted
- Calculating percentage of crossing point with staining to total crossing point

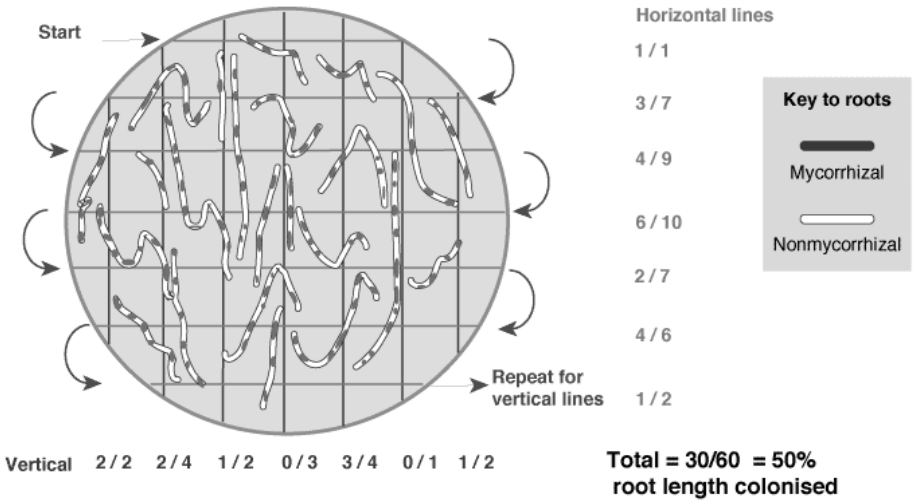

Figure 44 The calculation of percentage of root colonization under stereoscopic microscope by gridline-intersect method.

2. Catalase and Ascorbate peroxidase assay

- Ground 0.1 g of frozen leaf tissue to powder with liquid nitrogen
- Add 1 ml of extraction buffer and transfer to 1.5 microcentrifuge tube
- Centrifuge at 12,000 rpm, 4°C for 10 mins
- Collect supernatant

For Catalase assay:

Chemical	Reference	Test
50 mM Pi buffer	1,940 µl	1,740 µl
100 mM Hydrogen peroxide	-	200 µl
Plant extract	60 µl	60 µl
Total	2,000 µl	2,000 µl

Measurement: Read the absorbance at wavelength 240 nm every 15 s for total 90 s

Calculation: Units/mg protein = $\frac{(\Delta A240/min)*1000}{(43.6)(\mu l \text{ extract used})(mg \text{ protein}/\mu l \text{ extract})}$

For Ascorbate peroxidase assay:

Chemical	Reference	Test
50 mM Pi buffer	1,700 µl	1,500 µl
100 mM Hydrogen peroxide	200 µl	200 µl
500 mM EDTA	20 µl	20 µl
2 mM Ascorbate	-	200 µl
Plant extract	80 µl	80 µl
Total	2,000 µl	2,000 µl

Measurement: Read the absorbance at wavelength 290 nm every 12 s for total 60 s

Calculation: Units/mg protein = $\frac{(\Delta A290/min)*1000}{(2.8)(\mu l \text{ extract used})(mg \text{ protein}/\mu l \text{ extract})}$

For total protein assay:

Reaction:	Plant extract/BSA	50	µl
	Bio-rad preotein assay	50	µl
	H_2O	100	µl
	Total	200	µl

Stand for 5 mins and then read the absorbance at wavelength 595 nm

Standard graph:

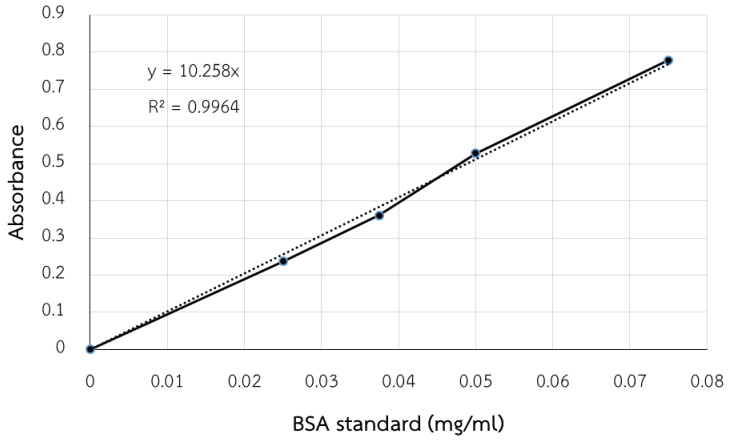

Figure 45 Protein standard (BSA) graph

4. Ascorbic acid determination

Extraction:

- Ground A gram of frozen leaf tissue with liquid nitrogen to powder
- Add 6% metaphosphoric acid and pour into 50 ml centrifuge tube
- Centrifuge at 6,000 rpm, 4°C for 15 mins
- Collect 1 ml supernatant (plant extract) into glass tube and add 50 µl of 2% 2,6-dichlorophenolindolphenol
- Incubate at room temperature for 1 hr.
- Add 2 ml of 2% thiourea and 1 ml of 2% DNPH
- Incubate in 60oC water bath for 3 hrs.

- Place on ice and slowly add 5 ml of 90% sulfuric acid
- Read the absorbance at wavelength 540 nm and calculate comparing to ascorbate standard graph

Ascorbate standard graph:

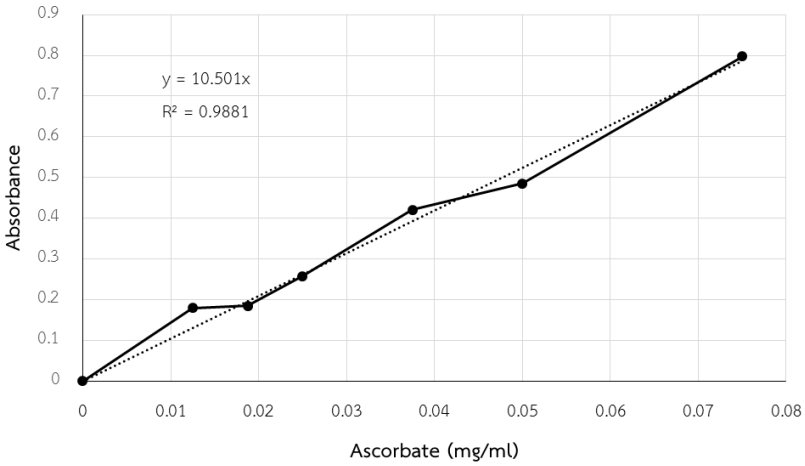

Figure 46 Ascorbate standard graph

5. Phenolic content determination and DPPH scavenging assay

Extraction:

- Ground A gram of frozen leaf tissue with liquid nitrogen to powder
- Add 10 ml of 80% Methanol and pour into 50 ml centrifuge tube
- Centrifuge at 9,000 rpm, 25°C for 5 mins
- Collect supernatant (plant extract)

For phenolic content determination:

Reaction:	Plant extract	100	µl
	1/3 Folin-ciocalteu	100	µl
	2% Na_2CO_3	300	µl
	H_2O	4.5	ml
	Total	5	ml

Mix and incubate in dark place for 2 hrs.

Read the absorbance at wavelength 670 nm and calculate comparing to gallic acid standard graph

Gallic acid standard graph:

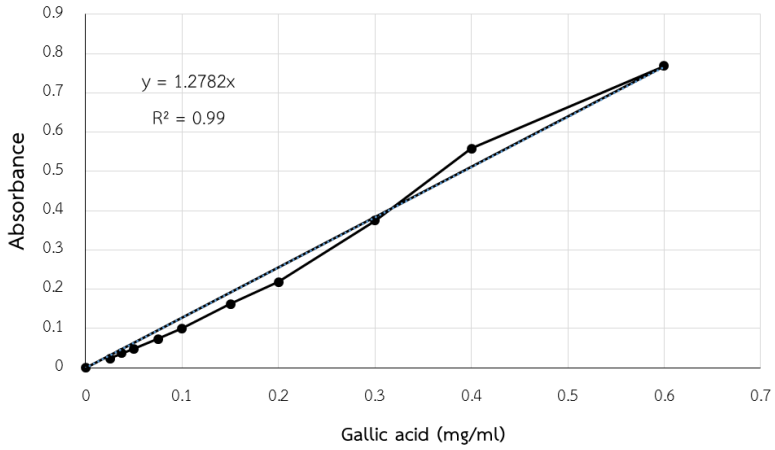

Figure 47 Gallic acid standard graph

6. Mo-Bio powerSoil DNA isolation protocol

- Weigh 0.5 g of soil and collect in Powerbead tube*, then gently mix with vortex
- Add 60 µl of Solution C1* then mix and incubate in water bath at 70°C for 10 mins
- Vortex horizontally at maximum speed or shake with bead beater machine at 3000 rpm for 10 mins
- Centrifuge at 9,000 rpm, for 30 s at room temperature
- Collect supernatant and add 250 µl of solution C2*
- Vortex for 5 s and incubate at 4°C for 5 mins
- Centrifuge at 9,000 rpm for 1 min at room temperature
- Collect supernatant and add 200 µl of solution C3*
- Vortex and centrifuge at 9,000 rpm for 1 min at room temperature

- Collect supernatant and add 1.2 ml of solution C4*
- Vortex for 5 s and load the mixture to spin filter*
- Centrifuge at 9,000 rpm for 1 min at room temperature
- Discard flow through and repeat loading and centrifuging until finish all the mixture
- Add 500 µl of solution C5* and centrifuge at 9,000 rpm for 1 min at room temperature
- Discard flow through and centrifuge at 9,000 rpm for 1 min at room temperature
- Place spin filter in new microcentrifuge tube and add 100 µl of solution C6*
- Centrifuge at 9,000 rpm for 30 s at room temperature
- Keep flow through and store at -20^{o}C

APPENDIX C

Statistical analysis

Table 16 Statistical analysis of relative abundance (mean) in phylum taxa of bacteria between each soil before transplantation (BA0), uninoculation (BAC) and *G. mosseae* inoculation (BAT) at harvesting time.

Phyla	BA0	BAC	BAT	*P* value
AD3	0.0934	0.0355	0.0247	0.0509
Acidobacteria	21.6231	15.8900	13.5912	**0.0273***
Actinobacteria	5.5957	3.6767	2.1799	**0.0273***
Armatimonadetes	0.5598	0.5668	0.4846	0.1133
BHI80 139	0.0000	0.0102	0.0113	0.0605
BRC1	0.0705	0.1915	0.2534	**0.0390***
Bacteroidetes	12.7089	14.1969	17.0546	**0.0390***
Chlamydiae	0.0401	0.1222	0.5713	**0.0273***
Chlorobi	0.1771	0.1643	0.4443	0.0608
Chloroflexi	5.2791	5.8049	5.8874	0.2881
Cyanobacteria	0.3846	0.7215	1.1107	**0.0273***
Elusimicrobia	0.0486	0.1421	0.1742	0.0509
FBP	0.0251	0.0382	0.0331	0.3292
FCPU426	0.0006	0.0546	0.1949	**0.0265***
Fibrobacteres	0.0108	0.0345	0.0469	0.0390
Firmicutes	2.0613	3.4239	2.8291	0.1133
GAL15	0.0397	0.0240	0.0149	0.1767
GN02	0.0016	0.0149	0.2151	**0.0273***
GN04	0.0007	0.0016	0.0793	0.0552
GOUTA4	0.0006	0.0000	0.0000	0.3679
Gemmatimonadetes	2.7110	2.1763	1.8496	0.0608
Kazan 3B 28	0.0000	0.0022	0.0018	0.2369
Lentisphaerae	0.0000	0.0000	0.0369	**0.0221***

Phyla	BA0	BAC	BAT	*P* value
MVP 21	0.0000	0.0000	0.0031	0.1054
NC10	0.0442	0.0259	0.0165	0.1133
NKB19	0.0040	0.0116	0.0695	**0.0273***
Nitrospirae	1.3158	1.0770	0.8695	0.1479
OD1	0.0468	0.2186	0.4577	**0.0273***
OP11	0.0026	0.0063	0.0189	0.0509
OP3	0.0048	0.0366	0.0536	**0.0390***
OP8	0.0000	0.0008	0.0016	0.3496
PAUC34f	0.0000	0.0000	0.0035	**0.0221***
Planctomycetes	3.2360	5.7602	6.4319	0.0509
Proteobacteria	25.7484	33.8957	33.7802	0.0608
SBR1093	0.0036	0.0048	0.1088	0.0665
SC4	0.0000	0.0007	0.0000	0.3679
SR1	0.0000	0.0055	0.0104	0.1199
Spirochaetes	0.0375	0.2518	0.3325	0.0509
TM6	0.0874	0.2723	0.4561	**0.0273***
TM7	0.0874	0.0608	0.0834	0.1931
Tenericutes	0.0188	0.0045	0.0141	0.1767
Thermi	0.0045	0.0022	0.0110	0.5611
Unclassified	0.5910	0.9777	1.7061	**0.0273***
Verrucomicrobia	17.1280	9.7528	8.1311	0.0608
WPS 2	0.0442	0.0158	0.0189	0.0608
WS2	0.0016	0.0119	0.0129	0.0608
WS3	0.1601	0.3129	0.3094	0.0665
WS4	0.0010	0.0007	0.0006	0.9392
ZB3	0.0000	0.0000	0.0094	**0.0221***

Table 17 Statistical analysis of relative abundance (mean) in genus taxa of bacteria between each soil before transplantation (BA0), uninoculation (BAC) and *G. mosseae* inoculation (BAT) at harvesting time.

Genera	BA0	BAC	BAT	*P* value
A17	0.0169	0.0905	0.1387	0.0509
Acholeplasma	0.0158	0.0000	0.0000	**0.0221***
Achromobacter	0.0139	0.0188	0.0132	0.4298
Acidisoma	0.0036	0.0000	0.0006	**0.0340***
Acinetobacter	0.0117	0.0968	0.0180	0.1479
Acrocarpospora	0.0000	0.0074	0.0059	0.5580
Actinoallomurus	0.0052	0.0037	0.0003	0.0594
Actinomadura	0.0231	0.0064	0.0019	**0.0390***
Actinomycetospora	0.0040	0.0092	0.0066	0.2019
Actinoplanes	0.0088	0.0224	0.0118	0.0608
Actinopolymorpha	0.0034	0.0009	0.0003	0.6884
Adhaeribacter	0.0371	0.0506	0.0862	0.2019
Aeromicrobium	0.0123	0.0013	0.0038	0.2019
Afifella	0.0074	0.0320	0.0373	0.0608
Agrobacterium	0.1526	0.1524	0.1099	0.3012
Agromyces	0.0043	0.0069	0.0041	0.1133
Alcanivorax	0.0610	0.0016	0.0013	0.1767
Alicyclobacillus	0.2749	0.4039	0.4911	0.0509
Ammoniphilus	0.0928	0.0807	0.0634	0.2019
Amycolatopsis	0.1108	0.0566	0.0194	**0.0273***
Anaerolinea	0.1064	0.0306	0.0238	0.0608
Anaeromyxobacter	0.2243	0.3896	0.1527	0.0582
Anaerospora	0.0035	0.0003	0.0003	0.3630
Aneurinibacillus	0.0101	0.0034	0.0063	0.1479
Aquicella	0.0899	0.2596	0.6999	**0.0273***

Genera	BA0	BAC	BAT	*P* value
Ardenscatena	0.0009	0.0119	0.0025	0.0879
Arthrobacter	0.0478	0.0464	0.0290	0.0992
Arthrospira	0.0007	0.0050	0.0036	0.3168
Asteroleplasma	0.0029	0.0045	0.0141	0.1133
Asticcacaulis	0.0328	0.0252	0.0153	0.1767
Azospirillum	0.0023	0.0037	0.0066	0.1767
B 42	0.0000	0.0019	0.0020	0.3496
Bacillus	0.1261	0.2522	0.0950	0.2881
Bacteriovorax	0.0044	0.0000	0.0006	0.1914
Bacteroides	0.0775	0.0572	0.0980	0.0509
Balneimonas	0.1746	0.1657	0.0858	0.0608
Bdellovibrio	0.1856	0.2301	0.2118	0.4298
Bifidobacterium	0.0334	0.0286	0.0457	0.0665
Bosea	0.0010	0.0047	0.0051	0.1133
Bradyrhizobium	0.3702	0.3836	0.2500	0.0608
Brevibacillus	0.0074	0.0126	0.0150	0.2881
Brevibacterium	0.0012	0.0000	0.0000	0.3679
Bryobacter	0.0000	0.0059	0.0022	**0.0349***
Burkholderia	0.1252	0.0531	0.0427	0.0992
Caldilinea	0.0029	0.0102	0.0084	0.0608
Caloramator	0.0036	0.0081	0.0006	**0.0265***
Candidatus Azobacteroides	0.0000	0.0007	0.0000	0.3679
Candidatus Glomeribacter	0.0014	0.0047	0.0056	0.4413
Candidatus Koribacter	0.5770	0.2735	0.1965	**0.0390***
Candidatus Methylomirabilis	0.0010	0.0007	0.0010	0.9187
Candidatus Protochlamydia	0.0029	0.0046	0.0038	0.5611
Candidatus Rhabdochlamydia	0.0049	0.0147	0.2630	**0.0273***
Candidatus Solibacter	0.4314	0.3306	0.2645	0.0794

Genera	BA0	BAC	BAT	*P* value
Candidatus Xiphinematobacter	0.4848	0.2729	0.3860	0.3932
Catellatospora	0.0179	0.0174	0.0082	0.1133
Caulobacter	0.0204	0.0679	0.0463	0.0992
Cellulomonas	0.0062	0.0181	0.0129	0.2492
Cellvibrio	0.0104	0.0950	0.0374	**0.0273***
Chelativorans	0.0054	0.0154	0.0070	0.1479
Chitinophaga	0.3378	0.0634	0.0814	0.0665
Chloronema	0.0013	0.0025	0.0046	0.4792
Chondromyces	0.0013	0.0174	0.0146	0.0665
Chryseobacterium	0.0277	0.0423	0.0149	0.4298
Chthoniobacter	0.0490	0.1921	0.2149	0.0608
Cloacibacterium	0.0003	0.0003	0.0007	0.9392
Clostridium	0.0010	0.0000	0.0000	0.3679
Cohnella	0.0095	0.0110	0.0064	0.3292
Constrictibacter	0.0000	0.0013	0.0007	0.2807
Corynebacterium	0.0006	0.0012	0.0006	0.5501
Crocinitomix	0.0003	0.0030	0.0013	**0.0379***
Cryptosporangium	0.0097	0.0052	0.0029	0.1133
Cupriavidus	0.1022	0.0568	0.0959	0.2521
Cystobacter	0.0138	0.0406	0.0434	0.0665
Cytophaga	0.0118	0.0258	0.0119	0.0665
DA101	13.9665	5.6758	3.4259	0.0509
Dactylosporangium	0.0261	0.0353	0.0462	0.7326
Dechloromonas	0.0669	0.1070	0.0429	0.2521
Deinococcus	0.0045	0.0003	0.0091	0.0594
Demequina	0.0019	0.0219	0.0047	0.0582
Desulfobacca	0.0023	0.0018	0.0012	0.8320
Desulfobulbus	0.0000	0.0063	0.0095	0.0552

Genera	BA0	BAC	BAT	*P* value
Desulfomonile	0.0027	0.0000	0.0013	0.1009
Desulfosporosinus	0.0130	0.0069	0.0076	0.0665
Desulfovibrio	0.0000	0.0094	0.0038	0.1258
Desulfovirga	0.0000	0.0000	0.0006	0.3679
Devosia	0.0164	0.0985	0.0486	**0.0390***
Dokdonella	0.0006	0.0163	0.0067	**0.0390***
Dunaliella	0.0003	0.0013	0.0009	0.8079
Dyadobacter	0.0525	0.0440	0.0514	0.6703
Edaphobacter	0.0121	0.0081	0.0044	0.1479
Ellin506	0.0172	0.0375	0.0326	0.0608
Emticicia	0.0029	0.0263	0.0108	0.0568
Erwinia	0.0000	0.0033	0.0000	0.3679
FFCH10602	0.0422	0.0245	0.0089	0.0794
Filimonas	0.0007	0.0051	0.0091	0.3168
Fimbriimonas	0.0870	0.1697	0.1110	0.0509
Flavihumibacter	0.0038	0.0307	0.0305	0.0665
Flavisolibacter	5.7098	2.4062	1.8591	**0.0273***
Flavobacterium	0.0288	0.3506	0.4854	0.0665
Fluviicola	0.0050	0.0202	0.0131	0.2521
GOUTA19	0.0036	0.0054	0.0035	0.7326
Gemmata	0.3162	0.2946	0.3528	0.5611
Gemmatimonas	0.0010	0.0146	0.0257	**0.0390***
Geobacillus	0.0003	0.0000	0.0061	**0.0347***
Geobacter	0.0414	0.2138	0.1162	0.1133
Geodermatophilus	0.0089	0.0077	0.0022	0.0608
Glycomyces	0.0003	0.0118	0.0029	0.1112
Gordonia	0.0053	0.0004	0.0016	0.0568
Gracilibacter	0.0016	0.0004	0.0009	0.4792

Genera	BA0	BAC	BAT	*P* value
Haererehalobacter	0.0503	0.0337	0.0309	0.5611
Haliscomenobacter	0.0000	0.0160	0.0247	**0.0459***
Halomonas	0.0006	0.0000	0.0000	0.3679
Hydrogenophilus	0.0007	0.0000	0.0000	0.3679
Hylemonella	0.1211	0.3055	0.2867	0.0665
Hymenobacter	0.0074	0.0151	0.0193	0.0608
Hyphomicrobium	0.0341	0.1790	0.2959	**0.0273***
Hyphomonas	0.0000	0.0034	0.0006	0.1988
Iamia	0.0000	0.0003	0.0016	0.0625
Inquilinus	0.0052	0.0227	0.0104	0.1767
Isosphaera	0.0000	0.0000	0.0019	0.1054
JG37 AG 70	0.0293	0.0236	0.0155	0.2521
Janthinobacterium	0.0105	0.0199	0.0216	0.6703
Kaistia	0.0072	0.0030	0.0054	0.1133
Kaistobacter	5.0422	1.7775	1.0381	**0.0273***
Kouleothrix	0.0013	0.0044	0.0019	0.1926
Kribbella	0.0237	0.0143	0.0031	0.0509
Labrys	0.0190	0.0235	0.0117	0.1479
Lacibacter	0.0010	0.0022	0.0054	**0.0496***
Lactobacillus	0.0003	0.0000	0.0010	0.5580
Lactococcus	0.0003	0.0011	0.0000	0.5580
Larkinella	0.0049	0.0218	0.0179	0.0992
Leadbetterella	0.0010	0.0000	0.0000	0.1054
Legionella	0.0270	0.0170	0.0119	0.1133
Leptolyngbya	0.0180	0.0108	0.0160	0.6703
Leptonema	0.0000	0.0034	0.0079	0.0539
Leptospira	0.0000	0.0042	0.0034	0.0552
Luteimonas	0.0062	0.0419	0.0044	0.1905

Genera	BA0	BAC	BAT	*P* value
Luteolibacter	0.0466	0.0653	0.0587	0.8371
Lutibacterium	0.0000	0.0017	0.0006	0.5580
Lysobacter	0.0959	0.0838	0.0562	0.2019
Magnetospirillum	0.0000	0.0049	0.0006	0.1988
Marinomonas	0.0571	0.0012	0.0016	0.0608
Megamonas	0.0087	0.0041	0.0144	**0.0390***
Mesorhizobium	0.0777	0.1180	0.0801	0.0608
Methylibium	0.0973	0.1856	0.1095	0.1133
Methylobacterium	0.0086	0.0247	0.0255	0.0665
Methylocaldum	0.0126	0.0155	0.0052	0.3292
Methylosarcina	0.0009	0.0336	0.0009	0.0628
Methylotenera	0.0003	0.0420	0.0146	**0.0265***
Microbacterium	0.0060	0.0093	0.0064	0.7326
Mucilaginibacter	0.0000	0.0021	0.0015	0.0775
Mycobacterium	0.0424	0.0501	0.0248	**0.0390***
Mycoplana	0.0003	0.0059	0.0022	0.0568
Nannocystis	0.0097	0.0109	0.0154	0.3292
Nevskia	0.0446	0.0099	0.0069	0.7326
Niabella	0.0016	0.0208	0.0099	0.0509
Niastella	0.5330	0.2566	0.2117	0.0608
Nitrospira	0.6247	0.3813	0.2680	0.0608
Nocardia	0.0035	0.0011	0.0010	0.2890
Nocardioides	0.0250	0.0145	0.0047	**0.0390***
Nonomuraea	0.0151	0.0620	0.0035	0.2881
Nostoc	0.0010	0.0019	0.0000	0.5580
Nostocoida	0.0016	0.0000	0.0000	0.1054
Novispirillum	0.0009	0.0000	0.0000	0.1054
Novosphingobium	0.0450	0.0179	0.0068	**0.0390***

Genera	BA0	BAC	BAT	*P* value
OR 59	0.0951	0.1897	0.2103	0.0665
Oceanibaculum	0.0000	0.0014	0.0000	0.3679
Ochrobactrum	0.0223	0.0087	0.0071	0.4911
Oleomonas	0.0038	0.0004	0.0000	0.0625
Olivibacter	0.0030	0.0003	0.0000	0.1988
Opitutus	0.2148	0.3918	0.2923	0.0509
Oscillochloris	0.0013	0.0027	0.0075	0.1767
Oscillospira	0.0000	0.0000	0.0006	0.3679
Owenweeksia	0.0009	0.0000	0.0000	0.1054
Oxobacter	0.0007	0.0000	0.0012	0.2807
Paenibacillus	0.0495	0.0944	0.0924	0.1767
Paludibacter	0.0060	0.0000	0.0000	**0.0221***
Pantoea	0.3916	0.1178	0.1108	0.0665
Parabacteroides	0.0020	0.0051	0.0026	0.1184
Paracoccus	0.0006	0.0026	0.0013	0.2404
Parapedobacter	0.0016	0.0267	0.0003	0.0519
Parasegitibacter	0.0164	0.0093	0.0059	0.1479
Parvibaculum	0.0267	0.0228	0.0104	0.0608
Patulibacter	0.0000	0.0000	0.0010	0.1054
Pedobacter	0.0069	0.0353	0.0056	0.0608
Pedomicrobium	0.0193	0.0458	0.1774	**0.0273***
Peredibacter	0.0125	0.0256	0.0403	0.0992
Perlucidibaca	0.0067	0.0128	0.0079	0.1133
Phaeobacter	0.0006	0.0000	0.0000	0.3679
Phaeospirillum	0.0180	0.0180	0.0192	0.9565
Phenylobacterium	0.2006	0.2104	0.1786	0.6703
Phormidium	0.0034	0.0104	0.0089	0.4268
Pigmentiphaga	0.0175	0.0091	0.0053	0.1133

Genera	BA0	BAC	BAT	*P* value
Pilimelia	0.0337	0.0335	0.0287	0.7326
Pimelobacter	0.0113	0.0000	0.0000	**0.0221***
Pirellula	0.2704	0.6314	0.5674	0.0608
Planctomyces	0.2709	0.8629	1.2458	**0.0273***
Planifilum	0.0016	0.0032	0.0173	0.0608
Pleomorphomonas	0.0046	0.0196	0.0137	**0.0390***
Plesiocystis	0.0057	0.0543	0.0360	0.0509
Prauserella	0.0000	0.0014	0.0000	0.3679
Prevotella	0.0019	0.0023	0.0022	0.9565
Promicromonospora	0.0007	0.0473	0.0116	**0.0379***
Prosthecobacter	0.0087	0.0296	0.0177	0.1479
Pseudanabaena	0.0000	0.0000	0.0006	0.3679
Pseudofulvimonas	0.0000	0.0130	0.0003	0.1988
Pseudomonas	0.0045	0.0321	0.1172	**0.0273***
Pseudonocardia	0.0167	0.0237	0.0066	**0.0273***
Pseudoxanthomonas	0.0289	0.0529	0.0277	0.1931
Pythium	0.0003	0.0010	0.0000	0.1988
Rathayibacter	0.0000	0.0014	0.0000	0.1054
Rhizobium	0.0305	0.0374	0.0883	0.0665
Rhodococcus	0.0000	0.0010	0.0007	0.5580
Rhodocytophaga	0.0030	0.0021	0.0046	1.0000
Rhodoplanes	0.7088	1.4675	2.0278	**0.0273***
Rubricoccus	0.0000	0.0128	0.0042	0.1914
Rubrivivax	0.0524	0.0766	0.0519	0.0665
Rubrobacter	0.0032	0.0010	0.0000	0.1009
Ruminococcus	0.0007	0.0000	0.0000	0.3679
Ruminofilibacter	0.0000	0.0007	0.0009	0.3496
Runella	0.0000	0.0063	0.0042	0.0605

Genera	BA0	BAC	BAT	*P* value
SC3 56	0.0043	0.0007	0.0000	**0.0347***
SMB53	0.0063	0.0008	0.0031	0.7557
Salinibacterium	0.0377	0.0527	0.0326	0.3932
Salinispora	0.0272	0.0084	0.0051	0.6703
Saprolegnia	0.0000	0.0078	0.0000	0.1054
Sediminibacterium	0.0633	0.0363	0.0276	0.0509
Segetibacter	0.0139	0.0045	0.0032	0.1133
Shewanella	0.0075	0.0065	0.0122	0.0992
Shimazuella	0.0007	0.0007	0.0016	0.5501
Sinomonas	0.0146	0.0105	0.0054	**0.0390***
Siphonobacter	0.0154	0.0000	0.0006	**0.0347***
Skermanella	0.0059	0.0022	0.0028	0.1931
Solitalea	0.0010	0.0068	0.0158	0.0665
Sorangium	0.0322	0.0154	0.0120	0.1767
Sphingobacterium	0.0190	0.0091	0.0044	0.5280
Sphingobium	0.0193	0.0070	0.0059	0.0665
Sphingomonas	0.2105	0.2144	0.1337	0.0665
Sphingopyxis	0.0000	0.0074	0.0029	0.0775
Spirochaeta	0.0336	0.0773	0.1626	**0.0390***
Spirosoma	0.0054	0.0124	0.0259	**0.0390***
Sporichthya	0.0007	0.0023	0.0013	0.8542
Sporobacter	0.0003	0.0031	0.0006	0.0605
Sporocytophaga	0.0305	0.0649	0.0591	0.0608
Sporomusa	0.0019	0.0023	0.0035	0.4298
Sporosarcina	0.8192	1.4977	1.1453	0.1133
Staphylococcus	0.0003	0.0004	0.0010	0.4999
Stenotrophomonas	0.0637	0.0706	0.0299	0.3932
Steroidobacter	0.3515	0.8796	0.4681	0.0582

Genera	BA0	BAC	BAT	*P* value
Streptomyces	0.1141	0.0840	0.0286	**0.0390***
Symbiobacterium	0.0052	0.0018	0.0019	0.1926
Syntrophobacter	0.0203	0.0206	0.0142	0.3292
Syntrophomonas	0.0013	0.0011	0.0013	1.0000
Tatlockia	0.0012	0.0003	0.0012	0.9392
Telmatospirillum	0.0000	0.0027	0.0047	**0.0349***
Tepidimicrobium	0.0000	0.0000	0.0010	0.3679
Terracoccus	0.0137	0.0136	0.0082	0.1931
Terribacillus	0.0003	0.0023	0.0016	0.1385
Terriglobus	0.0286	0.0078	0.0089	0.1133
Thermacetogenium	0.0000	0.0000	0.0019	**0.0221***
Thermobacillus	0.0000	0.0017	0.0003	0.5580
Thermobispora	0.0003	0.0000	0.0016	0.1988
Thermomonas	0.3291	0.0811	0.0677	**0.0390***
Thiobacillus	0.0000	0.0000	0.0013	**0.0221***
Treponema	0.0000	0.0007	0.0057	**0.0347***
Turicibacter	0.0006	0.0010	0.0006	0.6386
Turneriella	0.0019	0.0933	0.1019	0.0608
Uliginosibacterium	0.0260	0.0445	0.0456	0.4298
Unclassified	0.0000	0.0000	0.0028	**0.0221***
Variovorax	0.1531	0.2257	0.1397	**0.0273***
Veillonella	0.0061	0.0067	0.0066	0.5866
Vermamoeba	0.0010	0.0000	0.0000	0.3679
Virgisporangium	0.0219	0.0445	0.0489	0.1133
Vogesella	0.0013	0.0148	0.0032	0.1456
WCHB1 84	0.0000	0.0033	0.0003	**0.0347***
Xylanimicrobium	0.0013	0.0183	0.0111	0.0509
Zoogloea	0.0010	0.0025	0.0000	0.1988

Genera	BA0	BAC	BAT	*P* value
4 29	0.0384	0.0290	0.0195	0.0509
planctomycete	0.0007	0.0023	0.0006	0.9481

Table 18 Statistical analysis of relative abundance (mean) in species taxa of bacteria between each soil before transplantation (BA0), uninoculation (BAC) and *G. mosseae* inoculation (BAT) at harvesting time.

Species	BA0	BAC	BAT	*P* value
Acinetobacter rhizosphaerae	0.0062	0.0456	0.0118	0.3932
Acrocarpospora pleiomorpha	0.0000	0.0074	0.0059	0.5580
Actinomadura vinacea	0.0022	0.0003	0.0006	0.4660
Alicyclobacillus shizuokensis	0.0000	0.0007	0.0007	0.5580
Amycolatopsis thermoflava	0.0010	0.0083	0.0016	0.0657
Bacillus badius	0.0177	0.0134	0.0122	0.5611
Bacillus cereus	0.0798	0.2198	0.0693	0.1931
Bacteroides fragilis	0.0058	0.0056	0.0103	0.4298
Bdellovibrio bacteriovorus	0.0431	0.0161	0.0173	0.0665
Bosea genosp.	0.0010	0.0047	0.0051	0.1133
Brevibacterium aureum	0.0012	0.0000	0.0000	0.3679
Burkholderia bryophila	0.0068	0.0026	0.0067	0.1931
Candidatus Glomeribacter gigasporarum	0.0007	0.0047	0.0056	0.3373
Candidatus Koribacter versatilis	0.0059	0.0081	0.0028	0.3012
Clostridium acetobutylicum	0.0019	0.0004	0.0013	0.3808
Clostridium bowmanii	0.0000	0.0000	0.0009	0.3679
Clostridium butyricum	0.0094	0.0154	0.0054	0.0794
Clostridium cellulovorans	0.0034	0.0000	0.0003	**0.0347***
Clostridium intestinale	0.0009	0.0011	0.0000	0.3496
Clostridium neonatale	0.0012	0.0000	0.0006	0.5580
Clostridium thermopalmarium	0.0000	0.0000	0.0013	0.3679

Species	BA0	BAC	BAT	*P* value
Constrictibacter antarcticus	0.0000	0.0013	0.0007	0.2807
Desulfosporosinus meridiei	0.0029	0.0011	0.0025	0.3902
Desulfovibrio putealis	0.0000	0.0094	0.0038	0.1258
Desulfovirga adipica	0.0000	0.0000	0.0006	0.3679
Dunaliella tertiolecta	0.0003	0.0013	0.0009	0.8079
Edaphobacter modestum	0.0121	0.0081	0.0044	0.1479
Filimonas lacunae	0.0007	0.0051	0.0091	0.3168
Flavobacterium columnare	0.0003	0.0121	0.0101	0.0650
Flavobacterium succinicans	0.0023	0.0765	0.0767	0.0594
Haererehalobacter salaria	0.0503	0.0337	0.0309	0.5611
Inquilinus limosus	0.0039	0.0035	0.0028	0.9565
Lacibacter cauensis	0.0010	0.0022	0.0054	**0.0496***
Legionella nagasakiensis	0.0045	0.0007	0.0003	**0.0459***
Methylosarcina lacus	0.0009	0.0336	0.0009	0.0628
Methylotenera mobilis	0.0003	0.0420	0.0146	**0.0265***
Mucilaginibacter daejeonensis	0.0000	0.0021	0.0015	0.0775
Nannocystis exedens	0.0097	0.0079	0.0101	0.7326
Nostoc muscorum	0.0010	0.0019	0.0000	0.5580
Nostocoida limicola III	0.0010	0.0000	0.0000	0.3679
Oceanibaculum indicum	0.0000	0.0014	0.0000	0.3679
Paenibacillus chondroitinus	0.0127	0.0276	0.0227	0.2881
Paenibacillus edaphicus	0.0055	0.0059	0.0047	0.5611
Paenibacillus stellifer	0.0022	0.0000	0.0032	0.1289
Parasegitibacter luojiensis	0.0164	0.0093	0.0059	0.1479
Peredibacter starrii	0.0125	0.0256	0.0403	0.0992
Phaeospirillum fulvum	0.0164	0.0155	0.0176	0.8371
Prauserella rugosa	0.0000	0.0014	0.0000	0.3679
Prosthecobacter debontii	0.0043	0.0165	0.0126	0.1133

Species	BA0	BAC	BAT	*P* value
Pseudanabaena galeata	0.0000	0.0000	0.0006	0.3679
Pseudofulvimonas gallinarii	0.0000	0.0130	0.0003	0.1988
Pseudomonas alcaligenes	0.0013	0.0238	0.0641	**0.0265***
Pseudoxanthomonas kalamensis	0.0000	0.0015	0.0003	0.5580
Pseudoxanthomonas kaohsiungensis	0.0016	0.0321	0.0087	**0.0390***
Pythium ultimum	0.0003	0.0010	0.0000	0.1988
Ruminofilibacter xylanolyticum	0.0000	0.0007	0.0009	0.3496
Saprolegnia ferax	0.0000	0.0078	0.0000	0.1054
Siphonobacter aquaeclarae	0.0154	0.0000	0.0006	**0.0347***
Solitalea canadensis	0.0010	0.0068	0.0158	0.0665
Sphingobacterium multivorum	0.0190	0.0049	0.0032	0.2760
Sphingomonas suberifaciens	0.0000	0.0142	0.0040	0.0539
Sphingomonas wittichii	0.0811	0.0736	0.0514	0.2019
Spirochaeta aurantia	0.0336	0.0764	0.0499	0.2521
Sporobacter termitidis	0.0003	0.0031	0.0006	0.0605
Stenotrophomonas panacihumi	0.0147	0.0217	0.0264	0.5866
Streptomyces mirabilis	0.0151	0.0068	0.0053	0.0665
Streptomyces scabrisporus	0.0007	0.0000	0.0000	0.3679
Thermobacillus composti	0.0000	0.0017	0.0003	0.5580
Thermobispora bispora	0.0003	0.0000	0.0016	0.1988
Unclassified	0.0000	0.0000	0.0028	**0.0221***
Vermamoeba vermiformis	0.0010	0.0000	0.0000	0.3679
Virgisporangium ochraceum	0.0219	0.0445	0.0489	0.1133
planctomycete DWL3I2	0.0007	0.0023	0.0006	0.9481

Table 19 Statistical analysis of relative abundance (mean) in phylum taxa of fungi between each soil before transplantation (FU0), uninoculation (FUC) and *G. mosseae* inoculation (FUT) at harvesting time.

Phyla	FU0	FUC	FUT	*P* value
Ascomycota	81.0509	87.2842	81.3019	0.5611
Basidiomycota	0.5440	0.7608	0.9683	0.4911
Chytridiomycota	0.0000	0.0043	0.0121	0.2807
Glomeromycota	0.1177	0.2179	0.0997	0.4298
Unclassified	18.1033	11.6633	17.3820	0.7326
Zygomycota	0.1841	0.0695	0.2361	0.2881

Table 20 Statistical analysis of relative abundance (mean) in genus taxa of fungi between each soil before transplantation (FU0), uninoculation (FUC) and *G. mosseae* inoculation (FUT) at harvesting time.

Genera	FU0	FUC	FUT	*P* value
Acremonium	0.1356	0.2018	5.7654	0.0608
Alternaria	0.2119	0.0246	0.0626	0.0794
Angulomyces	0.0000	0.0000	0.0034	0.3679
Arnium	0.0294	1.0320	2.4652	0.0509
Arthrographis	0.0000	0.0021	0.0000	0.3679
Aspergillus	1.3419	1.2490	0.6744	0.0665
Auricularia	0.0139	0.0097	0.0078	0.9187
Basidiobolus	0.0211	0.0000	0.0137	0.5580
Bensingtonia	0.0000	0.0000	0.0032	0.3679
Berkleasmium	0.0000	0.0054	0.0000	0.1054
Blastobotrys	0.0000	0.0084	0.0074	0.2369
Camarosporium	0.0209	0.0041	0.0011	0.3630
Campylocarpon	0.0041	0.0000	0.0151	0.1988
Candida	0.5022	0.1171	0.2203	0.0509
Capnodium	0.0041	0.0436	0.0032	0.1100

Genera	FU0	FUC	FUT	*P* value
Ceratocystis	0.0161	0.0086	0.0043	0.1767
Cercophora	0.0000	5.9983	0.1997	0.5580
Chaetomium	1.6341	0.1894	0.1231	0.0608
Cladophialophora	0.0010	0.0032	0.0032	0.9392
Cladorrhinum	0.4070	0.4254	0.2130	0.4298
Cladosporium	0.1695	0.0589	0.0857	0.3932
Claviceps	0.0052	0.0021	0.0000	0.5580
Clonostachys	0.0031	0.0257	0.0300	0.0650
Cochliobolus	2.4614	0.3132	0.1868	**0.0273***
Codinaeopsis	0.0000	0.0086	0.0011	0.1988
Colletotrichum	0.3803	0.0075	0.0085	0.0665
Coniocessia	0.0148	0.0011	0.0042	0.3319
Conlarium	0.0029	0.0105	0.0120	0.2982
Coprinus	0.0000	0.0032	0.0011	0.5580
Corynespora	0.0000	0.0053	0.0042	0.2807
Cosmospora	0.5530	0.0127	0.0306	0.0608
Cryptococcus	0.0454	0.0064	0.0105	0.5280
Curvularia	0.1099	0.0253	0.0044	**0.0390***
Cyberlindnera	0.0041	0.0000	0.0000	0.3679
Cylindrocarpon	0.0060	0.0000	0.0000	0.1054
Cyphellophora	0.0000	0.0021	0.0000	0.3679
Dactylella	0.0000	0.0160	0.0090	0.2807
Devriesia	0.0355	0.0054	0.0032	0.3630
Dinemasporium	0.0317	0.0000	0.0021	**0.0347***
Dokmaia	0.2159	12.9176	19.6724	**0.0390***
Edenia	0.0717	0.0398	0.0576	0.7326
Emericella	0.0257	0.8194	0.3470	**0.0273***
Emericellopsis	0.0050	0.0021	0.0000	0.2807

Genera	FU0	FUC	FUT	*P* value
Entrophospora	0.1177	0.2179	0.0997	0.4298
Exidia	0.0077	0.0032	0.0000	0.2807
Exophiala	0.0180	0.0140	0.0160	0.8752
Exserohilum	0.0010	0.0000	0.0042	0.5580
Fellomyces	0.0000	0.0052	0.0011	0.5580
Fusarium	1.1796	0.2842	0.3851	0.1133
Ganoderma	0.0010	0.0000	0.0034	0.5580
Gibellulopsis	0.0010	0.0095	0.0158	0.1825
Graphium	0.0059	0.0000	0.0000	0.1054
Guehomyces	0.0000	0.0000	0.0077	0.1054
Heterochaete	0.0152	0.0021	0.0200	0.9392
Hyphodermella	0.0000	0.0075	0.0053	0.2369
Kappamyces	0.0000	0.0000	0.0021	0.3679
Kurtzmanomyces	0.0000	0.0097	0.0000	0.3679
Lasiodiplodia	0.5653	0.0532	0.0525	0.0665
Leptosphaeria	0.0125	4.3695	0.8985	0.0608
Leptosphaerulina	0.0051	0.0237	0.0053	0.7557
Leptospora	0.0000	0.0000	0.0101	0.3679
Leptoxyphium	0.0000	0.0000	0.0034	0.3679
Lophiostoma	0.0269	0.0257	0.0064	0.3168
Magnaporthe	0.0000	0.0062	0.0042	0.5580
Meliniomyces	0.0000	0.0000	0.0032	0.3679
Metarhizium	0.0574	0.0054	0.0176	0.0549
Meyerozyma	1.1197	0.0343	0.0457	0.0608
Microascus	0.0010	0.0425	0.0137	0.1051
Microdochium	0.0283	0.0191	0.0095	0.3168
Microsphaeropsis	0.0000	0.0000	0.0089	0.1054
Mortierella	0.1631	0.0695	0.2224	0.3012

Genera	FU0	FUC	FUT	*P* value
Myceliophthora	0.3467	0.2530	0.2428	0.9565
Mycosphaerella	0.0397	0.0031	0.0022	0.1825
Myrmecridium	0.1230	0.4492	0.2585	0.2019
Myrothecium	0.7816	0.3000	0.1746	0.0794
Nectria	0.0000	0.0083	0.0152	0.3496
Neurospora	0.1139	0.1028	0.0928	0.8752
Occultifur	0.0000	0.0041	0.0011	0.5580
Paecilomyces	0.0088	0.0043	0.0000	0.2369
Parascedosporium	0.0094	0.0085	0.0000	0.2369
Penicillium	0.3955	0.7073	2.7430	0.5611
Periconia	1.4078	0.6462	0.0993	0.0665
Pesotum	0.0029	0.0000	0.0000	0.1054
Pestalotiopsis	0.0042	0.0108	0.0054	0.7248
Phaeoacremonium	0.1730	0.0054	0.0053	0.0657
Phaeoisaria	0.0237	0.0000	0.0000	**0.0221***
Phaeoseptoria	0.0165	0.5033	0.5194	0.0665
Phaeosphaeria	0.0241	1.3295	2.2605	0.0608
Phaeosphaeriopsis	0.0115	0.2546	0.4533	**0.0273***
Phanerochaete	0.0000	0.0885	0.0056	0.5580
Phialemonium	0.0031	0.0000	0.0000	0.3679
Phoma	0.8805	0.0985	0.3123	0.1479
Pichia	0.0074	0.0000	0.0000	0.1054
Pisolithus	0.0145	0.0021	0.0000	0.5580
Plectosphaerella	0.0000	0.0093	0.0011	0.5580
Podospora	0.0521	0.1650	0.4105	0.0794
Preussia	0.0161	1.7428	0.8417	0.0509
Pseudallescheria	0.1587	0.0202	0.0366	**0.0390***
Pseudocercospora	0.0771	0.0053	0.0096	0.5775

Genera	FU0	FUC	FUT	*P* value
Psilocybe	0.0000	0.0041	0.0074	0.5580
Pyrenochaeta	0.0422	0.3735	0.0644	0.5584
Pyrenochaetopsis	0.0000	0.9702	1.6772	**0.0459***
Rasamsonia	0.0019	0.0022	0.0000	0.5580
Rhodosporidium	0.0512	0.0052	0.0043	0.4660
Rhodotorula	0.0170	0.0097	0.0097	0.7326
Russula	0.0000	0.0065	0.0042	0.5580
Sarocladium	0.0000	0.0000	0.0022	0.3679
Schizophyllum	0.0000	0.0000	0.0117	0.3679
Schizothecium	0.0000	0.0914	0.0032	**0.0347***
Scytalidium	0.0467	0.0263	0.0398	0.5775
Sebacina	0.0000	0.0000	0.0181	0.1054
Simplicillium	0.0165	0.0149	0.0618	0.5866
Sistotrema	0.0000	0.0010	0.0221	0.5580
Spegazzinia	0.0010	0.0698	0.0374	0.0594
Sporobolomyces	0.0000	0.0022	0.0000	0.3679
Stachybotrys	0.1680	0.2128	0.3560	0.2521
Strelitziana	0.0000	0.0140	0.0032	0.3496
Talaromyces	0.2045	0.1412	0.1762	0.6703
Thermomyces	0.0000	0.0823	0.0203	**0.0349***
Thielavia	12.1094	2.6381	1.4037	**0.0390***
Thozetella	0.0116	0.0076	0.0032	0.8542
Togninia	0.3418	0.0118	0.0085	0.0665
Torula	0.0000	0.0771	0.0244	0.0775
Trichoderma	0.0733	0.1559	0.0472	0.7326
Trichosporon	0.0049	0.0032	0.0000	0.2246
Unclassified	18.1033	11.6633	17.3820	0.7326
Verticillium	0.0308	0.0159	0.0244	0.8645

Genera	FU0	FUC	FUT	*P* value
Viridispora	0.0853	0.0288	0.0489	0.1479
Volutella	0.0076	0.0052	0.0021	0.9392
Waitea	0.0000	0.0010	0.0297	0.5580
Wallemia	0.0000	0.0042	0.0021	0.2807
Westerdykella	0.5629	0.1506	0.5483	0.1479
Xylaria	0.5650	0.2727	0.4497	0.2019
Xylogone	0.4690	0.2936	0.1158	0.0794
Xylomyces	0.1745	0.0586	0.0381	0.0509
Zopfiella	1.1048	2.1849	2.4529	0.1931

Table 21 Statistical analysis of relative abundance (mean) in species taxa of fungi between each soil before transplantation (FU0), uninoculation (FUC) and *G. mosseae* inoculation (FUT) at harvesting time.

Species	FU0	FUC	FUT	*P* value
Acremonium furcatum	0.0000	0.0032	0.0042	0.5580
Acremonium polychromum	0.0373	0.0096	0.0011	**0.0459***
Acremonium sp AR2 30	0.0048	0.0284	0.0362	0.2760
Acremonium sp R8 9	0.0000	0.1113	5.6208	**0.0241***
Acremonium sp SHW11	0.0935	0.0491	0.1030	0.1767
Alternaria alternata	0.2021	0.0139	0.0482	0.0582
Alternaria sp	0.0098	0.0107	0.0144	0.5501
Amphisphaeriaceae sp	0.1024	0.0183	0.0226	0.5866
Angulomyces argentinensis	0.0000	0.0000	0.0034	0.3679
Aplosporellaceae sp	0.0010	0.0032	0.0000	0.5580
Archaeorhizomycetes sp	0.0000	0.0000	0.0032	0.3679
Arnium sp	0.0294	1.0320	2.4652	0.0509
Arthrographis sp	0.0000	0.0021	0.0000	0.3679
Aspergillus aculeatus	0.0747	0.0349	0.0465	0.5866

Species	FU0	FUC	FUT	*P* value
Aspergillus carbonarius	0.0041	0.0000	0.0021	0.5580
Aspergillus cibarius	0.3312	0.7280	0.4336	0.0509
Aspergillus flavus	0.0812	0.0824	0.0467	0.5611
Aspergillus japonicus	0.1239	0.0021	0.0011	0.4128
Aspergillus niveus	0.0188	0.0075	0.0063	0.5501
Aspergillus terreus	0.0883	0.1201	0.0296	0.3932
Auriculariales sp	0.0000	0.1042	0.0000	0.3679
Bensingtonia musae	0.0000	0.0000	0.0032	0.3679
Berkleasmium sp BCC 17003	0.0000	0.0054	0.0000	0.1054
Bionectriaceae sp	0.0141	0.0054	0.0021	0.1509
Blastobotrys adeninivorans	0.0000	0.0084	0.0074	0.2369
Camarosporium sp	0.0209	0.0041	0.0011	0.3630
Candida aaseri	0.0000	0.0000	0.0021	0.3679
Candida blankii	0.0000	0.0000	0.0021	0.3679
Candida gosingica	0.0076	0.0000	0.0000	0.3679
Candida orthopsilosis	0.0058	0.0010	0.0000	0.2807
Candida tropicalis	0.4774	0.1160	0.2161	0.0794
Capnodium coffeae	0.0000	0.0232	0.0021	**0.0347***
Capnodium sp	0.0041	0.0204	0.0011	0.9392
Ceratobasidiaceae sp	0.0230	0.0000	0.0000	0.3679
Ceratocystis paradoxa	0.0161	0.0086	0.0043	0.1767
Cercophora caudata	0.0000	5.9983	0.1997	0.5580
Chaetomium erectum	0.0049	0.0000	0.0000	0.1054
Chaetomium incomptum	0.3678	0.0537	0.0383	0.0665
Chaetomium medusarum	0.0022	0.0000	0.0000	0.3679
Chaetomium sp 4 SA 2013	1.2294	0.1304	0.0729	0.0608
Cladophialophora immunda	0.0010	0.0032	0.0011	0.9392
Cladorrhinum bulbillosum	0.2070	0.1067	0.0489	0.0794

Species	FU0	FUC	FUT	*P* value
Cladosporium sphaerospermum	0.0011	0.0021	0.0042	0.7753
Claviceps fusiformis	0.0052	0.0021	0.0000	0.5580
Clonostachys sp	0.0031	0.0257	0.0300	0.0650
Cochliobolus lunatus	2.2709	0.2848	0.1076	**0.0273***
Codinaeopsis sp	0.0000	0.0086	0.0011	0.1988
Conlarium duplumascospora	0.0029	0.0105	0.0120	0.2982
Coprinus cordisporus	0.0000	0.0032	0.0011	0.5580
Cosmospora sp	0.5530	0.0127	0.0306	0.0608
Cryptococcus aff taibaiensis IMUFRJ 51982	0.0232	0.0021	0.0021	0.3630
Cryptococcus carnescens	0.0038	0.0043	0.0084	0.9481
Cryptococcus flavus	0.0180	0.0064	0.0769	0.4792
Cryptococcus friedmannii	0.0057	0.0000	0.0000	0.3679
Cryptococcus laurentii	0.0040	0.0000	0.0000	0.1054
Curvularia brachyspora	0.0057	0.0000	0.0000	0.3679
Curvularia sp 01M 1	0.0408	0.0178	0.0011	0.1825
Curvularia sp LH3	0.0633	0.0076	0.0034	0.4999
Cyberlindnera jadinii	0.0041	0.0000	0.0000	0.3679
Cylindrocarpon decumbens	0.0060	0.0000	0.0000	0.1054
Dactylella oxyspora	0.0000	0.0160	0.0090	0.2807
Devriesia shakazului	0.0115	0.0000	0.0000	0.3679
Dokmaia sp	0.0000	0.0053	0.0195	**0.0241***
Dokmaia sp 2 TMS 2011	0.0750	11.5412	17.6785	**0.0390***
Edenia sp AS 60	0.0000	0.0010	0.0127	0.1988
Emericella sp d01	0.0257	0.3766	0.0751	0.0794
Emericellopsis glabra	0.0050	0.0021	0.0000	0.2807
Entrophospora sp	0.1177	0.2179	0.0997	0.4298
Exophiala jeanselmei	0.0000	0.0000	0.0021	0.3679
Exophiala spinifera	0.0087	0.0118	0.0095	0.6611

Species	FU0	FUC	FUT	*P* value
Exserohilum neoregeliae	0.0010	0.0000	0.0042	0.5580
Fellomyces fuzhouensis	0.0000	0.0052	0.0011	0.5580
Fusarium nelsonii	0.4519	0.1332	0.0767	0.0794
Fusarium sp 419	0.0000	0.0000	0.0064	0.3679
Fusarium sp FRC S1936	0.1258	0.0456	0.0261	**0.0390***
Fusarium sp hz 8	0.2758	0.0744	0.2544	0.2881
Fusarium sp IBL 03157	0.0249	0.0053	0.0109	0.7943
Gibellulopsis sp YH 2012	0.0010	0.0095	0.0158	0.1825
Guehomyces pullulans	0.0000	0.0000	0.0077	0.1054
Heterochaete shearii	0.0152	0.0021	0.0200	0.9392
Hyponectriaceae sp	0.0165	0.0000	0.0021	0.0625
Kappamyces sp Barr 316	0.0000	0.0000	0.0021	0.3679
Leptosphaeria sp B86 8	0.0125	4.3695	0.8985	0.0608
Leptosphaerulina sp	0.0051	0.0237	0.0053	0.7557
Leptospora rubella	0.0000	0.0000	0.0101	0.3679
Leptoxyphium sp TMS 2011	0.0000	0.0000	0.0034	0.3679
Magnaporthe griffinii	0.0000	0.0062	0.0042	0.5580
Massarinaceae sp	0.1759	0.1032	0.0641	0.8752
Meliniomyces sp	0.0000	0.0000	0.0032	0.3679
Metarhizium anisopliae	0.0150	0.0000	0.0021	0.2807
Metarhizium sp V259	0.0424	0.0054	0.0155	0.1052
Meyerozyma guilliermondii	1.1197	0.0343	0.0457	0.0608
Microascus sp r250	0.0010	0.0425	0.0137	0.1051
Microsphaeropsis arundinis	0.0000	0.0000	0.0089	0.1054
Montagnulaceae sp	0.0011	0.0075	0.0211	0.5011
Mortierella ambigua	0.1589	0.0587	0.2016	0.3012
Mortierella wolfii	0.0042	0.0108	0.0088	0.6177
Myceliophthora fergusii	0.0000	0.0032	0.0053	0.2369

Species	FU0	FUC	FUT	*P* value
Myceliophthora lutea	0.3467	0.2498	0.2375	0.9565
Mycosphaerella sp AA 2012	0.0397	0.0031	0.0022	0.1825
Myrmecridium schulzeri	0.1230	0.4492	0.2585	0.2019
Myrothecium cinctum	0.0672	0.0417	0.0191	0.1479
Myrothecium gramineum	0.3254	0.0396	0.0604	0.1184
Myrothecium inundatum	0.0550	0.0609	0.0482	0.9565
Myrothecium sp HKC11	0.0010	0.0104	0.0032	0.3319
Nectria mauritiicola	0.0000	0.0062	0.0074	0.5580
Nectria rigidiuscula	0.0000	0.0021	0.0078	0.5580
Occultifur sp TMS 2011	0.0000	0.0041	0.0011	0.5580
Parascedosporium putredinis	0.0094	0.0085	0.0000	0.2369
Penicillium capsulatum	0.0000	0.0104	0.0021	0.2807
Penicillium citrinum	0.0781	0.0293	0.0320	0.4298
Penicillium ilerdanum	0.0000	0.5573	2.6229	0.0552
Penicillium implicatum	0.0000	0.0000	0.0032	0.3679
Penicillium oxalicum	0.0431	0.0085	0.0063	0.8712
Penicillium parvulum	0.0041	0.0011	0.0000	0.5580
Penicillium pimiteouiense	0.0140	0.0042	0.0032	0.5501
Penicillium sp 0410ARD6F 2	0.0100	0.0000	0.0022	0.1988
Penicillium sp NRRL 35186	0.0130	0.0022	0.0000	**0.0340***
Penicillium sp NRRL 35203	0.0185	0.0374	0.0147	0.5775
Penicillium spinulosum	0.0000	0.0000	0.0056	0.3679
Periconia sp	1.4021	0.5890	0.0951	0.0665
Periconia sp 9 MU 2012	0.0000	0.0572	0.0000	0.3679
Pesotum australi	0.0029	0.0000	0.0000	0.1054
Pestalotiopsis theae	0.0000	0.0086	0.0021	0.5580
Phaeoacremonium fuscum	0.1586	0.0022	0.0022	0.0605
Phaeoacremonium rubrigenum	0.0010	0.0010	0.0032	0.9392

Species	FU0	FUC	FUT	*P* value
Phaeoisaria clematidis	0.0237	0.0000	0.0000	**0.0221***
Phaeoseptoria sp FF 2011	0.0165	0.5033	0.5194	0.0665
Phaeosphaeria sp HKA27	0.0241	1.3208	2.2605	0.0608
Phaeosphaeria sp HKC12	0.0000	0.0086	0.0000	0.3679
Phaeosphaeriopsis sp	0.0000	0.0032	0.0000	0.3679
Phaeosphaeriopsis sp C652	0.0115	0.2513	0.4533	**0.0273***
Phanerochaete australis	0.0000	0.0000	0.0056	0.3679
Phanerochaete chrysosporium	0.0000	0.0885	0.0000	0.3679
Phialemonium dimorphosporum	0.0031	0.0000	0.0000	0.3679
Phoma medicaginis	0.0506	0.0192	0.0042	0.2760
Phoma sp FF 2011	0.8105	0.0739	0.3016	0.1133
Pichia myanmarensis	0.0074	0.0000	0.0000	0.1054
Plectosphaerella citrulli	0.0000	0.0093	0.0011	0.5580
Pleurostomataceae sp	0.0324	0.0000	0.0000	0.1054
Podospora dakotensis	0.0000	0.0032	0.0189	0.3496
Podospora sp	0.0456	0.0216	0.0000	0.1009
Preussia pseudominima	0.0086	0.0000	0.0000	0.3679
Preussia sp EAL24	0.0000	0.0720	0.0011	0.5580
Preussia terricola	0.0010	0.0095	0.0000	0.1988
Pseudallescheria angusta	0.1587	0.0202	0.0366	**0.0390***
Pyrenochaetopsis leptospora	0.0000	0.9702	1.6772	**0.0459***
Rasamsonia emersonii	0.0019	0.0022	0.0000	0.5580
Rhodosporidium paludigenum	0.0172	0.0031	0.0021	0.9392
Rhodosporidium toruloides	0.0340	0.0021	0.0022	0.3630
Rhodotorula cassiicola	0.0067	0.0000	0.0063	0.3496
Rhodotorula lamellibrachiae	0.0021	0.0064	0.0000	0.0625
Rhodotorula mucilaginosa	0.0000	0.0000	0.0034	0.3679
Rhodotorula philyla	0.0083	0.0000	0.0000	0.3679

Species	FU0	FUC	FUT	*P* value
Russula xerampelina	0.0000	0.0065	0.0042	0.5580
Sarocladium strictum	0.0000	0.0000	0.0022	0.3679
Schizophyllum commune	0.0000	0.0000	0.0117	0.3679
Schizothecium carpinicola	0.0000	0.0914	0.0032	**0.0347***
Scytalidium circinatum	0.0000	0.0000	0.0053	0.3679
Scytalidium sp	0.0467	0.0263	0.0346	0.6460
Sistotrema brinkmannii	0.0000	0.0010	0.0221	0.5580
Spegazzinia sp	0.0010	0.0698	0.0374	0.0594
Sporidiobolales sp	0.0163	0.0000	0.0000	0.3679
Sporobolomyces aff jilinensis MCA 3774	0.0000	0.0022	0.0000	0.3679
Stachybotrys cf elegans HGUP 0208	0.0817	0.0395	0.0151	**0.0390***
Stachybotrys microspora	0.0000	0.0192	0.0011	**0.0347***
Stachybotrys sp HGUP 0201	0.0269	0.0176	0.0147	0.4792
Talaromyces flavus	0.1710	0.1327	0.1635	0.7326
Talaromyces islandicus	0.0115	0.0063	0.0021	0.4029
Thermomyces lanuginosus	0.0000	0.0823	0.0203	**0.0349***
Thielavia terricola	12.1094	2.6381	1.4037	**0.0390***
Togninia minima	0.3418	0.0118	0.0085	0.0665
Torula sp	0.0000	0.0771	0.0244	0.0775
Tremellales sp	0.0000	0.0000	0.0021	0.3679
Trichoderma brevicompactum	0.0000	0.0022	0.0000	0.3679
Trichoderma ghanense	0.0000	0.0032	0.0000	0.3679
Trichosporon asahii	0.0030	0.0021	0.0000	0.3496
Unclassified	0.0000	0.0000	0.0121	0.1054
Verticillium leptobactrum	0.0308	0.0159	0.0244	0.8645
Viridispora sp	0.0853	0.0288	0.0489	0.1479
Volutella sp	0.0076	0.0052	0.0021	0.9392
Waitea circinata var circinata	0.0000	0.0010	0.0297	0.5580

Species	FU0	FUC	FUT	*P* value
Westerdykella angulata	0.0021	0.0076	0.0053	0.9481
Westerdykella dispersa	0.0363	0.0043	0.3794	**0.0390***
Westerdykella ornata	0.1029	0.0128	0.0306	**0.0390***
Westerdykella sp WQ63	0.0033	0.0043	0.0180	0.9392
Xylaria badia	0.0000	0.0032	0.0000	0.3679
Xylaria psidii	0.0000	0.0054	0.0000	0.3679
Xylaria sp D61	0.0000	0.0041	0.0000	0.3679
Xylaria sp WR1	0.5531	0.2556	0.4497	0.2019
Xylomyces sp	0.1745	0.0586	0.0381	0.0509
Zopfiella marina	0.9527	1.3265	2.3968	0.1133
Zopfiella sp M2 XS 2012	0.0011	0.0124	0.0147	0.9392

VITA

Miss Patai Charoonnart was born on April 3rd, 1984 in Bangkok, Thailand. In 2006, she graduated with bachelor's degree in genetic program from Department of Botany, Faculty of Science, Chulalongkorn University. Subsequently, she studied the application of chitosan on postharvest of vegetable for master's degree of science in program in biotechnology, Chulalongkorn University with the thesis entitled "Chitosan Usage to Delay Senescence and Extend Postharvest Storage of Asparagus Asparagus officinalis" and graduated in 2007. After that, she was granted from ChulalongKorn University Graduate Scholarship to Commemorate the 72nd Anniversary of His Majesty King Bhumibol Adulyadej in 2010 for doctor's degree and continuously worked with vegetable technology in Biological Science program at the same university since 2009.