EFFECTS OF *Bacillus salmalaya* STRAIN 139SI ON OIL PALM (*Elaeis guineensis* Jacq.) GROWTH AND YIELD

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EFFECTS OF *Bacillus salmalaya* STRAIN 139SI ON OIL PALM (*Elaeis guineensis* Jacq.) GROWTH AND YIELD

ABSTRACT

Plant-microbe interactions in the rhizosphere are one of the most important determining factors that could influence soil fertility and plant growth. Thus, a pre-nursery to field-scale experiment was conducted to study the potential of a new strain of plant growth-promoting rhizobacteria (PGPR), *Bacillus salmalaya* strain 139SI on soil fertility, oil palm nutrient uptake, physiology and fresh fruit bunch (FFB) yield. The early screening on the plant growth-promoting features of the *B. salmalaya* strain 139SI showed that the strain was positive for indole acetic acid (IAA) and siderophores production. The strain was also involved in biological nitrogen fixation (BNF) and able to solubilize phosphate. Analysis of strain 139SI colonization showed that the strain colonized and attached to the root surface by forming a biofilm. The strain 139SI was identified as endophytic bacteria as it showed the ability to colonize plant rhizosphere and penetrate into the plant internal root tissue. The plant growth promoting features of strain 139SI were further confirmed by growth enhancement of oil palm seedling inoculated with this strain in the nursery experiment. Analysis of soil nutrient content found that inoculation of 139SI increases totals N content in the soil. In addition, the results of the nursery experiment also revealed the synergistic effects of 139SI inoculation with chemical fertilizer. Addition of strain 139SI inoculation to the fertilized palm produces the best results for plant growth and significantly enhanced nutrient uptake. Ultimately, enhanced in palm nutrients uptake has directly increased the photosynthetic activity. A one year of field experiment found that inoculation of *B. salmalaya* strain 139SI produced higher palm fresh fruit bunch (FFB) yield over the untreated. Integrating the 139SI inoculant with inorganic fertilizer resulted in more substantial FFB yield than palm received recommended inorganic fertilizer rate. Enhancement of N level in soil

samples from field site and nutrient uptake was also recorded in strain 139SI inoculated palm. While the number of bunches produced by palm, the oil extraction rate, and fatty acid profile shows comparable reading among all treatments. The overall findings of this study suggest that associations of this novel strain with oil palm at the early stage of growth could enhance growth quality of oil palm seedlings, hence, enable better adaptation of the seedlings to the environmental conditions of the planting site. The potential of strain 139SI in enhancing oil palm yield as evidenced in the field experiment, for the first time provides the information on the potential of integrating PGPR in oil palm agronomic practice. Furthermore, the synergistic effect of this strain in optimizing the fertilizer use efficiency could lead to more sustainable agriculture practice in oil palm industry.

Keywords: Plant growth-promoting rhizobacteria, soil fertility, nutrient uptake, field trial, endophyte.

KESAN - KESAN *Bacillus salmalaya* STRAIN 139SI KEATAS PERTUMBUHAN DAN HASIL KELAPA SAWIT (*Elaeis guineensis* Jacq.)

ABSTRAK

Interaksi diantara tumbuhan-mikrob di dalam rhizosphere merupakan antara salah satu faktor penentu penting yang boleh mempengaruhi kesuburan tanah dan tumbesaran tumbuhan. Oleh itu, satu kajian bermula dari peringkat pra-nurseri hingga ke sekala lapangan telah dijalankan untuk mengkaji potensi strain baru rhizobacteria penggalak-tumbesaran tumbuhan (PGPR), *Bacillus salmalaya* strain 139SI terhadap kesuburan tanah, pengambilan nutrien, fisiologi tumbuhan dan hasil tuaian kelapa sawit. Penyaringan awal terhadap ciri – ciri penggalak-tumbesaran tumbuhan *B. salmalaya* strain 139SI mendapati strain baru ini memberikan keputusan positif untuk penghasilan asid indole asetik (IAA) dan siderophores. Strain 139SI ini juga didapati terlibat dalam proses pengikatan nitrogen secara biologi (BNF) dan mampu melarutkan fosfat. Analisis terhadap corak pengkolonian strain 139SI menunjukkan bahawa strain ini mampu untuk mengkoloni permukaan akar dengan membentuk biofilm. Strain 139SI juga dikenalpasti sebagai bakteria endophytic kerana ia menunjukkan keupayaan untuk mengkoloni rhizosphere dan menembusi ke dalam tisu dalaman akar tumbuhan. Ciri-ciri penggalak-tumbesaran tumbuhan strain 139SI telah disahkan lagi oleh peningkatan tumbesaran anak benih kelapa sawit yang menerima inokulasi strain ini dalam kajian di peringkat nurseri. Analisis kandungan nutrien tanah mendapati bahawa inokulasi 139SI meningkatkan jumlah kandungan N dalam tanah. Di samping itu, hasil kajian di peringkat nurseri juga menunjukkan kesan sinergi inokulasi strain 139SI dengan baja inorganik. Pemberian inokulasi strain 139SI kepada anak benih kelapa sawit yang menerima baja inorganic memberikan hasil yang terbaik untuk tumbesaran tumbuhan dan mempertingkatkan pengambilan nutrien dengan ketara. Akhirnya, peningkatan dalam pengambilan nutrien oleh tumbuhan ini didapati secara langsung telah

meningkatkan aktiviti fotosintesis. Kajian lapangan selama setahun mendapati inokulasi strain *B. salmalaya* 139SI menghasilkan tuaian tandan buah segar (FFB) yang lebih tinggi berbanding pokok kelapa sawit yang tidak dirawat. Mengintegrasikan pemberian inokulasi strain 139SI bersama baja inorganik memberikan peningkatan hasil FFB yang lebih tinggi berbanding pokok kelapa sawit yang hanya menerima baja inorganik pada kadar yang disyorkan. Peningkatan nilai N dalam sampel tanah yang diambil dari tapak kajian lapangan dan pertambahan kadar pengambilan nutrien juga direkodkan oleh pokok sawit yang menerima inokulasi strain 139SI. Manakala jumlah tandan yang dihasilkan oleh setiap pokok, kadar perahan minyak, dan profil asid lemak dari minyak yang dihasilkan menunjukkan bacaan yang setara antara semua kumpulan rawatan. Secara keseluruhannya, keputusan dari kajian ini mendapati hubungan mutual strain baru ini dengan anak pokok kelapa sawit pada peringkat awal tumbesaran dapat meningkatkan kualiti pertumbuhan anak benih, dengan itu, membolehkan anak benih tersebut menyesuaikan diri dengan lebih baik terhadap keadaan persekitaran di ladang tanaman. Potensi strain 139SI dalam meningkatkan hasil kelapa sawit seperti yang dibuktikan dalam kajian lapangan, buat pertama kalinya menyediakan maklumat tentang potensi mengintegrasikan PGPR dalam amalan agronomi di ladang kelapa sawit. Tambahan pula, kesan sinergistik strain baru yang digunakan dalam kajian ini dalam mengoptimumkan kecekapan penggunaan baja boleh membawa kepada amalan pertanian yang lebih lestari dalam industri kelapa sawit.

Kata kunci: Rhizobakteria penggalak-tumbesaran tumbuhan, kesuburan tanah, pengambilan nutrient, kajian lapangan, endophyte.

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LIST OF ABBREVIATIONS

%	=	Percentage
>	=	More than
°C	=	Degree celcius
ANOVA	=	Analysis of Variance
BHI	=	Brain heart infusion
Ca	=	Calcium
$Ca_3(PO_4)$	=	Calcium phosphate
$CaCl_2 \cdot 2H_2O$	=	Calcium chloride dihydrate
CAS	=	Chrome azurol-S
CFU	=	Colony forming unit
cm	=	Centimetre
cm3	=	Centimeter
Cu	=	Copper
et al.	=	et alia (and other)
Fe^{2+}	=	Ferrous Ion
Fe^{3+}	=	Ferric Ion
$FeCl_3$	=	Ferric chloride
$FeCl_3.6H_2O$	=	Iron(III) chloride hexahydrate
FFB	=	Fresh fruit bunch
F_M	=	Maximal fluorescence
F_O	=	Minimal fluorescence
F_V	=	Variable fluorescence

g	=	Gram
h	=	Hour
HCl	=	Hydrochloric acid
ICP-OES	=	Inductively Coupled Plasma Optical Emission Spectrometry
K	=	Potassium
K_2HPO_4	=	Dipotassium phosphate
KCl	=	Potassium chloride
kg	=	kilogram
KOH	=	Potassium hydrate
Mg	=	Magnesium
mg	=	Milligram
$MgSO_4.7H_2O$	=	Magnesium sulfate heptahydrate
min	=	Minute
ml	=	Milliliter
mm	=	Millimeter
mM	=	Millimolar
$MnSO_4 \cdot H_2O$	=	Manganese(II) sulfate monohydrate
$Na_2MoO_4 \cdot 2H_2O$	=	Sodium molybdate dihydrate
NaCl	=	Sodium chloride
NBRIP	=	National Botanical Research Institute's Phosphate
Nfb	=	Nitrogen free semisolid medium
NH_4Cl	=	Ammonium chloride
nm	=	Nanometer
P	=	Phosphorus

PBS	=	Phosphate-buffered saline
PGPR	=	Plant growth-promoting rhizobacteria
ppm	=	Parts per million
rpm	=	Revolution per minute
s	=	Second
S	=	Sulfur
W/V	=	Weigth per volume
µg	=	Microgram
µl	=	Microlitre
µM	=	Micromolar

CHAPTER 1: INTRODUCTION

1.1 Application of Chemical Fertilizer and Its Environmental Impacts

Maintaining soil quality is one of the biggest challenges in agriculture industry. Soil nutrients that are removed continuously through the harvested yield or absorbed for plant growth need to be replaced. Soil fertility can be restored effectively through fertilization. However, indiscriminate use and over-fertilization, especially with chemical fertilizer, will cost adverse effect on the ecosystems. Many long term studies have concluded that routine inorganic fertilization has led to the net loss of soil organic matter (Kotschi, 2013). The amount of soil organic matter made up of plant and animal residues at various stages of decomposition, cells and tissues of soil organisms, and substances synthesized by soil organisms is one of soil fertility determinant. This is because soil organic matter improves water-holding capacity and aeration, enhances absorption and release of nutrients, and makes soil less susceptible to leaching and erosion (Baldock & Nelson, 2000). Thus, Loss of soil organic matter could decreases soil quality and the agronomic efficiency of fertilizer, consequently larger amounts of fertilizer is needed for optimum yield (Mulvaney *et al.,* 2009).

Intensification of agricultural production involving the application of inorganic fertilizers is also one of the sources of ground and surface waters contamination. Water contamination caused by nitrate leaching from fertilizers has become a major concern worldwide (Di & Cameron, 2002). A high concentration of nitrate in drinking water can cause interference of oxygen transportation in blood circulation system, particularly for infants less than 1 year of age. Nitrate leaching from excessive fertilizer application drained into water sources was also found to be the element that causes deterioration of surface water quality, resulting in eutrophication, algal bloom, and fish poisoning (Howarth, 1988).

Currently, fertilizer use in the agriculture industry is expanding globally in line with growing demands for food and fuel from world population. However, this practice is considered one of the main causes for the increase in greenhouse gas N_2O emissions (Mosier, 2001). The concentration of N_2O in the atmosphere has increased from 270 parts per billion (ppb) in the preindustrial period to approximately 319 ppb in 2005 (IPCC, 2007). The increased of N_2O emissions into the atmosphere is of great concern due to its high global warming potential: 298 times greater than CO_2 (Forster *et al.,* 2007). Hence, the fertilizer-used efficiency through best management practices in the agriculture industry plays a large role in minimizing residual soil N_2O, which helps lower the risk of increased greenhouse gas emissions (Snyder *et al.,* 2009)

1.2 Role of Plant Growth-Promoting Rhizobacteria (PGPR) in Agronomic Practices

Due to the aforementioned reasons, plant growth-promoting rhizobacteria (PGPR) has emerged as an important component of the integrated nutrient supply system in agronomic practices in recent years. A huge attention was given to PGPR since it holds great potential in improving soil fertility and crop yields, but contrary to chemicals fertilizer, it is through more eco-friendly means. This scenario is contributed by the advances in the understanding the role of the plant-microbe interactions in improving crop productivity.

Generally, the plant growth-promoting mechanism that can favor a reduction in agro-chemical use and support eco-friendly crop production exhibited by PGPR can be divided into direct and indirect mechanism. The direct mechanisms include modulating plant hormone levels, facilitating nutrients acquisition via N_2-fixation and mobilization of nutrients by production of phosphatases and siderophores. Indirectly, the bacteria may exert plant growth-promoting effects by decreasing the inhibitory effects of various pathogenic organisms by inducing host resistance to the pathogen or acts as biocontrol

agent by suppressing the growth of pathogenic organisms that inhabit in the immediate vicinity of the root (Ahemad & Kibret, 2014). The details on the mechanisms of soil bacteria in promoting plant growth will be further elaborated in the literature review chapter.

1.3 Applications of PGPR in Oil Palm Agriculture Industry

Oil palm is well known for having a high fertilizer input demand to sustain high yields. This is one of the major challenges faced by oil palm grower to meet the growing global demand for oil and fats while at the same time, keeping the environmental impacts of oil palm cultivation at the lowest level (Comte *et al*., 2012). Although PGPR have a long success story and now widely used in the agriculture industry, its application on oil palm is still scarce. Several findings from previous research have concluded that PGPR effectively enhances nutrient uptake and oil palm growth at different stages of palm development. Azlin *et al.* (2009) reported that introduction of PGPR to oil palm tissues during the *in vitro* micropropagation process successfully inducing root formation and improved the growth of the oil palm. The same observation was reported by Noor Ai'shah *et al.* (2013) in which indole acetic producing-rhizobacteria enhances root development and growth of in vitro oil palm shoots.

A nursery experiment was conducted by Amir *et al.* (2005) showed that oil palm seedlings inoculated with *Azospirillum* and *Bacillus* spp produce a higher accumulation of nutrients in the plant tissues and leads to enhanced growth and leaf chlorophyll content. The only field experiment involving PGPR and oil palm was conducted by Zakry *et al.* (2012). In their studies, inoculation of *Bacillus sphaericus* strain UPMB-10 increased the N and dry matter yields of the palm leaflets and rachis significantly. Meanwhile, *Pseudomonas aeruginosa* GanoEB1, *Burkholderia cepacia* GanoEB2, and *Pseudomonas syringae* GanoEB3 isolated from oil palm root tissues reduce the

percentage of disease incidence, severity of foliar symptoms and dead in seedlings that were pre-treated with those bacteria (Ramli *et al.,* 2016)

1.4 Research Gap and Objectives of the Study

Over the last few decades, the effectiveness of PGPR as an alternative to reduce agro-chemical activities and at the same time enhancing crop yield has been widely reported such as on wheat, rice, maize, potato, and many others (Kuan *et al.,* 2016; Kumar *et al.,* 2014; Lavakusha *et al.,* 2014; Ekin *et al.,* 2009) . However, no information regarding the beneficial effects of PGPR on oil palm yield is quite surprising in view of the promising potential offered by PGPR based on numerous studies reported earlier. Moreover, the environmental impacts due to fertilizers used needs a serious action in order to reduce its application as recommended by The Roundtable on Sustainable Palm Oil (RSPO) (Klaarenbeeksingel, 2009). Thus, a pre-nursery to field-scale study was designed to fill this knowledge gap and pave a way to explore the potential of PGPR application in oil palm plantation industry. The objectives of this study were:

i. To determine the plant growth-promoting features of soil bacteria, *Bacillus salmalaya* strain 139SI.
ii. To study the plant-microbe interaction between *B. salmalaya* strain 139SI and oil palm in rhizosphere niche by analyzing the distribution and root colonization pattern of *B. salmalaya* strain 139SI.
iii. To study the effects of *B. salmalaya* strain 139SI inoculation on soil fertility and the synergistic effects of this rhizobacteria inoculant with inorganic fertilizer on plant physiology and nutrients uptake under nursery and field condition.
iv. To study the effects of *B. salmalaya* strain 139SI inoculant on the palm yield

v. To analyze the oil extraction rate (OER) and the fatty acid composition of crude palm oil (CPO) as means to determine the quality of oil produced from palm tree inoculated with *B. salmalaya* strain 139SI.

CHAPTER 2: LITERATURE REVIEW

2.1 Rhizosphere

Rhizosphere is referred to the narrow region of soil directly influenced by the root system and associated soil microorganisms (Walker *et al.,* 2003; Dobbelaere *et al.,* 2003). Plant roots synthesize and secrete a diverse kind of compounds, generally called as root exudates, such as amino acids, organic acids, sugars, enzymes and vitamins (Table 2.1).

Table 2.1: Various compounds in root exudates (Dakora & Phillips, 2002).

Chemical groups	Compounds
Amino acids	a-Alanine, b-alanine, asparagines, aspartate, cystein, cystine, glutamate, glycine, isoleucine, leucine, lysine, methionine, serine, threonine, proline, valine, tryptophan, ornithine, histidine, arginine, homoserine, phenylalanine, c-Aminobutyric acid and a-Aminoadipic acid
Organic acids	Citric acid, oxalic acid, malic acid, fumaric acid, succinic acid, acetic acid, butyric acid, valeric acid, glycolic acid, piscidic acid, formic acid, aconitic acid, lactic acid, pyruvic acid, glutaric acid, malonic acid, tetronic acid, aldonic acid and erythronic acid
Sugars	Glucose, fructose, galactose, ribose, xylose, rhamnose, arabinose, desoxyribose, oligosaccharides, raffinose and maltose
Vitamins	Biotin, thiamin, pantothenate, riboflavin and niacin
Purines/ nucleosides	Adenine, guanine, cytidine and uridine
Enzymes	Acid/alkaline-phosphatase, invertase, amylase and protease
Inorganic ion and gaseous molecules	HCO^-_3 ,OH^-,H^+ and CO_2.H_2

Production of different compounds of exudates by plant roots influences the solubility and hence availability of nutrients. Such products can influencing rhizosphere properties by altering rhizosphere pH and the activity of microbes including PGPR (Table 2.2).

Table 2.2: Root exudates and their roles in the rhizosphere (Jones *et al.,* 2004)

Compounds	Activity
Phenolics	Chelating nutrients with little solubility (e.g., Fe), source of nutrients, increasing microbial growth, inducing or inhibiting rhizobial Nod genes, signals attracting microbes and controlling pathogens.
Phytosideropores and amino acids	Source of nutrients and signals attracting microbes.
Organic acids	Source of nutrients, signals attracting microbes, chelating nutrients with little solubility (e.g., Fe) and inducing Nod genes.
Purines	Source of nutrients.
Vitamins	Increasing the growth of plants and microbes and as a source of nutrients.
Enzymes	Enhancing P solubility from organic molecules and increasing the of mineralization rate of organic products.
Sugars	Source of nutrients and increasing microbial growth.
Root cells	Controlling cell cycling and gene expression by producing signals, enhancing microbial growth, producing chemo attractants, producing proteins and mucilage, production of molecules to increase the rhizosphere immunity.

These compounds secreted by plant roots could also act as a chemical attractant for a vast number of diverse and actively metabolizing soil microbial communities. This is because the exudation of a wide range of chemical compounds by plant root in the rhizosphere zone could modify the chemical and physical properties of the soil and thus, regulates the structure of soil microbial community in the immediate vicinity of root surface (Dakora & Phillips, 2002). The rhizosphere zone also is rich in nutrients for microbes when compared to the bulk soil. This is well reflected by the number of bacteria that are present around the roots of the plants are generally higher than in the bulk soil (Weller & Thomashow, 1994). Thus, in contrast to other microenvironments of plant-soil niche, the rhizosphere is characterized by a high microbial abundance and activities. The corresponding microbial community associated to plant roots is referred as the rhizosphere microbiome (Chaparro *et al*., 2013). The rhizosphere microbiome composition is distinctive from that of the microbial community of the surrounding bulk soil (Bulgarelli *et al.,* 2013; Chaparro *et al.,* 2013).

2.2 Plant-Microbe Interactions Mediated by Root Exudates

Interestingly, the composition of root exudate may also changes along the different parts of the root system, stages of plant development or according to plant genotype and consequently, the soil-microbe composition also differs accordingly (Berg & Smalla, 2009; Bulgarelli *et al.,* 2013; Chaparro *et al.,* 2013). In such way, a specific composition of root exudates may create a niche that influences which soil microbial communities are to colonize plant root immediate vicinity (Grayston *et al.,* 1998). The influence of root exudate produced by plant towards microbial communities in rhizosphere can be classified into positive and negative plant-microbe interaction.

2.3 Positive Plant-Microbe Interactions

An example of positive plant-microbe interactions promotes by root exudate is endosymbiotic rhizobium bacteria with plants of the legume family. Flavonoids present in the root exudates of legumes have been shown to play an important role in initiate legume-rhizobium symbiosis (Coronado *et al.,* 1995; Zhang *et al.,* 2009). A study describes the changes of root exudates along the different parts of the root system of *Avena barbata* as reported by Jaeger *et al.* (1999) showed that the availability of tryptophan (the precursor for a major auxin, indole 3-acetic acid) was mainly near the root tip region. This finding suggesting that auxin-producing bacteria, such as plant growth promoting rhizobacteria (PGPR) could exploit tryptophan from root exudate for various precursors of growth regulators and encourage the beneficial plant-microbe symbiotic interactions (Nardi *et al.,* 2000).

2.4 Negative Plant-Microbe Interactions

Conversely, some of the exudates act as repellents against microorganisms. This phenomenon has been reported previously by Bais *et al.* (2002) in which they had identified rosmarinic acid (RA), a kind of caffeic acid ester in the root exudates of hairy root cultures of sweet basil (*Ocimum basilicum*), elicited using fungal cell wall extracts from *Phytophthora cinnamoni*. The studies by Brigham *et al.* (1999) with *Lithospermum erythrorhizon* hairy roots reported the production of pigmented naphthoquinones by specific root cell upon elicitation, and other biological activity against soil-borne bacteria and fungi. These findings provide valuable insights into the biological importance of root exudates in defending the rhizosphere against pathogenic microorganisms.

2.5 Plant Growth Promoting Rhizobacteria (PGPR)

The term of plant growth promoting rhizobacteria was first introduced by Kloepper and Schroth (1978) to refer to the bacteria that colonize the roots of plants (rhizosphere) and enhance plant growth. PGPR involved in promoting plant growth and development via production and secretion of various regulatory chemicals in the vicinity of rhizosphere, facilitating nutrient acquisition and acts as biocontrol agents by decreasing the inhibitory effects of various plant pathogens (Glick, 2012). There are three distinct characteristics that differentiate PGPR from other rhizosphere mirobiome. The most distinctive feature of PGPR is they must promote plant growth. they must be proficient to colonize the rhizophere and they must be able to multiply and compete with other microbiota in order to suvive in the rhizophere, at least for the time needed to express their plant growth promotion or protection activities (Kloepper, 1994).

2.6 Classification of PGPR

Generally, a vast species of PGPR can be categorized based on their mechanism in promoting plant growth i.e. directly by either facilitating nutrient uptake (nitrogen, phosphorus and essential minerals) or modulating plant growth hormone levels, or indirectly by acting as biocontrol agents in decreasing the inhibitory effects of various pathogens on plant growth and development. Alternatively, PGPR can also be classified based on their functional activities i.e. as biofertilizers by increasing the availability of nutrients to plant, phytostimulators through phytohormones production, rhizoremediators by degrading organic pollutants or as a biopesticides by the production of antibiotics and antifungal metabolites Somers *et al.* (2004). Gray and Smith (2005) have classified PGPR based on their association's range in the degree of bacterial proximity to the root and intimacy of association. Based on their classification, PGPR can be separated in to two different groups, (i) extracellular (ePGPR) - PGPR existing in the rhizosphere, on the rhizoplane, or in the spaces between cells of the root cortex and

(ii) intracellular (iPGPR) - PGPR exist inside root cells or generally known as endophytic PGPR (Figueiredo *et al.,* 2011).

Table 2.3: PGPR and their plant growth-promoting features

PGPR	Plants	Plant growth-promoting traits	References
Azospirillum spp., *Azoarcus* spp. and *Azorhizobium* spp.	*Triticum aestivum* L	Nitrogen fixation	Dal Cortivo *et al.* (2017)
Bacillus licheniformis, *Micrococcus luteus* and *Pseudomonas fluorescens*	*Vitis vinifera* L	Siderophore production, phosphate solubilization and nitrogen fixation	Pinter *et al.* (2017)
Pseudomonas putida	*Eruca sativa*	Production of IAA and siderophore	Kamran *et al*. (2016)
Pseudomonas Fluorescens, Bacillus subtilis, and *Azospirillum brasiliense*	*Brassica napus* L and *Festuca ovinia* L	Production of IAA, siderophore, phosphate solubilization, nitrogen fixation	Grobelak *et al.* (2015)
Pseudomonas aeruginosa	*Brassica compestris*	Production of IAA, siderophore and phosphate solubilization	Ahemad and Khan (2012)

Table 2.3, continued.

PGPR	Plants	Plant growth promoting traits	References
Achromobacter xylosoxidans and *Psychrobacter sp. SRS8*	*Brassica juncea*	Heavy metal mobilization	Ma *et al.* (2011)
Pseudomonas aeruginosa 4EA		Production of siderophore	Naik and Dubey (2011)
Bradyrhizobium sp. 750	*Lupinus luteus*	Heavy metal mobilization	Dary *et al.* (2010)
Stenotrophomonas maltophilia	*Saccharum officinarum*	Production of IAA and phosphate solubilization	Mehnaz *et al.* (2010)
Rhizobium phaseoli	*Vigna radiata L.*	production of IAA	Zahir *et al.* (2010)
Pseudomonas aeruginosa, Pseudomonas Siderophores fluorescens, Ralstonia metallidurans		Production of siderophore	Braud *et al.* (2009)
Proteus vulgaris	*Glycine max*	Production of siderophore	Rani *et al.* (2009)
Proteus vulgaris	*Brassica juncea*	Production of IAA, siderophore, phosphate solubilization and heavy metal mobilization	Rajkumar and Freitas (2008)

2.7 Mechanisms of Plant Growth Promotion

In most studied cases, a single species of PGPR will often reveal multiple modes of action. As mentioned earlier, the mechanism of action of PGPR in promoting plant growth can be classified into direct or indirect mechanisms.

2.7.1 Direct Mechanisms

2.7.1.1 Nitrogen Fixation

Besides water and climate changes, the availability of nutrients in the soil are the environmental factors that most strongly affecting plant growth. One of the most vital nutrients for plant growth and productivity is nitrogen (N). Although, there is about 78% of N_2 in the atmosphere, its state in a form of gaseous make it unavailable for plant's metabolic purposes. PGPR can increase plant N uptake by processes such as the symbiotic and non-symbiotic N fixation. Nitrogen fixing microorganisms can convert N_2 from the atmosphere into plant-utilizable forms of ammonia (NH_3) by a process called biological nitrogen fixation (BNF). The biochemical machinery required for BNF process is known as nitrogenase enzyme system (Kim & Rees, 1994). The structure of nitrogenase enzyme consists two-component metalloenzyme: (i) the homodimeric iron protein (Fe-protein or azo-ferrodoxin or component 1), a reductase which has a high reducing power and is responsible for the supply of electrons and (ii) the heterotetrameric iron-molybdenum protein (Fe-Mo-protein, or component II), a nitrogenase which uses the electrons provided to reduce N_2 to NH_3.

Structurally, N_2-fixing system varies among different bacterial genera. There are three different types of N fixing systems found in various nitrogen-fixing bacteria: molybdenum (Mo) nitrogenase, vanadium (V) nitrogenase, and iron (Fe) nitrogenase. Molybdenum nitrogenase, which can be found in diazotrophs and legumes, is the nitrogenase that has been studied the most extensively and thus is the most well characterized (Bishop & Jorerger, 1990). Nitrogen fixing bacteria that forms symbiotic

N_2 fixation with plants are including members of the family rhizobiaceae which forms symbiotic interaction with leguminous plants such as rhizobia (Ahemad & Khan, 2012; Zahran, 2001) and with non-leguminous plants (e.g. Frankia). While free living, associative and endophytes bacteria such as cyanobacteria (*Anabaena, Nostoc*), *Azospirillum, Azotobacter, Gluconoacetobacter diazotrophicus* and *Azocarus* forms non-symbiotic nitrogen fixation (Bhattacharyya & Jha, 2012). Symbiotic nitrogen fixing bacteria provide approximately 80% of total BNF and the remaining small amount of the fixed nitrogen comes from free living nitrogen fixation (Glick, 2012). Thus, BNF could reduce chemical fertilizers used in the present day agriculture since BNF imparts 180 x 10^6 metric tons of N per year globally (Adesemoye *et al.,* 2009).

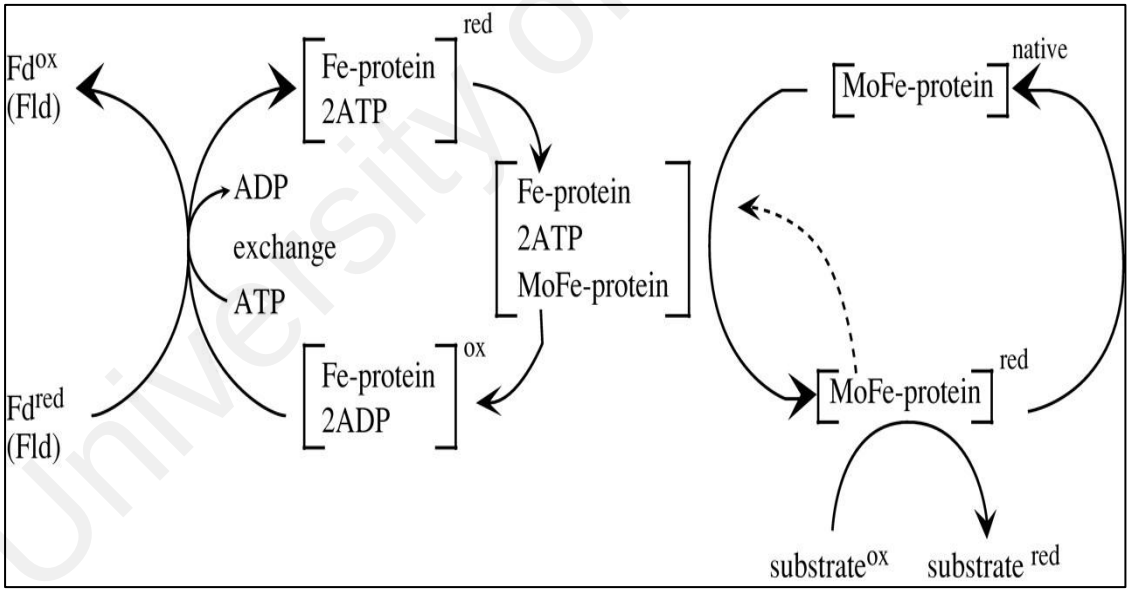


Figure 2.1: Schematic of the nitrogenase turnover cycle, illustrating the flow of electrons from electron carriers such as ferredoxin (Fd) or flavodoxin (Fld) to the Fe-protein (left), the transfer of electrons from the Fe-protein to the MoFe-protein coupled to the hydrolysis of ATP (centre) and the subsequent reduction of substrates coupled with return of the MoFe-protein to the resting redox state (right) (Rees *et al.,* 2005).

2.7.1.2 Phosphorus Solubilization

Phosphorus (P) is one of the major essential macronutrients for plant growth and development. In comparison with N, P is absorbed at much less amounts by plant, as it acts differently in the soil, which is due to its chemical properties (Miransari, 2013). Accordingly, P is subjected to processes such as precipitation or desolubilization and hence its availability to plant would significantly decrease. Phosphorus in soil is present in two main forms: mineral forms, such as apatite ($Ca_5(PO_4)_3(F,Cl,OH)$, hydroxyapatite ($Ca_5(PO_4)_3(OH)$ and oxyapatite ($Ca_5(PO_4)_3(F)$, and organic forms including inositol phosphate (soil phytate), phosphomonoesters, phosphodiesters and phosphotriesters (Bishnoi, 2015). Meanwhile, the precipitation of P in acidic soils occur with active forms of aluminum ion such as $Al^{3+,}$ $Al(OH)^{2+,}$ $Al(OH)_2^{+}$) and iron ion like Fe^{2+} and Fe^{3+}). Phosphorus is also sorbed on the surface of calcium carbonate or precipitated with calcium ion (Ca^{2+}) in calcareous soils (Bohn *et al*., 1985). The predominance of these P precipitation depends on the degree of soil weathering and soil pH. The availability and solubility of organic P in the soil is more higher than mineral P. Organic P is also subjected to different microbial activities such as solubilization and mineralization by phosphate-solubilizing bacteria (PSB) and this microbial activities are determining factor for its availability to the plant (Marschner, 1995). Solubilization and mineralization of P by PSB are among the most important bacterial physiological traits in soil biogeochemical cycles as well as in plant growth promotion by PGPR (Richardson, 2001; Jeffries *et al.,* 2003; Rodriguez *et al.,* 2006).

Most soil bacteria can solubilize insoluble phosphates, particularly bacterial belong to genera like *Azotobacter, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Microbacterium, Pseudomonas, Rhizobium* and *Serratiaare* reported as the most significant PSB (Bhattacharyya & Jha, 2012). Besides that, P-solubilizing PGPR species such as *Azotobacter chroococcum, Bacillus circulans,*

Cladosporium herbarum, Bradyrhizobium japonicum, Enterobacter agglomerans, Pseudomonas chlororaphis, Pseudomonas putida and *Rhizobium leguminosarum* are also reported associated with a large number of agricultural crops for examples wheat, potato, tomato, pulses, and radish are (Antoun *et al.,* 1998; Chabot *et al.,* 1998; Cattelan *et al.,* 1999).

Typically, the mechanisms involved in the solubilization of inorganic P by microbial activities occurs as a consequence of the action of low molecular weight organic acids and the release of protons to the soil solution (Figure 2.2) (Zaidi *et al.,* 2009). Organic acid synthesized by PSB such as gluconic acid, formic acid, butyrate and propanedioic acid were found involved in solubilizing P (Table 2.4).

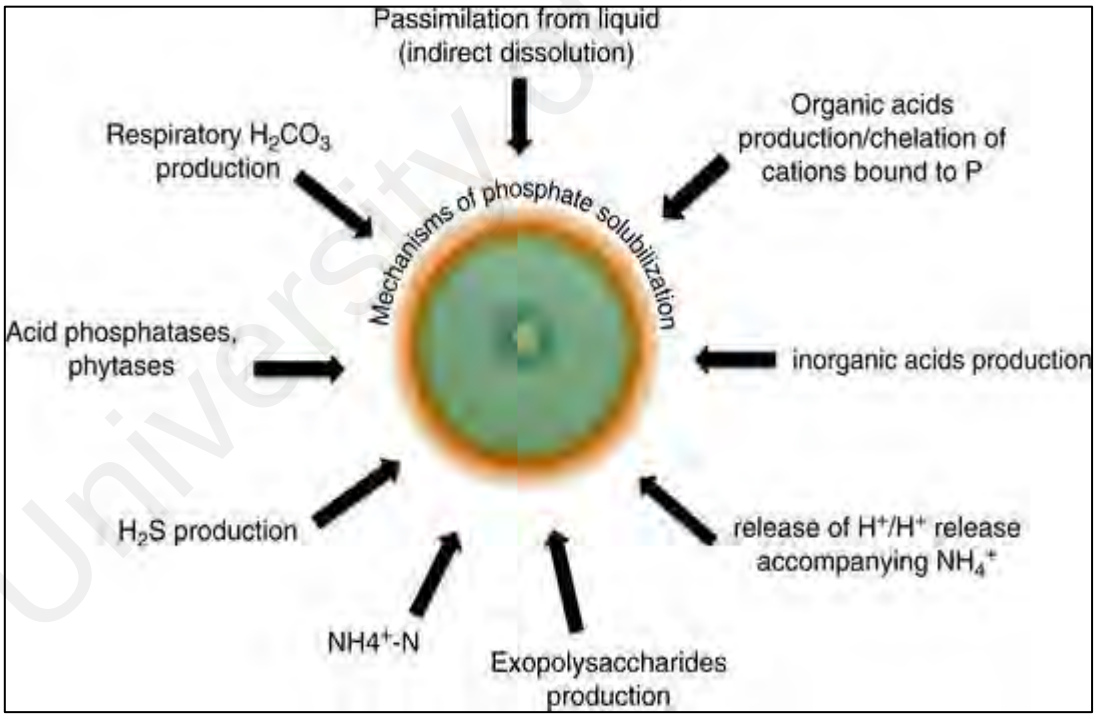


Figure 2.2: Mechanisms of P-solubilization by phosphate solubilizing bacteria (Zaidi *et al.,* 2009)

Table 2.4: Organic acids involved in P-solubilization and produced by PS-bacteria

Bacterial communities	Organic acids	References
Serratia sp.	Malic acid, lactic acid and acetic acid	Baheera *et al.* (2017)
Pseudomonas sp. PSB12	Gluconic acid, formic acid, butyrate, and propanedioic acids	Chen *et al.* (2016)
Burkholderia cepacia DA23	Gluconic acid	Song *et al.* (2008)
Pseudomonas corrugata (NRRL B-30409)	Gluconic, 2-ketogluconic acid	Trivedi and Sa (2008)
Citrobacter sp. DHRSS	Acetic and gluconic acid	Patel *et al*. (2008)
Burkholderia, Serratia, Ralstonia and *Pantoea*	Gluconic acid	Elizabeth *et al.* (2007)
Bacillus, Rhodococcus, Arthrobacter, Serratia and one *Chryseobacterium, Delftia, Gordonia, Phyllobacterium, Arthrobacter ureafaciens, Rhodococcus erythropolis* and *Delftia* sp	Citric acid, gluconic acid, lactic acid, succinic acid, propionic acid	Chen *et al.* (2006)
Enterobacter intermedium	2-ketogluconic	Hwangbo *et al.* (2003)
Bacillus amyloliquefaciens, B. licheniformis, B. atrophaeus, Penibacillus macerans, Vibrio proteolyticus, Kluyvera cryocrescens, Pseudomonas aerogenes and *Chryseomonas luteola*	Lactic, itaconic, isovaleric, isobutyric, acetic	Vazquez *et al.* (2000)

Conversely, the mineralization of organic phosphorus occurs through the synthesis of a variety of different phosphatases, catalyzing the hydrolysis of phosphoric esters (Glick, 2012). Phosphate solubilization and mineralization can coexist in the same bacterial strain (Tao *et al.,* 2008), besides providing P to the plants, the PS bacteria also augment the growth of plants by stimulating the efficiency of BNF, enhancing the availability of other trace elements (such as iron, zinc). The possibility of enhancing P uptake of crops by inoculation with P-solubilizing strains of PGPR presents a promising approach towards recovering the reservoirs of insoluble phosphorus from the soil and thus minimizing the external application of phosphate fertilizers to the soil.

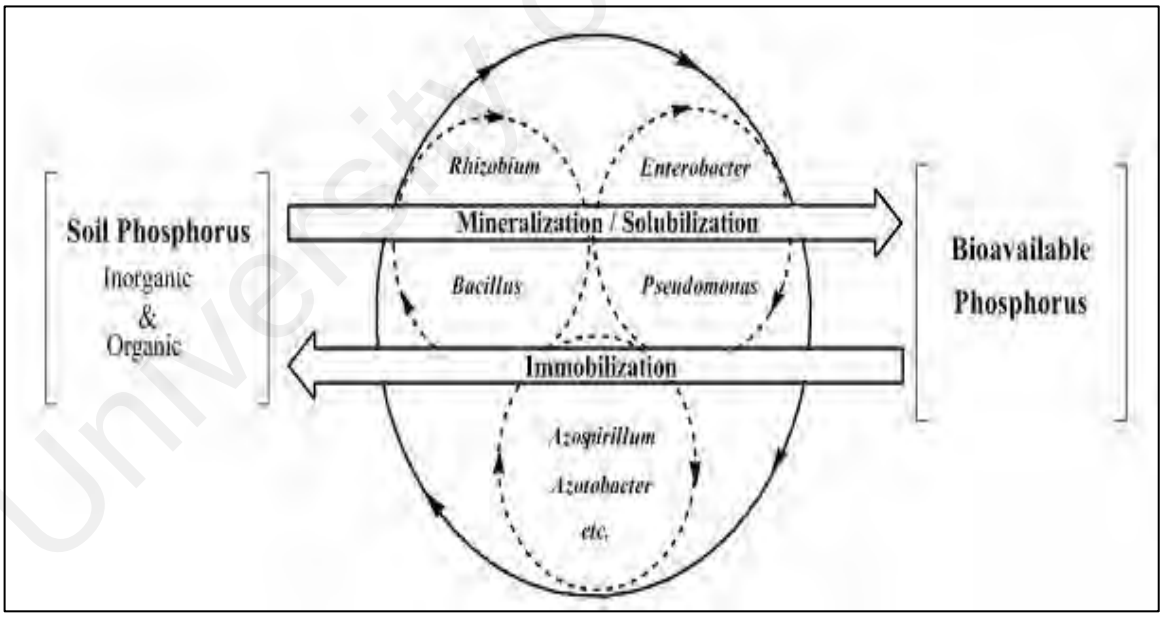


Figure 2.3: A simplified schematic representation of the role of phosphate-solubilizing bacteria (PSB) to P availability in soil and uptake by plants (Richardson & Simpson, 2011)

2.7.1.3 Absorption of Iron

Iron is an important micronutrient for almost all forms of life. It is essential for plants as it serves as a cofactor of many enzymes activities in physiological processes such as N_2 fixation, photosynthesis, respiration, and nutrient acquisitions (Ahemad & Kibret, 2013). Although iron is among the bulk minerals present in soil, yet it generally inaccessible to plants. This is because iron is commonly present in nature as Fe^{3+} and is likely to form hydroxides and oxyhydroxides thus making it highly insoluble for plant absorption (Rajkumar *et al.,* 2010). Plants perform two types of strategies to chelate insoluble iron under iron-deficient conditions. Strategy I plants produce organic products such as phenolic compounds to acidify the rhizosphere and resulting in the activation of ferric chelate reductase and Fe(II) transporter in plant roots. Meanwhile, in order to meet their iron requirement, strategy II plants have evolved specific mechanisms to chelate insoluble iron through the release of low-molecular mass iron chelators referred to as siderophores and uptake of iron-siderophore complexes through specific outer membrane receptor proteins (Sharma & Johri, 2003). The siderophores produced by plant roots can significantly increase Fe availability due to its high affinity to chelate Fe^{3+} and hence increase the iron absorption efficiency by plant roots (Chen *et al.,* 1998).

Similarly, PGPR are also able to produce siderophores, which increase the solubility and hence the availability of iron for the use of plant (Jalili *et al.,* 2009; Abbas-Zadeh *et al.,* 2010; Zabihi *et al.,* 2011). A lot of studies have successfully isolated siderophore-producing bacteria belonging to the various genera such as *Bradyrhizobium, Pseudomonas, Rhizobium, Serratia* and *Streptomyces* from the rhizosphere (Kuffner *et al*., 2008; Nagata *et al.,* 2013). Plants use different mechanisms to assimilate iron from bacterial siderophores for instance by the direct uptake of siderophore-iron complexes, chelate and release of iron, or by a ligand exchange

reaction (Schmidt, 1999). Studies done by Yang and Crowley (2000) have clearly shown that the iron nutrition status of the plant influences the microbial community structure in rhizosphere. Under iron-deficient conditions, plant may produce more root hairs and root exudates including organic acid, amino acids as well as the secondary metabolites. The production of these root exudates can affect the combination of soil microbes in the rhizosphere and affect the solubility and availability of different nutrients in the plant rhizosphere (Lamont, 2003; Badri & Vivanco, 2009). Jin *et al.* (2010) found that the number of soil microbes which produce siderophores increases in the rhizosphere of plants with iron deficiency.

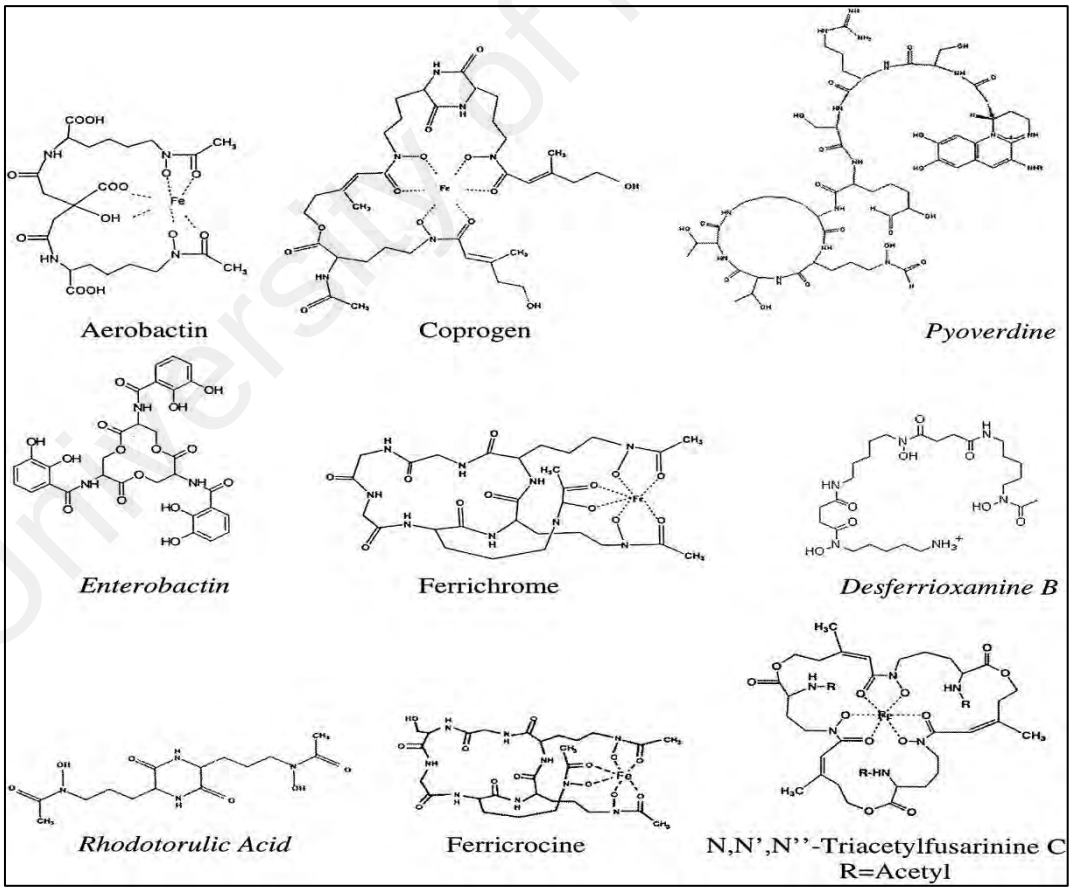


Figure 2.4: Different types of siderophores structures (Fluckinger *et al*., 2004)

Numerous studies have reported the positive effects on plant growth as the result of inoculation of siderophore-producing *Pseudomonas, Rhizobiumand Azospirillumin* iron-limited conditions (Nagata *et al.,* 2013; Carrillo-Castaneda *et al.,* 2002). For example, Nagata *et al.* (2013) demonstrated that the *Pseudomonas fluorescens* synthesizes siderophores called pyoverdine, which have a high affinity for ferric iron improved bioavailability of iron in strategy I plants that generally cannot synthesize pyoverdine or take up ferric iron. Similarly, Vansuyt *et al.* (2007) showed that Fe-pyoverdine complex synthesized by *Pseudomonas fluorescens* C7 can change physiological responses in *Arabidopsis thaliana*, leading to an increase of iron inside plant tissues and to improved plant growth. Works done by Sharma *et al.* (2003) revealed that *Vigna radiate* plant inoculated with the siderophore-producing *Pseudomonas strain* GRP3 produced higher chlorophyll a and chlorophyll b content and showed lower chlorotic symptoms, a condition in which leaves produce insufficient chlorophyll due to iron deficiency. Crowley and Kraemer (2007) also found a siderophore mediated iron transport system in oat plants and inferred that siderophores produced by rhizosphere microorganisms deliver iron to oat. In addition, other bacteria such as *Azadirachta indica*, which produce ferrioxamines, could contribute into plant iron nutrition and promote the root and shoot growth (Siebner-Freibach et al*.,* 2003; Verma et al*.,* 2011).

Siderophore-producing PGPR in the rhizosphere are also frequently associated with biocontrol activities hence suggested to be an environmentally friendly alternative to hazardous pesticides (Vessey, 2003). Iron is essential for cellular growth and metabolism, thus the ability of rhizosphere microbiome to compete for iron with other microorganisms in the rhizosphere by produce siderophore plays an important role in determining their survival in rhizosphere and competitive fitness to colonize plant roots (Crowley, 2006). In that way, siderophore-producing PGPR can prevent the

proliferation of pathogenic microorganisms by making iron unavailable to them, thereby restricting their growth (Loper & Henkels, 1999). For example, siderophores produced by *Bacillus subtilis* had a significant role in the biocontrol of *F. oxysporum*, which causes the *Fusarium* wilt of pepper (Yu *et al*., 2011). Pyoverdine siderophores produced by pseudomonads were found to control *Gaeumannomyces graminis*, which causes a deficiency of wheat and barley growth (Voisard *et al.,* 1989). Schippers *et al.* (1987) found that Pyoverdine effectively acts as a biocontrol agent to suppress the wilt diseases of potato caused by *Fusarium oxysporum*. Furthermore, pyoverdine siderophores produced by pseudomonads also involved in the biocontrol of the phytopathogens in peanuts and maize (Pal *et al.,* 2001).

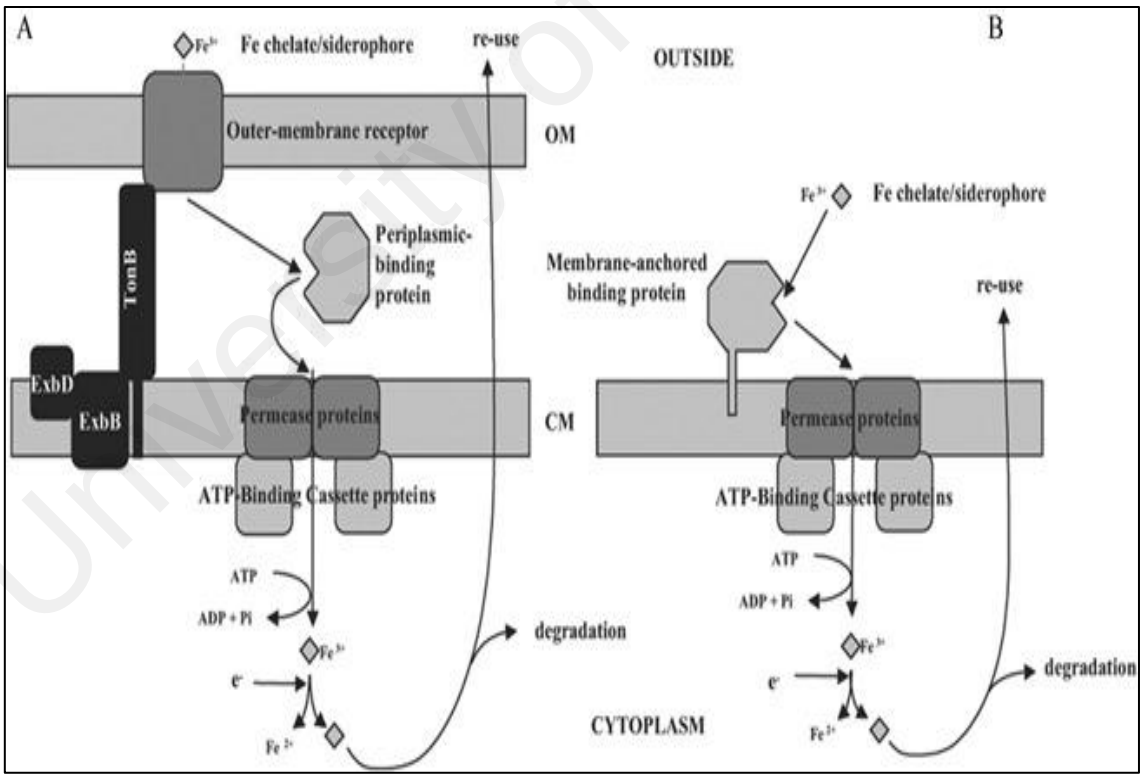


Figure 2.5: Schematic representation of siderophore-mediated iron uptake in gram-negative (A) and gram-positive (B) bacteria (Andrews *et al*., 2003).

Siderophores are also able to form a stable complexes with other heavy metals that are of environmental concern, such as Al, Cd, Cu, Ga, In, and Pb as well as with radionuclides including U and Np (Neubauer *et al.,* 2000; Kiss & Farkas, 1998). The siderophore-heavy metal complexes increase the solubility of the heavy metal concentration, hence, help to remove the contaminants in soils by phytoremediation (Rajkumar *et al.,* 2010).

2.7.1.4 Phytohormone Production

One of the prominent modes of action for plant growth promotion by PGPR is by means phytostimulator through the production of plant hormones. Plant hormones, also known as phytohormones are organic substances that regulate growth processes in plants. Auxin is one of the most phytohormones produced by PGPR. Most of PGPR isolated from the rhizosphere of various crops showed the ability to produce and release auxins as secondary metabolites (Patten & Glick, 1996). Charles Darwin was the first person suggested the existence of the plant hormone auxin. By using canary grass (*Phalaris canariensis* L.) plants, he showed that coleoptile growth towards a light source is regulated by a plant-derived chemical substance (Taiz & Zeiger, 2010).

PGPR are able to synthesize phytohormone auxin (indole-3-acetic acid/ indole acetic acid/ IAA) in the presence of the precursor tryptophan and its plays an important role in modulating the level of IAA biosynthesis (Zaidi *et al.,* 2009). Interestingly, anthranilate, a precursor for tryptophan biosynthesize, reduces IAA synthesis. By this mechanism, IAA biosynthesis is fine-tuned because an increase in tryptophan level will inhibits anthranilate formation by a negative feedback regulation on the anthranilate synthase (Spaepen *et al.,* 2007). Even though, many works reported that supplementation of culture media with tryptophan increases the IAA production by most of the rhizobacteria (Spaepen & Vanderleyden, 2011).

Although tryptophan is the most important precursor for IAA synthesis in tryptophan side chain pathway, certain PGPR are able to produce the hormone through several tryptophan-independent pathways includes indole-3-acetamide pathway, indole-3- pyruvic acid pathway, tryptamine pathway and indole-3- acetonitrile pathway (Figure 2.6) (Kumar *et al.,* 2015; Spaepen *et al.,* 2007). The tryptophan side chain pathway is most common in plants and also found in *azospirilla* and *cyanobacteria.* IAA biosynthesis via indole-3-acetamide formation is reported for phytopathogenic bacteria *Agrobacterium tumefaciens, Pseudomonas syringae*, and *E. herbicola; saprophytic pseudomonads* like (e.g. *Pseudomonas putida* and *P. fluorescens*).

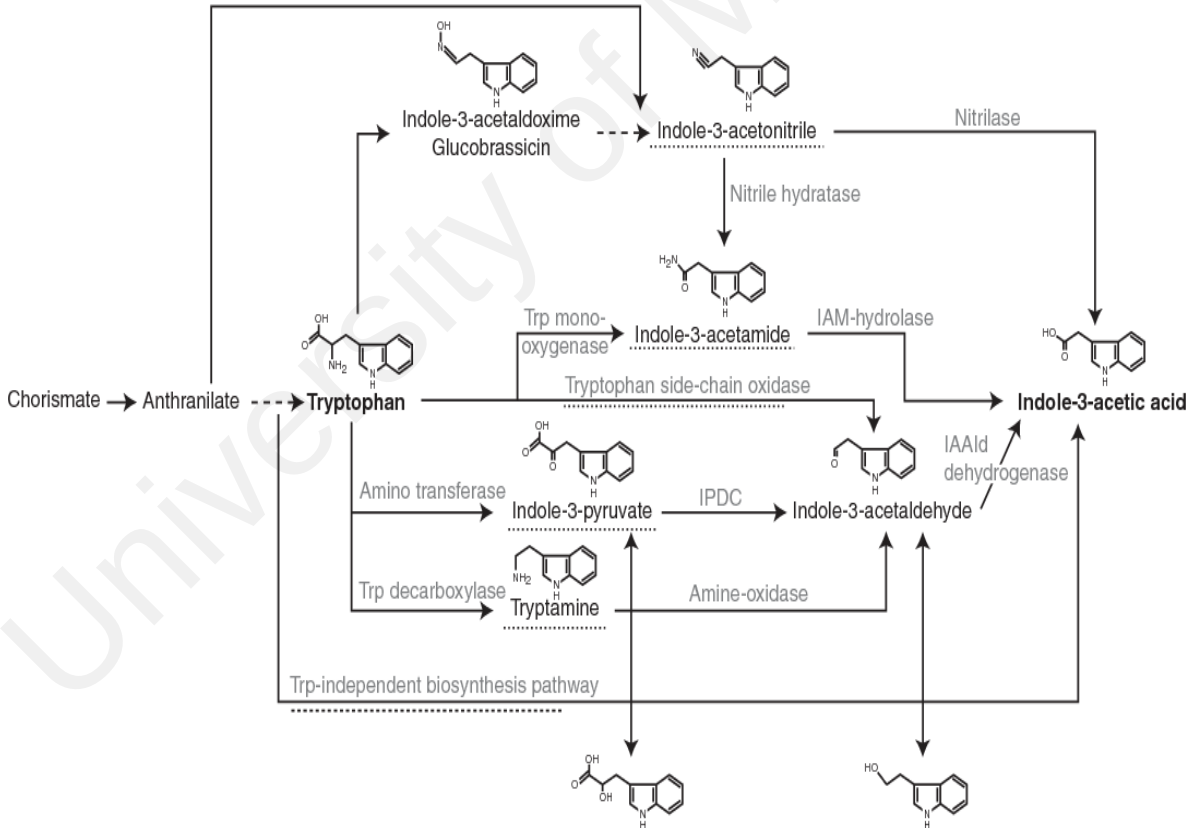


Figure 2.6: Overview of the different pathways to synthesize IAA in bacteria. The intermediate referring to the name of the pathway or the pathway itself is underlined with a dashed line. IAAld, indole-3-acetaldehyde; IAM, indole-3-acetamide; IPDC, indole-3-pyruvate decarboxylase; Trp, tryptophan (Spaepen *et al*., 2007).

IAA formation via indole-3-pyruvic acid is found in a majority of bacteria like, *Erwinia herbicola*; saprophytic species of the genera *Agrobacterium* and *Pseudomonas*; certain bacteria from *Bradyrhizobium, Rhizobium, Azospirillum, Klebsiella*, and *Enterobacter.* The biosynthesis of IAA by tryptamine pathway was reported in some *pseudomonads* and *azospirilla.* While the production of IAA that involves tryptophan conversion into indole-3-acetonitrile is found in the *cyanobacterium* like *Synechocystis* sp (Ahemad & Kibret, 2013). Generally, IAA influences cell division, extension, and differentiation processes in plant like initiates lateral and adventitious root formation, hence, increases root surface area and length to provide the plant with greater access to soil nutrients. In addition, it also affects photosynthesis, controls vegetative growth, biosynthesis of various metabolites, plant's resistance to stressful conditions and ultimately increased growth rate of plant (Frankenberger, 1995). IAA synthesized by PGPR can affect these physiological processes since the acquisition of IAA secreted by the PGPR could change the endogenous pool of plant IAA (Glick, 2012; Spaepen *et al.,* 2007).

IAA also plays a very important role in rhizobacteria-plant interactions by loosens plant cell walls and as a result facilitates an increasing amount of root exudation that provides additional nutrients to support the growth of rhizosphere bacteria (Glick, 2012). IAA is also involved in in multiple processes of nodule formation by rhizobia in legume plants, such as founder cell specification (auxin transport inhibition mainly by flavonoids), nodule initiation and differentiation (auxin accumulation), vascular bundle formation, and nodule numbers. Because many rhizobia are capable of producing IAA via different pathways, it is assumed that bacterially produced auxin can alter the auxin balance inside the plant. In addition, rhizobia can also indirectly influence the auxin homeostasis by interfering with plant auxin transport (Mathesius, 2008).

2.7.2 Indirect Mechanisms

The major indirect mechanism of plant growth-promotion effect by PGPR is through suppressing the plant pathogens, thus, lessen or prevent its deleterious effects. PGPR are widely known to possess several mechanisms to suppress the plant pathogens such as competing for fundamental niche and nutrients especially in rhizosphere (Elad & Baker, 1985; Elad & Chet, 1987), producing antibiotics metabolite that can kill plant pathogens (Bhattacharyya & Jha, 2012) and interaction of some rhizobacteria with the plant roots also can result in induced systemic resistance (ISR) to pathogenic bacteria, fungi, and viruses (Lugtenberg & Kamilova, 2009).

As mentioned earlier, PGPR can act as a good source of siderophores which chelate the iron in the root vicinity to limit the availability of iron necessary for the growth of plant pathogens. Under highly competitive conditions, the ability to acquire iron via siderophores may determine the outcome of competition for different nutrient sources available (Crowley, 2006). Thus, siderophore production confers competitive advantages to PGPR that can colonize roots and exclude plant pathogens from this ecological niche (Haas & Défago, 2005).

The indirect mechanism of plant growth-promotion effect by PGPR is also contributed by the ability of certain PGPR in producing a wide range of chemical compounds with antimicrobial activity such as antibiotics, lytic agents, exotoxins and bacteriocins (Riley & Wertz, 2002). Previous studies have revealed that PGPR produce antibiotics metabolite such as hydrogen cyanide (HCN), phenazines, pyrrolnitrin, 2,4-diacetylphloroglucinol, pyoluteorin, viscosinamide and tensin as a defense system (Bhattacharyya & Jha, 2012; Senthilkumar *et al.,* 2007a, b; Pierson & Thomashow, 1992). Hill *et al.* (1994) reported *Pseudomonas fluorescens* BL915 strain is able to prevent the damage of *Rhizoctonia solani* during damping-off of cotton plants by producing antibiotic substance called pyrrolnitrin. Paulin *et al.* (2017) demonstrated that

inoculation of tomato plants with 2,4-diacetylphloroglucinol (DAPG) and hydrogen cyanide-producing *Pseudomonas brassicacearum* LBUM300 could significantly reduce bacterial canker symptoms caused by *Clavibacter michiganensis*. While *Pseudomonas fluorescens* DR54 showed antagonistic properties against plant pathogenic *Pythium ultimum* and *Rhizoctonia solani* both in vitro and in planta by producing viscosinamide (Nielsen *et al.,* 1999) Beside that, PGPR also synthesis hydrolytic enzymes such as chitinases, glucanases, proteases, and lipases, that can lyse pathogenic fungal cells (Neeraja *et al.,* 2010; Maksimov *et al*., 2011)

Plants can develop an enhanced defensive ability upon triggered by pathogenic and non-pathogenic microorganisms or termed as induced systemic resistance (ISR). This induced disease resistance is generally expressed as a restriction of pathogen growth and reduction of symptom development (Hammerschmidt, 1999). In recent years, several studies have been carried out to evaluate the potential of PGPR as an inducer to elicit induced systemic resistance (ISR) in plants against different pathogens under field conditions. The ability to develop ISR in response to rhizobacteria has been documented for many plant species (Table 2.5).

Table 2.5: List of some studies conducted to show PGPR-induced systemic resistance

PGPR	Host plant	Plant pathogen	References
Bacillus sp	Cotton	*Spodoptera exigua*	Zebelo *et al.* (2016)
P. aeruginosa	Soya bean	Soya bean stunt virus	Khalimi & Suprapta (2011)
P. fluorescens	Brinjal	*Ralstonia solanacearum*	Chakravarty & Kalita (2011)
P. putida	Lentil	*M. javanica*	Siddiqui *et al.* (2007)
P. fluorescens(PFV, PFP, PSV) and *Bacillus subtilis*(BSV, BSP)	Tea	*Exobasidium vexaus*	Saravanakumar *et al.* (2007)
P. fluorescens (P-112)	Cucumber	*etranychus urticae*	Tomczyk (2006)
P. fluorescens (Pf1) and *Bacillus subtilis*	Chillies	*Colletotrichum capsici*	Bharathi *et al.* (2004)
P. fluorescens (FP7)	Mango	*Colletotrichum gloeosporioides*	Vivekananthan *et al.* (2004)

2.8 Potential of *B. salmalaya* Strain 139SI as a Plant Growth-Promoting Rhizobacteria

A novel strain of soil bacteria, *Bacillus salmalaya* strain 139SI (Gen Bank accession No: JF825470.1; ATCC BAA-2268) used in this study was originally isolated from soil obtained from agricultural sites in Selangor, Malaysia (2.99917°N 101.70778°E) (Ismail & Dadrasnia, 2015). The bacterial cells were characterized as Gram-positive, facultatively anaerobic, endospore-forming bacteria and straight rods of 0.4–0.5 μm x 2.0–2.5 μm in size. Colonies grown on BHI-blood agar were observed as grey color, large, rough, and irregular edged with a size of 2-3 mm in diameter exhibiting a strong hemolytic activity. The optimum growth was observed on BHI agar at 37°C.

Results from our previous experiments implicate the presence of growth-transforming proteins or genetic elements in this novel strain that could act as a growth-promoting agent (Salmah *et al.,* 2012). In other studies, we found that the strain 139SI can produce dehydrogenase enzyme (Dadrasnia *et al*., 2016), an enzyme produced by PGPR that play a very significant role in plant growth promotion particularly to protect plants from biotic and abiotic stresses (Reddy *et al.,* 2014). Furthermore, results from our preliminary study also demonstrated the plant growth promotion features of this strain on several crops (data not published).

2.9 Oil Palm (*Elaeis guineensis* Jacq.)

2.9.1 Taxonomy of Oil Palm

Elaeis guineensis Jacq. which is commonly known as the oil palm is the most important species in the genus *Elaeis*. The scientific classification of oil palm is as follows:

Kingdom	: Plantae – Plants
Subkingdom	: Tracheobionta – Vascular plants
Superdivision	: Spermatophyta – Seed plants
Division	: Magnoliophyta – Flowering plants
Class	: Liliopsida – Monocotyledons
Subclass	: Arecidae
Order	: Arecales
Family	: Arecaceae – Palm family
Genus	: *Elaeis* Jacq. – Oil palm
Species	: *Elaeis guineensis* Jacq. – African oil palm

2.9.2 The Botany of Oil Palm

The genus of *Elaeis,* a diploid (2n = 32) oleaginous tropical perennial crop, contains two main species*: E. guineensis* or African oil palm, and *E. oleifera* or American oil palm (Dransfield *et al.*, 2005). *E. oleifera* is not widely commercialized due to significantly lower in oil-to-bunch content compared to its African counterpart but valuable for production of interspecfic hybrids with *E. guineensis*. Oil palm tree is characterized by a single-stemmed palm that bears a single vegetative shoot apical meristem. The apical meristem is actively producing a new leaf every month during the first 6 months old of the seedling. Later, under favorable soil and climatic conditions, the number of leaves produced increases to 30-40 per year at 5-6 years old and later

declines to 18-25 per year (Adam *et al.,* 2005). The early growth of from the seedlings results in the formation of a wide stem (stipe) base. Roots arise from the base of the hypocotyl and later from the basal of the stem. Primary roots descend deeply from the base of the trunk, but remain short when the water table is high. Otherwise, they produce secondary, tertiary and quaternary roots that form a dense mat in the immediate neighborhood of the tree. Most roots are found in the top 15cm of the soil, with a main concentration near the palm and a secondary concentration 1.5 to 2m from the base (Verheye, 2010).

Male and female inflorescences of oil palm occur on the same tree in alternated cycles of the same sex, and are only differentiated after approximately two years. This process is influenced by moisture and temperature conditions, fertilization and other secondary ecological factors. The development of the inflorescence to the fruit regime takes 42 months, including 10 months from establishment to initial sexual differentiation, 24-26 months between sex development and flowering and 4-5 months from flowering to yield. Hence, ecological conditions which affect earlier phases of inflorescence and flowering appear only in the yields 18 to 24 months afterwards (Verheye, 2010).

Oil palms are cross pollinated. In the past, oil palm was thought to be wind pollination and owing to the low level of natural pollination. Assisted pollination is a standard management practice in plantations as effort to increase the yield. Later, this practice was discontinued following the discovery that oil palm was insect pollinated and the introduction of *Elaeidobius kamerunicus* from the Cameroons to Malaysia (Hai, 2002).

According to Verheye (2010), cultivars of *E. guineensis* in the strict sense do not occur. This is due to the fact that oil palm is monoecious and cross-pollinated, individual palms are usually very heterozygous. However, there are three main cultivars namely Dura, Pisifera and Tenera. The current classification of cultivars is mainly based on fruit structure and commercial value:

- Dura: shell 2-8mm thick, comprising 25-55% of weight of fruit, medium mesocarp content of 35-55% by weight, less productive.
- Pisifera: shell-less or have no endocarp, with small pea-like kernels in fertile fruits and about 95% mesocarp. This cultivar has little commercial value, because of its high abortion ratio, but important for cross-breeding commercial palms.
- Tenera: shell 0.5-3mm thick; comprising 1-32% of weight of fruit; medium to high mesocarp content of 60-95%, but occasionally as low as 55%; this variety is the result of a hybridization of Dura and Pisifera, and has a high commercial value.

Most commercial plantations are established on the basis of Tenera palms due to its high commercial value. Oil palms may live up to 200 years, but their commercial yield rapidly decreases after 30 years of age (Latiff, 2000).

2.9.3 The Products of Oil Palm

There are two types of edible vegetable oil produced from the fruit of the oil palm. Palm oil is derived from the fibrous mesocarp of the fruit, while palm kernel oil is edible oil extracted from the kernel of the oil palm. Palm oil and palm kernel oil are chemically and nutritionally different. Palm oil is naturally reddish in color because of high beta-carotene content. Both palm oil and palm kernel oil are semi-solid at room temperature. However, palm kernel oil contains higher saturated fatty acid than palm oil. Fractionation of crude palm oil and crude palm kernel oil by crystallization process at controlled temperatures will separate the liquid portion from the solid portion of the oil. Palm olein, the liquid fraction obtained from the fractionation process is suitable for frying and cooking products include salad and cooking oils in households, industrial frying fat of instant noodles, potato chips, doughnuts and condensed milk. Meanwhile, the solid fat portion, called palm stearin, is commonly used to formulate trans-free fats such as margarine, shortening, vegetable ghee and as vegetable fat in soap industry. Palm oil olein also could be further fractionated to produce a more liquid fraction called palm super olein. This palm super olein could withstand colder temperature than palm olein before they could be solidified.

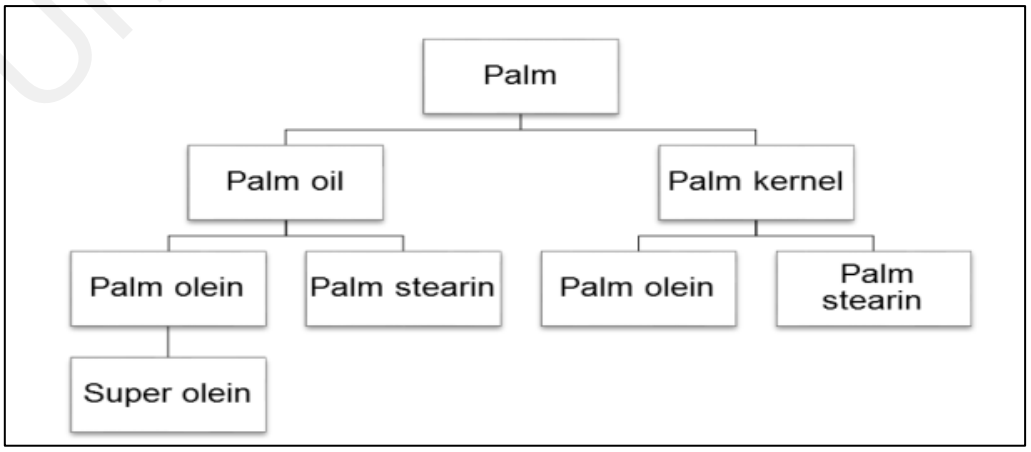


Figure 2.7: Different types of vegetable fat produced from palm oil

The comparative of fatty acid compositions in the crude palm oil (CPO) and crude palm kernel oil (CPKO) are presented in Table 2.6. CPO has a balanced ratio of saturated and unsaturated fatty acids while CPKO has mainly saturated fatty acids which are broadly similar to the composition of coconut oil. Palmitic acid (C16:0) is the major fatty acid found in CPO. It accounts for approximately 44% of the fatty acid content in CPO. The other major fatty acids are oleic acid (C18:1), linoleic acid (C18:2) and stearic acid (C18:0) accounting for approximately 40%, 10% and 5%, respectively (Tan & Man, 2000). Meanwhile, the high content of saturated fatty acid in CPKO is attributed to the presence of lauric acid (C12:0), myristic acid (C14:0) and oleic acid (C18:1).

Those fatty acids are representing for approximately 50, 15, and 10 % of total fatty acid content in CPKO. The high level of saturated fatty acid content in palm kernel indicated that it is suitable to be used for cooking as well as for other applications. Similar to other vegetable oils, CPO and its fractions, namely palm olein; palm stearin; and superolein are comprised of mixed triacylglycerols (TAGs) and partial acylglycerols, such as diacylglycerols (DAGs) and monoacylglycerols (MAGs). DAGs and MAGs are hydrolysis products of TAGs that can affect the melting point and crystallization behavior of the oil (Foster *et al.,* 2009).

Table 2.6: Fatty acid compositions in the crude palm oil (CPO) and crude palm kernel oil (CPKO) (Dubois *et al.,* 2017; Kok *et al*., 2011; O'Brien, 2010; Firestone, 2006).

Fatty acid composition	Range percentage (%) of fatty acid	
	Crude palm oil	Crude palm kernel oil
Saturated fatty acid	44.00 – 55.00	82.83 – 84.25
Monounsaturated fatty acid	36.00 – 44.00	9.54 – 15.15
Polyunsaturated fatty acid	6.50 – 12.00	2.02 – 5.47
Caprylic (C 8:0)	–	1.11 – 3.25
Capric (C 10:0)	–	4.00 – 5.73
Lauric (C 12:0)	0.10 – 1.00	45.25 – 53.73
Myristic (C 14:0)	0.50 – 2.00	14.46 – 18.10
Palmitic (C 16:0)	40.00 – 48.00	7.32 – 9.20
Stearic (C 18:0)	3.50 – 6.50	1.90 – 3.30
Oleic (C 18:1)	36.00 – 44.00	9.54 – 15.15
Linoleic (C 18:2)	6.50 – 12.00	2.00 –2.77
Arachidic (C 20:0)	0.00 – 1.00	2.00 – 2.71
Gadoleic (C 20:1)	–	0.1 – 0.73

2.9.4 Palm Oil Industry in Malaysia

From its origin in West Africa, the oil palm has spread throughout the tropics and is now grown in 16 or more countries. The seeds of the Malaysian oil palm were first arrived on Malaysian shores as ornamental plants in 1887. These were derived from the famous four palms in Java's Bogor botanical garden (Yee & Chandran, 2005). As of 2015, Malaysia had 4.7 million hectares of oil palm plantations and it has also received worldwide attention in a number of success stories dealing with poverty alleviation and the equitable distribution of wealth (Ferdous *et al.,* 2015). The success of the oil palm industry in Malaysia was attributed to many factors, which include favorable climatic conditions, well-established infrastructure, good management skills, and advanced in technology for oil palm cultivation. Nevertheless, to stay economically competitive and to ensure the industry environmentally sustainable, appropriate R&D in various disciplines were also strategically planned and implemented. Both the public and private sectors carry out oil palm research and development (R&D) in Malaysia. Palm Oil Research Institute of Malaysia (PORIM) was set up in 1974 and was merged with the Palm Oil Licensing Authority (PORLA) in 2000 to form the Malaysian Palm Oil Board (MPOB). The MPOB now deals with all aspects of oil palm and palm oil development and provides regulatory, training and technical advisory services to all sectors of the industry (Wahid *et al*., 2005).

Overall, the palm oil industry in Malaysia has recorded an amazing statistics. The total oil palm planted area in the country increased by 18.4 % to 5.74 million hectares in 2016 compared 4.85 million hectares in 2010. Private estate remained the largest oil palm plantation owner. 61.2 % of total planted area was contributed by the private estate followed by 16.3 and 12.3 % by independent smallholders and Federal Land Development Authority (FELDA) respectively. The production of CPO reached a record 17.32 million tonnes in 2016, an increase of 0.55 million tonnes or 3.28 % from

2010. Total exports of oil palm products, consisting of palm oil, palm kernel oil, palm kernel cake, oleochemicals, biodiesel and finished products increased by 1.00 % or 0.23 million tonnes to 23.29 million tonnes in 2016 from 23.06 million tonnes recorded in 2010. Total export earnings also rose by 8.01% or RM 4.79 billion to RM 64.58 billion compared to the RM59.79 billion achieved in 2010 because of higher export prices. Biodiesel is a new emerging sector in the palm oil industry. In 2016, 17 plants have been established with total annual biodiesel installed capacity of 2.07 million tonnes. An additional three biodiesel plants with an increased annual capacity of 0.37 million tonnes compared to 2008. However, it is important to mention that the production of fresh fruit bunch in 2016 was severely affected by El Nino, in which consequently, the Malaysian palm oil output is seen dropping approximately by 10%.

2.9.5 Oil Palm Agronomy and Nutrition Requirements

The oil palm is recognized as having a high demand for nutrients; not surprising in view of its high dry matter production. Nutrients that are removed continuously through the harvested FFB or sequestered in the standing biomass need replacing if soil nutrient reserves are not to become depleted. From previous studies, it has been estimated that for Malaysian soils between 0.5 and 1.1 kg/palm /year of N, 0.7 and 1.1 kg/palm/year of P_2O_5, and 0.5 to 2.0 kg/palm/year of K_2O are needed to replace soil nutrients after taking into account expected losses of the applied nutrients (Tarmizi, 2000). In addition, it is important that for yields to be maximized, the nutrients applied are balanced. Past studies have shown that empty fruits bunch (EFB) mulching significantly improved oil palm yield (Hamdan *et al.*,1998). EFB generally contains 0.80 % N, 0.22 % P_2O_5, 2.90 % K_2O, and 0.30 % MgO on a dry weight basis. Yield improvement ranging from 5 % to 23 % has been achieved depending on soil type. Hamdan *et al.* (1998) showed that nutrients from 60 tonnes/hectare/year of EFB, without any inorganic fertilizer, were sufficient to support palm growth.

Nevertheless, because of the high C/N ratio, a lower rate of EFB application is made with supplements of inorganic fertilizer. This approach maintains soil productivity through better soil structure and reduces fertilizer cost for immature palms by as much as 58 %, and by 5 % for mature palms. The high cost of inorganic fertilizer encourages best-developed practices designed to optimize fertilizer use and minimize nutrient losses. For example, it is routine to base fertilizer recommendation on foliar analysis so that observed deficiencies can be corrected and an appropriate balance maintained between different elements. Foliar analysis may be supplemented by analysis of rachis tissue (which acts as a nutrient store) and soil. Using such information, application rates and fertilizer sources are objectively determined, often with the aid of customized computer programs such as the MPOB Oil Palm Nutrient System (OPENS). The technology for producing a fertilizer management map has been developed for oil palm and current investigation is on variable rate technology (VRT) required for the implementation of precision agriculture. An innovative replanting technique has been developed where young palms are planted directly amongst old crop residue piles to improve accessibility and efficiency of nutrient utilization (Khalid *et al.,* 2000). This technique offers greater synchrony between nutrient release and plant uptake in terms of space and time compared to the standard practice. The residues contain 642 kg N, 58 kg P, 1384 kg K and 156 kg Mg per ha. In terms of inorganic fertilizers, this is equivalent to 3.06 tonnes of sulphate of ammonia, 0.37 tonnes of Christmas Island rock phosphate, 2.77 tonnes of muriate of potash and 1.0 tonne of kieserite.

The oil palm manifests nutrient deficiencies with typical and visible diagnostic symptoms as well described by Turner and Gillbanks (1974). A wide spread of elements is found in oil palm tissues but not all deficient in nutrient give rise to characteristic symptoms in foliage. Deficiency symptoms for P, S, Cl and Mn are indistinct while hunger signs for N, K, Mg, B, Cu and Zn are well defined.

Table 2.7: Nutrients deficiency symptoms in oil palm leaflets (Ng, 2012).

Elements	Distinct Symptoms
N	Chlorosis
K	Orange spotting/orange blotch
Mg	Orange frond,
B	Hook leaf/crinkle leaf, white stripe (associated with high N, low K)
Cu	Mid-crown chlorosis
Zn	Yellow, shortened narrow pinnae

CHAPTER 3: MATERIALS AND METHODS

3.1 Promotion of Oil Palm Seedling (*Elaeis guineensis* Jacq.) Growth by *B. salmalaya* Strain 139SI

Agriculture area cultivated with oil palm (*Elaeis guineensis* Jacq.) in Malaysia has expanded tremendously in recent years. First introduced as an ornamental tree in early 1870's, oil palm has since undergone significant leaps in production and planted areas to become the most important industrial crop. Palm oil production has risen by more than 2 million tonnes in the past 10 years from 15.8 million tonnes in 2007 to 17.3 million tonnes in 2016. The total area planted with oil palm had increased from 4.3 million hectares in 2007 to 5.7 million hectares in 2016, an increase of 32.6% (MPOB, 2017). Various factors have contributed to spurring oil palm industry occupying its present position, including the introduction of high-quality planting material through genetic improvements and also the implementation of good agronomic practices (Wahid *et al.,* 2005).

Breeding and selection of high-quality planting material for oil palm plantation industry are not only focusing on achieving high fresh fruit bunch yield, but the quality of the oil produced and desirable vegetative characters such as reduced rates of trunk extension and long bunch stalks are also taken into account (Basri *et al.,* 2003). Strong demands for high quality planting material has initiated the efforts to propagate the oil palm through tissue culture. Vegetative propagation of oil palm by tissue culture allows rapid multiplication of uniform planting materials with desired characteristics (Sogeke, 1998).

Besides that, inoculation of plant growth-promoting rhizobacteria (PGPR) at the early stage of oil palm seedling growth is one of the promising alternative strategies as a measure to produce high-quality planting material. Inoculation of PGPR to oil palm seedling at the early stage of growth would enable early associative interactions between the bacteria and the seedling. These early associations would enhance adaptation of the seedlings to the environmental conditions, hence, increase the survival rate (Sturz & Nowak, 2000; Azlin *et al.,* 2007). Previous studies have shown that inoculation of PGPR at the early stage of plant growth could significantly enhance survival rates of the host plants (Pandey *et al.,* 2000). Thus, in this study, the potential of a new strain of soil bacteria, *B. salmalaya* strain 139SI, as plant growth promoting bacteria were evaluated. Subsequently, the effects of strain 139SI inoculation at the early stage of oil palm seedling growth and the colonization of the strain on palm root were also determined.

3.1.1 Bacterial Strain Preparation

B. salmalaya strain 139SI (Gen Bank accession No: JF825470.1; ATCC BAA-2268) used in this study was originally isolated from soil obtained from the private oil palm plantation in Selangor, Malaysia (2.99917°N 101.70778°E). The stock culture of 139SI was prepared by sub-culturing the bacteria in universal bottles containing BBL™ Brain Heart Infusion (BHI) slants and maintained at 4°C until required.

3.1.2 Quantification of Indole Acetic Acid (IAA)

IAA content produced by the strain 139SI was measured spectrophotometrically using the method described by Gordon and Weber (1951). The strain 139SI was cultured in two sets of flasks, one containing 20 mL of Brain Heart Infusion (BHI) medium supplemented with 0.1% (w/v) L-Tryptophan and the other without L-Tryptophan. The culture was then incubated on orbital shaker (150 rpm) at 30°C for 96 h. After 96 h of incubation, the cultures were centrifuged at 5000 rpm for 20 min and 1

mL cell suspension was transferred into a tube and mixed vigorously with 2 mL of Salkowski's reagent. The mixtures were then incubated at room temperature for 25 min and the formation of a pink color was measured using a spectrophotometer at 535 nm. The quantity of IAA in the culture filtrate was determined from a standard curve prepared with known concentrations of IAA.

3.1.3 Evaluation of Phosphate Solubilizing Ability of the Strain 139SI

The phosphate solubilizing ability of 139SI was evaluated with the National Botanical Research Institute's Phosphate (NBRIP) plate culture [composition per liter: glucose 10 g, $Ca_3(PO_4)$ 5 g, $MgSO_4.7H_2O$ 1 g, KCl 0.2 g, NaCl 1 g, NH_4Cl 5 g and 2% general purpose agar-agar, pH 7.0 (Nautiyal, 1999)]. Freshly grown of strain 139SI cultures was inoculated on NBRIP medium and incubated at 30^{o}C for 96 h. The phosphate solubilizing ability of 139SI was shown by the formation of clear zone around the bacterial colony. The ratio of the diameter of the clear zone to the diameter of the bacterial colony was calculated as a phosphate solubilization index (SI) of the strain 139SI.

3.1.4 Siderophore Production

Production of siderophore by strain 139SI was determined qualitatively following the method of Schwyn and Neilands (1987) by using the universal CAS agar plate assay. Composition of CAS-reagent was: 1 mM CAS, 10 ml $FeCl_3.6H_2O$ (1 mM stock) made in 10 mM HCl and 2 mM N,N-cetyl trimethyl ammonium bromide (CTAB). The CAS-reagent solution was autoclaved separately and added to 500 ml of NA medium. Freshly grown of strain 139SI cultures was spot inoculated on Chrome-Azurol-S (CAS) agar medium and incubated for four days at 30°C. The color changes of CAS reagent from blue to of yellowish or orange zone around bacterial colony was considered positive for siderophore production.

3.1.5 N_2 Fixation

The ability of strain 139SI to fix atmospheric N_2 was determined qualitatively with N-free semisolid medium (Baldani *et al.,* 2014). N-free semisolid medium contained per L: 5 g malic acid, 0.5 g K_2HPO_4, 0.2 g $MgSO{\cdot}7H_2O$, 0.1 g NaCl, 0.02 g $CaCl{\cdot}2H_2O$,1 g $Na_2MoO_4{\cdot}2H_2O$, 1.175 g $MnSO_4{\cdot}H_2O$), 4.0 g KOH, 1.4% agar, 2 mL bromothymol blue (5% sol KOH), and the pH was adjusted at pH 7. The strain 139SI was grown in BHI liquid medium for 48 h at 30ºC and 140 rpm. Then, 10 mL of strain 139SI culture was centrifuged for 5 min at 5000 rpm, and the cell pellets were collected and re-suspended in physiological solution (0.8% NaCl). After that, an aliquot of 10 µL was transferred into vials containing semi-solid N-free media. The ability of strain 139SI to fix atmospheric N_2 is evidenced by the formation of a veil-like pellicle near the surface of the semi-solid N-free media.

3.1.6 Growth Promotion of Oil Palm Seedling by *B. salmalaya* Strain 139SI

3.1.6.1 Bacterial Inoculum Preparation

Bacterial inoculum was produced by transferring two loops of strain 139SI from 2 day-old cultures on to BHI plate followed by incubation at 30°C for 48 hours. Bacterial cells were scraped from the plate and suspended in PBS buffer (10 mM NaH_2PO_4 containing 0.8% NaC1, pH 6.5). The inoculum concentration was adjusted to approximately 1 x 10^9 CFU/ml with PBS, based on the optical density at 600 nm, and was confirmed by plate counting.

3.1.6.2 Seedling Preparation and Experimental Design

Aseptically grown oil palm seedlings were used in this study. The oil palm seeds were sprayed with a mist sprayer of distilled water to raise the moisture content required for germination. The moist seeds were kept in 40 cm x 40 cm polythene bags for heat-treatment at 60 days durations at temperature of 39 ± 1°C in a germinator (Nicolas, 2010). Seeds of oil palm (*Elaeis guineensis* Jacq.) that have undergone heat treatment to stimulate germination were surface-disinfected by briefly washing in 70% ethanol, followed by 5% sodium hypochlorite before it was rinsed several times with sterile distilled water. The distilled water from the last rinse was cultured on BHI medium plates to make sure the seeds were fully sterilized. The sterilized seeds, then immersed in sterile distilled water to raise the moisture content required for germination. Subsequently, the seeds were kept in the germination room at a temperature 30 ºC for 60 days (Green *et al.,* 2013).

After 60 days, the germinated seeds with 1.0 -1.5 cm of epicotyl and 2.0 – 2.5 cm of root were selected for this experiment. The oil palm seedlings were washed with sterile distilled water several times and the residue from the last rinse was streaked on BHI plates to ensure the seedling free of any microbe before transferred into glass bottle (15 cm x 5 cm) filled with a 100 g mixture of sterilized peat and vermiculite (1:10, v: v) as a medium to grow the seeds. The growing media were analyzed for pH and macro and micronutrient contents. The pH of the dried soil was measured using a pH meter (soil to water ratio of 1:4) (Thomas, 1996). The amount of nitrogen was determined by Kjedahl method (Bremner & Mulvaney, 1982). While the quantities of other nutrients in soil were determined using an Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) (Jones, 2001). The pH of soil was = 6.78 ± 0.16. Percentage of elements in 100g soil sample were nitrogen: 0.11% ± 0.06, phosphorus: 0.10% ± 0.02, potassium: 0.06% ± 0.01, magnesium: 0.04% ± 0.02, calcium: 0.08% ± 0.01, sulfur:

0.09% ± 0.01). The growth media previously was sterilized by autoclaving three times for 20 min at 121°C with 24 hour intervals. The seedlings were allowed to grow for a week to make sure they were stable before treatment.

The seedlings were then inoculated with 5 ml of $1x10^9$ CFU ml^{-1} strains 139SI in PBS. The inoculum was mixed into the soil using a sterile metal spatula. Bacterial suspension was replaced with sterile PBS buffer in the control group. Each treatment was comprised 60 replications. Plant vegetative growth and bacterial colonization on a plant's root was assessed by sampling the plants on day 7, 14, and 21 after inoculation. 10 replicates were used for analyzing 139SI colonizing ability and the other 10 replicates were used for vegetative growth measurement. The glass bottle that contained the seedling was placed in a nursery under natural light and temperature condition [mean temperature: 31/25°C (day/ night)].

3.1.6.3 Vegetative Growth Measurement

Plants were removed from growth medium and roots were gently shaken to remove root-adhering soil. Shoots length, stem diameter, root length and lateral root number were determined as growth parameters. Shoot and root were separated and oven-dried at 70ºC until constant weight was achieved to determine the dry weight.

3.1.6.4 Bacterial Population in Rhizosphere

Roots and rhizosphere vermiculite covered the root from each bottle were weighed and 1 g of samples were placed in a tube containing 10 ml of sterile PBS water. Then, the samples were shaken vigorously using vortex mixture for two minutes. Resulting suspensions were serially diluted and 100 µl aliquots were streaked on BHI plates. The Bacterial growth was recorded after two days incubation at 30ºC in the dark.

3.1.6.5 Endophytic Colonization

To determine endophytic populations of strain 139SI, 1 g of root segments from inoculated and non-inoculated plants were surface sterilized with 70% ethanol for 30 sec followed by 1% commercial bleach for 30 sec and then washed three times in distilled water (this step was repeated for several times). Root samples were then aseptically macerated in 10 ml PBS with a mortar and pestle. Homogenates were serially diluted and plated on BHI to determine the microbial populations inside surface-sterilized roots. The bacterial colonies were counted after 4 days of incubation at 30°C (Chanway, 2000)**.**

3.1.6.6 Evaluation of the Efficacy of Surface Sterilization Methods

To determine the efficiency of sterilization, 100 µl of distilled water from the last rinse was streaked on BHI medium plates. The surface-sterilized root samples were randomly selected and then submerged in BHI broth media (Baldani *et al.,* 2014). The surface sterilization of root samples was achieved when there was no growth on the media after incubated at 30°C for 3 days. Only data from the fully surface-sterilized root samples were selected to be analyzed.

3.1.6.7 Observation of the Bacterial Colonization on Root Surface

Root samples from inoculated and non-inoculated plants were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate, pH 7.4, for 2 hours, and post fixed in 1% (w/v) osmium tetroxide in the same buffer for 2 hours. Samples then were washed and dehydrated in ethanol and then were followed by critical point drying with liquid carbon dioxide using the CPD 030 (BAL-TEC, Germany). The samples were then mounted on stubs for SEM, spattered with gold (Sputter Coater SCD, 005, BAL-TEC, Germany) and examined with a scanning electron microscope.

3.2 Synergistic Effects of *B. salmalaya* Strain 139SI Inoculation with Inorganic Fertilizer on Soil Fertility and Oil Palm Seedling Growth

Palm oil is one of the most consumed vegetable oil in the world. Malaysia currently stood as the second largest palm oil producer with total area cultivated with oil palm in 2016 reached 5.74 million hectares, an increase of 1.7% as against 5.64 million hectares recorded in the previous year (MPOB, 2017). There are many factors that determine the productivity of an oil palm plantation, the most important of which is the quality of the transplanted oil palm seedlings produced at the nursery stage. Besides the significant genetic improvement of the seedling derived from the cross-pollination of selected parent palms, proper agronomic practice, particularly through fertilizer application could enhance the production of high-quality planting materials (Khusairi *et al.*, 2001). However, fertilizer inputs are one of the major costs in oil palm nursery management due to the high demand for nutrient by the seedlings (Wahid *et al.,* 2005). Thus, the best practice has to be designed to optimize the fertilizer use efficiency and mitigate the impacts of the high cost of inorganic fertilizer.

The integration of fertilizer application with organic materials such as plant growth promoting rhizobacteria (PGPR) is one option that can benefit both agronomic and ecosystems. Various studies have documented the mechanisms of action of PGPR in promoting plant growth and development. The role of PGPR in biological nitrogen fixation (BNF), nutrient chelation, and solubilization, production of growth hormone and biocontrol agents can improve soil fertility and facilitate the plant growth (Ahemad & Kibret, 2013). There were several researchers have reported about the potential of various species of PGPR inoculant in reducing chemical fertilizer used without compromising plant growth and yield (Lugtenberg & Kamilova, 2009).

However, the effectiveness of PGPR inoculation with the presence of inorganic fertilizer needs to be further investigated as many previous studies reported that PGPR

gave several different effects on nutrient uptake and plant growth. For example, Adesemoye *et al.* (2009) found that PGPR such as *Bacillus amyloliquefaciens* and *Bacillus pumilus* could reduce application rates of chemical fertilizers to the plant by increasing fertilizer use efficiency. On the other hand, Freitas *et al.* (1997) reported that inoculation of phosphate solubilizing bacteria consists of *Bacillus brevis, Bacillus megaterium, Bacillus polymyxa, Bacillus sphaericus, Bacillus thurigiensis* and *Xanthomonas maltophilia* to the P-deficient soil amended with rock phosphate fertilizer have an insignificant effect on P uptake of the plant. Meanwhile, some PGPR affects fertilizer use efficiency depends on the rate of fertilizer applied. For instance, the efficacy of *Pseudomonas fluorescens* in enhancing fertilizer use efficiency reduced with the increasing rates of fertilizer added to the soil as mentioned by Shaharoona *et al.* (2008).

Nonetheless, the synergistic effects of this beneficial soil microbe inoculant with inorganic fertilizer application need more studies in order to reveal its potential for the enhancement of nutrients uptake of oil palm seedlings at the nursery stage. Early introduction of this microbe to the rhizosphere soil through inoculation can help increase its population, thus help achieve the full establishment of the microbe to the root system. This could offer protection for the seedling against the various soil stress conditions after transplanting to the plantation area. In the previous study, we found that *B. salmalaya* strain 139SI exhibited several promising plant growth-promoting features such as involved in biological nitrogen fixation (BNF), positive for phosphate solubilizing activity, produce plant growth hormone of IAA and iron chelation compound called siderophore. The result of scanning electron microscope (SEM) analysis also found that this strain has shown their ability to travel from rhizosphere soil to colonize plant root (Azri *et al.,* 2018). Based on the above-mentioned information, we hypothesized that inoculation of this strain could increase nutrient availability for

plant absorption in the immediate vicinity of the root, thus, enhance plant growth. This nursery stage of research was also designed to investigate the synergistic effects of *B. salmalaya* strain 139SI inoculant with inorganic fertilizer on nutrients uptake and plant physiology. On top of that, it is also important to determine whether the promising features of this strain in enhancing nutrient availability by BNF, solubilizing insoluble phosphate and production of the iron chelator found based on the laboratory results can be replicated in the real unsterilized nursery condition or not.

3.2.1 Bacterial Strain 139SI Preparation

B. salmalaya strain 139SI was maintained in BBL™ Brain Heart Infusion (BHI) slants at 4°C until required. For inoculum preparation, two loops of the strain from 2 day-old cultures were transferred on to BHI plate followed by incubation at 28°C for 48 hours. Bacterial cells were scraped from the plate and suspended in PBS buffer (10 mM NaH_2PO_4 containing 0.8% NaC1, pH 6.5). The inoculum was diluted with PBS to a final concentration approximately 1 x 10^9 CFU/ml, based on the optical density at 600 nm, and was confirmed by plate counting.

3.2.2 Plants Growth Conditions

A total of 40 oil palm seedlings (Dura x Pesifera), all three months of age, were obtained from Sime Darby Seed Sdn. Bhd, Malaysia. The seedlings were transplanted into a bigger polythene bag (38 cm x 51 cm) containing a horticultural soil formulation of 3:2:1 of peat, clay and sand. The growing media were analyzed for pH and macro and micronutrient contents. The pH of the dried soil was measured using a pH meter (soil to water ratio of 1:4). The amount of nitrogen was determined by Kjedahl method (Bremner & Mulvaney, 1982). While the quantities of other nutrients in soil were determined using an Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). The soil was pH = 6.89: percentage of elements in 100g soil sample: calcium 0.34% ± 0.02, potassium: 0.06% ± 0.01, magnesium: 0.03% ± 0.02, phosphorus: 0.1% ±

0.03, sulfur: 0.09% ± 0.01, nitrogen: 0.10% ± 0.07. Watering was done once daily throughout the experimental period. All seedlings were allowed to stabilize with the nursery climate conditions for 30 days after transplanted in the polythene bag.

3.2.3 Plants Treatment

After acclimatization, a total of 40 palm seedlings were selected for the experiment based on their uniformity in size and free from any disease. The seedlings were clustered into 4 groups of treatments. Palm in treatment 1 (C1) was untreated. Inorganic fertilizer was given to the palm in treatment 2 (C2). The other two groups of treatment consisted of palm inoculated with strain 139SI (T1) and palm inoculated with strain 139SI + inorganic fertilizer (T2). Palm seedlings in C1 and C2 were served as control. The inoculum was given at 15 ml/ plant and each bacterized palm in the T1 and T2 were re-inoculated at weekly intervals for four months. An inorganic fertilizer regime comprising ammonium sulfate as the N source (1.0 g N $plant^{-1}$), di-ammonium phosphate as the P source (0.5 g P $plant^{-1}$), and muriate of potash as the source of K (2.0 g K $plant^{-1}$) were given to the palm in T2 and T4 monthly. The experiment was conducted in the Institute of Biological Sciences nursery which is situated in the Faculty of Science, University Of Malaya (3° 7'24.63"N 101°39'15.61"E) for four months [mean temperature: 31/25°C (day/ night), relative humidity: 66–81%]. The plants were laid out in a completely randomized design (CRD) with 10 replications.

3.2.4 Growth and Vegetative Measurements

The parameters taken to determine the plant growth were plant height, stem diameter, number of leaves, leaf surface area, and dry weights of shoots and roots. Plant height and stem diameter were taken monthly for four months. Plant height was measured from the plant's base to the top of newest fully developed leaf. Stem diameter of plants was measured approximately 1 cm above the soil level using vernier caliper (Brown Sharpe, USA). Number of leaf and leaf surface area were taken at the end of

treatment. To measure leaf surface area, leaf image was first captured by a Casio Exilim 6 megapixels digital camera and the leaf surface area was determined by analyzing the scaled-photo of the leaves using the ImageJ image processing program. At the end of experiment, the plants were carefully uprooted and were cut to separate shoot and root. Each shoot and root was labeled according to the treatment group and oven-dried at 70ºC until constant weight was achieved. The total plant biomass was determined by calculating the sum of the plant's upper parts and roots dry weight.

3.2.5 Chlorophyll Content Measurement

Chlorophyll content was determined using a portable chlorophyll meter (SPAD-502, Minolta Co., Japan). The portable chlorophyll meter determines the relative amount of chlorophyll present by measuring the absorbance of the leaf in the blue (400 – 500 nm) and red (600 – 700 nm) regions. Using these two absorbance, the meter calculates a numerical SPAD value which is proportional to the amount of chlorophyll present in the leaf. The chlorophyll content was measured in five fully expanded leaves from the top of the plants and replicated ten plants per treatment at the end of the experiment.

3.2.6 Photosynthetic Rate Measurement

Photosynthetic rate was measured at the end of experiment in five fully expanded leaves per plant, replicated ten plants per treatment using the portable photosynthesis system (Model LI-6400XT, LICOR, USA) equipped with the built-in light source set at 1,800 μmol photons m^{-2} s^{-1}. The level of CO_2 supplied to the leaf was controlled by using the built-in CO_2 injection system of the photosynthesis unit and was adjusted at approximately 400 μmol mol^{-1}. The photosynthesis rate was measured between 11:00 a.m. and 15:00 p.m. at a leaf temperature between 28 - 30ºC. The portable photosynthesis system utilizes gas exchange principles to measure the

photosynthetic rates of plants. Net photosynthesis rates are expressed as rates of CO_2 uptake ($\mu mol\ m^{-2}\ s^{-1}$).

3.2.7 Chlorophyll Fluorescence Measurement

The light energy absorbed by chlorophyll molecules can be re-emitted as light or called chlorophyll fluorescence. The chlorophyll fluorescence was measured in five fully expanded leaves from the top of the plants, replicated ten plants per treatment at the end of the experiment using a chlorophyll fluorometer (Model LH36/2R, Hansatech Instrument Ltd., England). A clip, oriented with the shutter plate, was attached to the leaf for dark adaptation. After 30 minutes of dark adaptation, the fluorescence signal was counted after the light was applied to the leaf by removing the shutter plate and the quantum yield or photosynthetic yield (temperature = 28ºC and time range 10 μsec^{-3}) was measured. The maximum chlorophyll fluorescence yield (F_M) was determined by saturating light pulse and minimum chlorophyll fluorescence yield (F_O) values was obtained with modulated low light. The yield of variable fluorescence (F_V) was calculated as the difference between F_M and F_O, and the F_V/F_M ratio represents the relative state of photosystem 2 (PSII) was calculated.

3.2.8 Analysis of Nutrient Content in Soil and Plant Samples

Plant and soil samples for nutrient content determination were collected at the end of the study. Plant samples were uprooted, cleaned of any dirt and shoots were separated from the roots. The soil samples were taken from 0 – 20 cm depths. The plant and soil samples were oven-dried at 70°C until a constant weight was achieved. Then, the dried leaf and soil samples were ground to pass a 20 mesh screen. Nitrogen content in plant and soil samples were determined using Kjeldahl method (Bremner, 1996). 1 g of plant and soil sample was added with potassium sulfate (K_2SO_4), copper sulfate pentahydrate ($CuSO_4.5H_2O$) and 3.5 mL of concentrated sulfuric acid (H_2SO_4) before digested at temperature 390°C for 180 minutes. After the digestion process complete,

46.5 mL DI water was added to each tube to make a final volume of mixture to 50 ml. The mixture then filtered before analyzed for total nitrogen content by using Flow Injection Analysis Colorimetry.

The analysis of phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca) and sulfur (S) were determined by Inductively Coupled Plasma Optical Emission Spectrometry ICP-OES. 1 g of plant and soil samples was digested with 10 mL HNO_3 at temperature 115°C for 120 minutes. After the digestion process complete, the tubes were allowed about 1 hour for cooling. Then 5 mL of HCL was added to the samples and were digested again at same temperature for more 120 minutes. 1% of HNO_3 v/v solution was added to each tube to make a final volume of mixture to 100 ml. The mixture then filtered before analyzed for nutrient content by using ICP-OES (Jones, 2001).

3.2.9 pH Determination of the Soil Samples

Five gram of soil sample from each treatment group was suspended in 25 ml of Milli-Q water in a separate 50 ml tube. Then the tubes were vigorously shaken and left to stand for 5 hours. The pH of the soils was measured with scientific pH meter.

3.3 Effects of *B. salmalaya* Strain 139SI Inoculation on Yield and Palm Oil Quality

Palm oil, the oil derived from the mesocarp of a tropical crop of *Elaeis guineensis* Jacq, is of one the most consumed vegetable oil in the world. Oil palm was introduced in Malaysia in early 1870's as an ornamental plant, since then, it has undergone expansion and modernization to become the most important commodity crop in Malaysia with total area cultivated with oil palm in 2016 reached 5.74 million hectares (MPOB, 2017). Many factors involved in spurring the oil palm industry, of which, agronomic practice plays one of the most important roles. This is because oil palm growth and yield are recognized for highly responsive to fertilizer input. A high demand for nutrients, especially from fertilizer input is not surprising, in view of its high dry matter production (Wahid *et al.,* 2005).

Based on previous studies, a hectare of oil palm plantation is estimated to require approximately between 100 to 200 kg/ year of nitrogen (N), 50 to 100 kg/ year of phosphate (P), and 200 to 300 kg/ year of potassium (K) for optimum yield production (Wahid *et al.,* 2005; Bakar *et al.,* 2011; Lee *et al.,* 2014). As a comparison, a hectare of wheat required nutrient inputs of N, P, and K at 100 to 120, 60 to 75 and 50 to 60 kg/ year, respectively (Rana *et al.,* 2012; Khalid *et al.,* 2004). While rice needed 100 to 180 kg/ year of N, 50 to 100 kg/ year of P and 60 to 120 kg/ year of K for every hectare (Xu *et al.,* 2015; Sagarika *et al.,* 2015; Hoseinzade *et al.,* 2016). These huge amounts of fertilizer are needed by oil palm to replace the nutrients that are removed continuously through the harvested fresh fruit bunch (FFB). However, nutrient leaching, precipitation, erosion, volatilization and denitrification could cause low fertilizer use efficiency since the major portion of the applied inorganic fertilizers is not available to the plants (Powell *et al*., 2010). Furthermore, over-fertilization may result in undesired economic and environmental problems, including underground water contamination due

to nitrate leaching into waterways, increased gaseous emissions of ammonia and nitrous oxide to the atmosphere and soil degradation that could cause a decline in crop yields (Diacono & Montemurro, 2010; Zaman *et al.,* 2015).

As attempts to address this problem, the best agronomic practices that are efficient, sustainable and less harmful to the environment have to be developed. One of the best options is integrating the application of inorganic fertilizer with microbial inoculant such as plant growth-promoting rhizobacteria to optimize the use of fertilizer and minimize nutrient losses. As reported by previous research, integrated nutrient management with inorganic fertilizers and microbial inoculant can improve crop productivity as well as soil fertility (Tiyagi *et al.,* 2015; Hoseinzade *et al,* 2016; Thilagar *et al*., 2016). The beneficial effects of these bacteria have been attributed to their ability in assisting resource acquisition through enhancement of nutrient availability in soils and production of numerous plant growth regulators that promote root development resulting in more efficient uptake of nutrients and water (Ahemad & Kibret, 2014).

Surprisingly, there is no report related to the effects of bacterial inoculant on oil palm yield and subsequently, the quality of oil produced. Hence, this one-year of field study was initiated to discover the potential of integrating the bacterial inoculant with inorganic fertilizer in the oil palm plantation industry.

3.3.1 Bacterial Strain Preparation

B. salmalaya strain 139SI was provided by Molecular and Bacteriology Labroratory, University of Malaya. The strain 139SI originally isolated from rhizosphere soil obtained from the agricultural farm. The species classification of this strain was based on phenotypic characteristics, phylogenetic analysis and 16S rRNA G+C characterization (Gen Bank accession No:JF825470.1; ATCC BAA-2268) (Ismail *et al.,* 2015). The strain 139SI was maintained in BBL^{TM} Brain Heart Infusion (BHI) slants at 4°C until required. The strain was also tested for plant growth-promoting features and was found able to produce indole acetic acid (IAA) (18.5 ± 0.4 µg/ml), based on the method described by Gordon and Weber (1951), positive for N_2-fixing activity using the method of Döbereiner (1995), phosphate solubilization was evaluated with the National Botanical Research Institute's Phosphate (NBRIP) plate culture and siderophore production base on the chrome azurol S approach. For inoculums preparation, bacterial cells from 2 day-old cultures on BHI plate were scraped from the plate and suspended in PBS buffer (10 mM NaH_2PO_4 containing 0.8% NaC1, pH 6.5). The strain 139SI suspension was then diluted with PBS to a final concentration approximately 1 x 10^9 CFU/ml, based on the optical density at 600 nm, and was confirmed by plate counting.

3.3.2 Trial Site and Experimental Design

The field trial was conducted in Batu Pahat, Johor (2°21' N, 102°40'E) from June 2015 until May 2016. [Mean temperature: 30/23°C (day/ night), relative humidity: 60 –75%,]. Average rainfall 220 mm. Soil chemical properties of the 0–30 cm layer: N = 0.11%, P = 0.05%, K = 0.08%, Ca = 0.15%, Mg = 0.1%. The study was conducted on 10-year-old of *Dura* x *Pisifera* oil palm progeny, planted in a triangular system with a distance of 9m x 9m x 9m at a density of 148 palms per hectare. The treatment involved were: (T1) Untreated palm, (T2) palm received inorganic fertilizer, (T3) palm

inoculated with *B. salmalaya* strain 139SI inoculum and (T4) palm given a combination of inorganic fertilizer and inoculated with strain 139SI. Each treatment plot had 64 palms, with 4 replications, in a randomized complete block design. Recordings were made from 36 palms for each plot. Palms in the outermost rows served as a buffer (Figure 3.1).

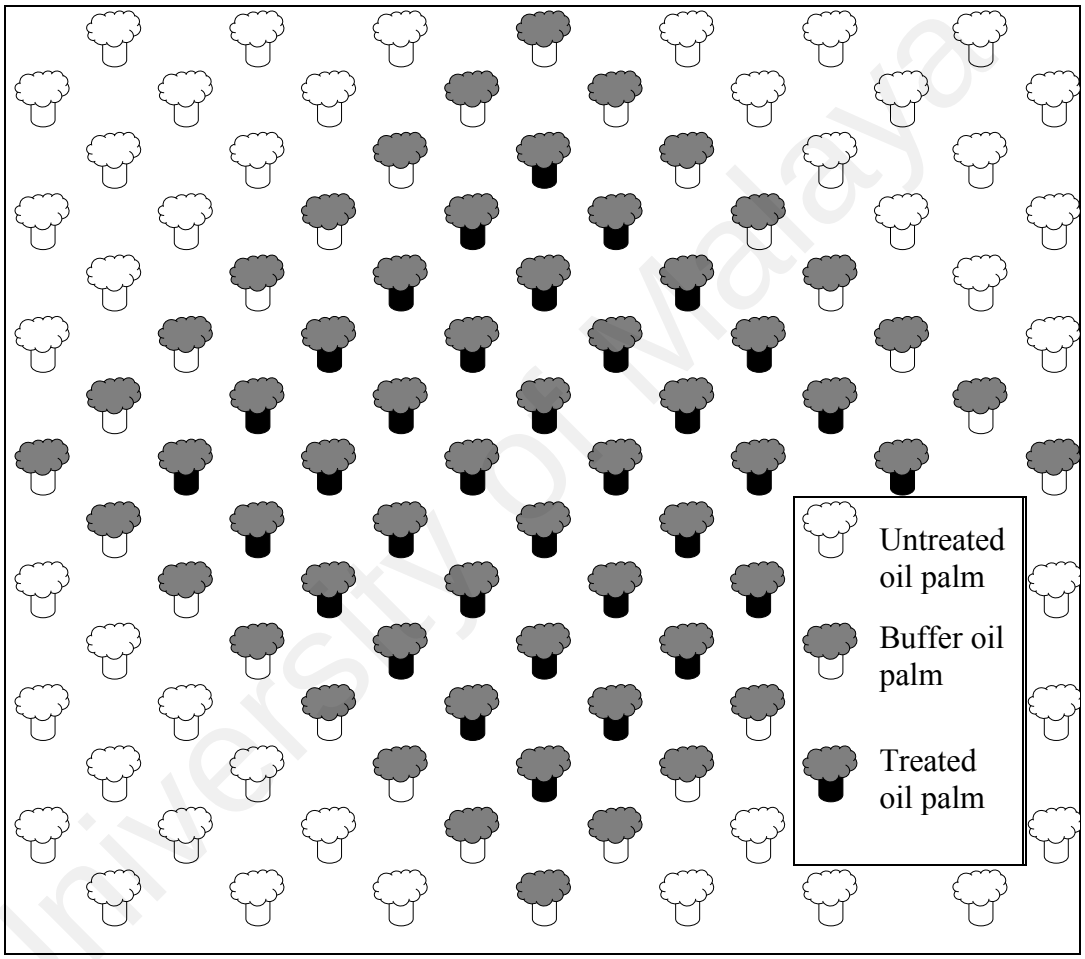


Figure 3.1: A plot with 64 oil palms and the outermost rows serving as a buffer. The buffer oil palms were receiving treatment same as the recording palm. They served as buffering zone to prevent cross-contamination between plots.

The oil palm in T1 and T2 were served as control. The oil palm in T3 and T4 received *B. salmalaya* 139SI inoculation at the rate of 1 L palm^{-1}month^{-1}. The bacterial inoculum was applied around the tree within 50 cm radius. An inorganic fertilizer regime comprising ammonium sulfate as the N source, di-ammonium phosphate as the P source and muriate of potash as the source of K was applied to the palm in T2 and T4. The fertilizer regime was applied thrice a year at the rate as tabulated in Table 3.1.

Table 3.1: Types and quantity of nutrient applied to the palm

	Fertilizer applied (kg hectare^{-1}year^{-1})		
Amount of nutrient	N	P	K
	111.00	59.94	179.82

3.3.3 Analysis of Nutrient Contents in Soil and Plant Samples

Palm nutrient status was determined according to the method described by Lee *et al.* (2014). Frond from the treatment palm was sampled at approximately 20 cm long and was cut into small pieces to facilitate the drying process. Soil samples were collected around 0.5 m from the base of the palm tree with an auger at depths of 0 – 40 cm. Rachis and soil samples were collected from each individual palm to make sure the data precisely represent the nutrient status of the palm and soil. All the rachis and soil samples from one treatment plot were bulked into one bag respectively. The samples were then dried at 80^{o}C for 3 days before finely ground to pass through a 1 mm sieve. The total nitrogen content of the palm and soil samples was estimated by micro-Kjeldahl method. While the analysis of phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca) and sulfur (S) was determined by Inductively Coupled Plasma Optical Emission Spectrometry ICP-OES as described previously.

3.3.4 Fresh Fruit Bunch (FFB) Yield

The oil palm FFB of each treatment was harvested at regular intervals of 14 days or two rounds per month. Data on FFB number and bunch weight produced from each treatment plot were recorded during the harvesting rounds and present in monthly basis. The data recordings were taken for one year period. The yield of FFB numbers and weights per hectare were calculated based on the following formula:

$$\text{FFB number or weight hectare}^{-1} = \frac{\text{Average FFB number or weight plot}^{-1}}{\text{36 palm}} \times \text{148 palm hectare}^{-1}$$

3.3.5 Crude Palm Oil (CPO) Content

Approximately 1 kg of oil palm fruitlets from FFB of each treatment plot were randomly sampled and bulked into one bag during harvesting rounds. Then, the CPO extraction procedure was done according to the method described by Junaidah *et al.* (2015). The oil palm fruitlets samples were placed in a laboratory-scale autoclave and subjected to sterilization process at temperature 110^{o}C for 20 min to deactivate the biological factors that can deteriorate the quality of oil produced. For CPO extraction, 100 g of oil palm fruitlets mesocarp of each treatment plot was peeled off from the nut before submerged into boiling water for 10 minutes. The soften mesocarp was meshed by using a commercial blender and then was pressed through a 30-mesh screen to facilitate oil extraction. Subsequently, the mixture was subjected to centrifugation at 10000 rpm and 45^{o}C for 10 minutes. The upper layers consist of oil, was carefully transferred into a beaker and subjected to vacuum drying process using vacuum oven (15 inHg max; 80^{o}C) for 150 min.

3.3.6 Fatty Acid Analysis of Palm Oil

3.3.6.1 Preparation of Fatty Acid Methyl Esters (FAME)

The fatty acids were converted to fatty acid methyl esters (FAME) according to the method as described in AOCS Official Method Ce 2-66 and Ce 1-62 (AOCS, 1994). Approximately 0.5 g of palm oil sample was introduced into a 50 ml flask. Then, 6.0 ml of 0.5N methanolic NaOH and boiling chip were added into the palm oil sample before the mixture was heated on a steam bath for about 5 – 10 minutes or until fat globules mix into the solution. Precisely, 7.0 ml of boron trifluoride (BF3) -Methanol was added and the mixture was heated for another 2 minutes. Subsequently, 5.0 ml of heptane was added into the mixture and heat was turned off after 1 minute. After the mixture was cool down to room temperature, sufficient amount of saturated NaCl was added into the mixture until the level of liquid reaches the neck of the flask. About 1 ml of heptane solution at the upper layer of the mixture was transferred into a test tube containing a small amount of anhydrous Na_2SO_4.

3.3.6.2 Chromatographic Analysis of Fatty Acids

Fatty acids were detected using gas chromatograph (GC) (Agilent Technologies, Wilmington, DE) equipped with a flame ionization detector (FID). One microliter of the FAMEs in *n*-hexane was loaded into automatic liquid injector. Separation of fatty acids was carried out on a BPX-70 a capillary column (30 m length, 0.32 mm i.d., 0.25 μm film thickness). Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The oven temperature was held initially at 140ºC for 5 min and then increased to 240ºC for 5 min with a gradient of 4ºC/min. The temperature of injection port and the detector was set at 260ºC. Identification of FAME was achieved by comparing the retention times of the peaks with those authentic standard mixtures. The results were expressed as relative percentages of total fatty acids.

3.4 Statistical Analysis

Results were expressed as means + standard deviation. The vegetative growth results obtained from the plants grown in sterilized condition were analyzed by student t-test. Data for strain 139SI population density were analyzed using one-way ANOVA with posthoc Tukey's test with the level of significance established at $p < 0.05$. Correlations between strain 139SI population density in rhizosphere and growth parameters were also analyzed. All data obtained from nursery and field trial were also analyzed by one-way analysis of variance (ANOVA) performed by using SPSS software version 22. Significant differences between means were compared using a Tukey range test at $P \leq 0.05$.

CHAPTER 4: RESULTS

4.1 Promotion of Oil Palm Seedling (*E. Guineensis* Jacq.) Growth by *B. salmalaya* Strain 139SI

4.1.1 Quantification of Indole Acetic Acid (IAA)

Screening results of plant growth promoting traits of strain 139SI are depicted in Table 4.1. IAA is a growth hormone that PGPR most commonly produce. Colorimetric analysis based on the colour changes of Salkowski reagent was used to measure the IAA production. Measurement of IAA produced by strain 139SI was done with and without the presence of tryptophan, a biochemical precursor of IAA production in bacteria. The strain 139SI was found to be able to produce about 28.5 ± 0.4 µg/ml of IAA and higher concentration of IAA were produced in the presence of L-tryptophan (46.5 ± 1.2 µg/ml).

Table 4.1: Quantification of IAA produced by strain 139SI

IAA production with tryptophan (µg/ml)	IAA production without tryptophan (µg/ml)
36.5 ± 1.2	18.5 ± 0.4

The experiments were performed in triplicate and individual values are depicted as mean ± standard deviation.

4.1.2 Evaluation of Phosphate Solubilizing Ability of Strain 139SI

Phosphate solubilizing bacteria can solubilize insoluble P in the soil into soluble form by producing organic acids. This process increases the availability of P that can be absorbed by plant roots. Calcium phosphate present in the growth medium is water-insoluble and used to determine the ability of the strain 139SI to turn the insoluble P to a soluble form. The 139SI strain was observed able to grow well on NBRIP agar medium and developed halo zone, a clear indication that this strain is active in phosphate solubilization process (Figure 4.1). The ratio of halo zone diameter to colony diameter of 139SI on NBRIP is 1.6 ± 0.03.

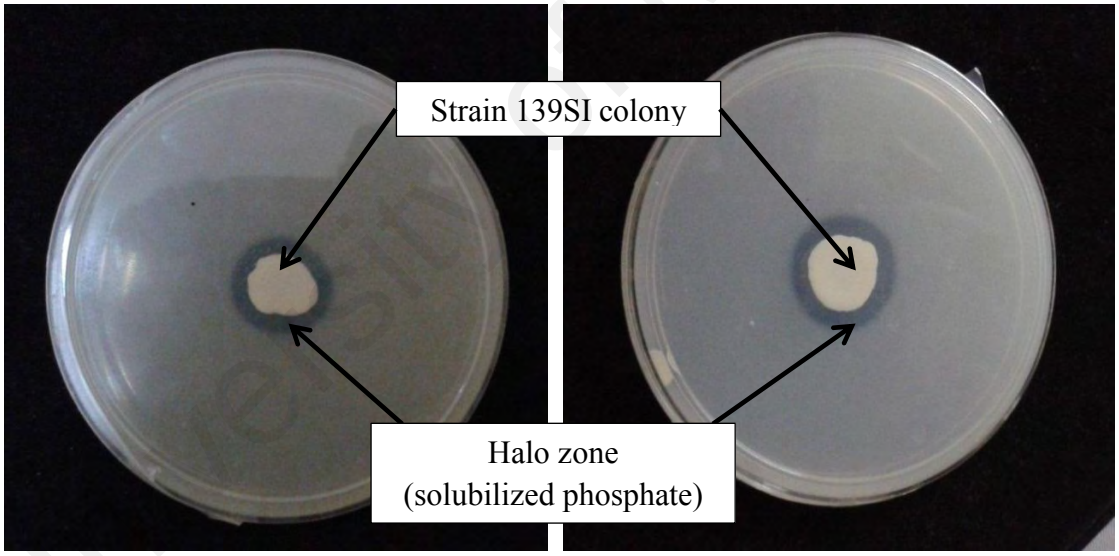


Figure 4.1: The clear zone around the colony is result of solubilization of phosphate on Pikovskaya's agar medium by *B.salmalaya* strain 139SI.

4.1.3 Siderophore Production

Some bacteria are also able to secret a high-affinity iron-chelating compounds called siderophore to facilitate the process of ferric iron uptake in the environment. Plants are able to utilize iron-loaded siderophores produced by the microbial in the rhizosphere, which they themselves might not synthesize. This process is necessary for the plants to obtain the environmental iron needed for essential processes. The universal CAS agar plate assay developed by Schwyn and Neiland (1978) is using chrome azurol S (CAS) and hexadecyltrimethylammonium bromide (HDTMA) as indicators. The CAS - HDTMA complexes bind tightly with ferric iron to produce a blue color. When a strong iron chelator such as a siderophore removes iron from the dye complex, the color changes from blue to orange. The result of this study shows that inoculation of 139SI strain resulting in color changes of CAS reagent from blue to orange, a positive result for siderophore production (Figure 4.2).

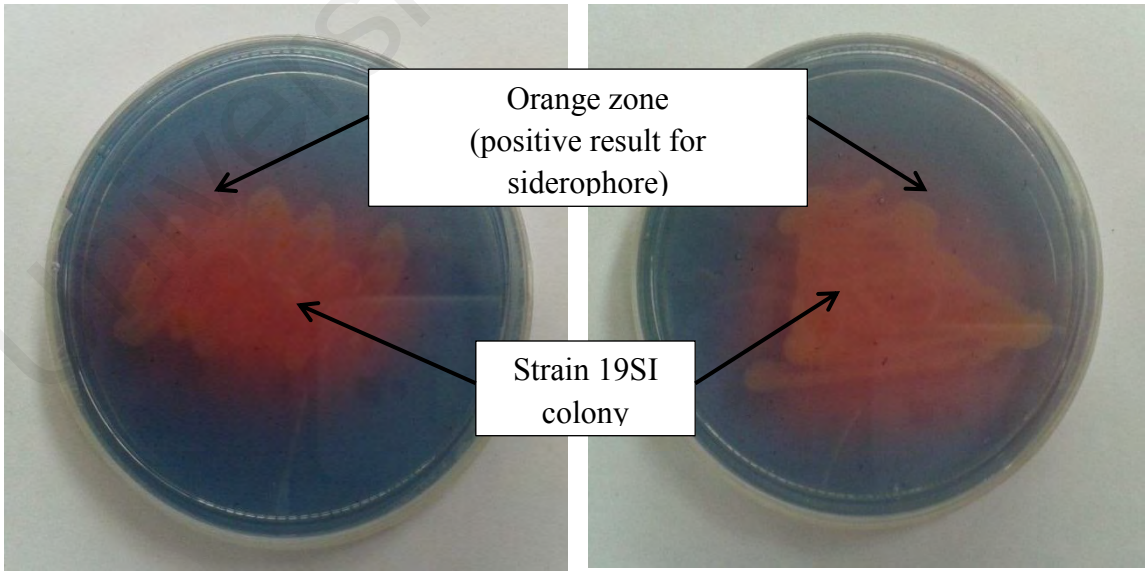


Figure 4.2: Orange halo around the colony of *B. salmalaya* 139SI indicating the ability of this strain to excrete siderophores

4.1.4 N_2 Fixation

The ability of strain 139SI to fix the atmospheric nitrogen was qualitatively evaluated by the growth of the strain in semi-solid NFb media. Result from the study revealed that the strain 139SI was able to grow in semi-solid NFb media after 3 days of incubation at 30°C. The strain 139SI growth was evidenced by the formation of a veil-like pellicle near the surface of the semi-solid NFb media (Figure 4.3). The strain 139SI from semi-solid NFb media was re-inoculated into a fresh semi-solid NFb media with the aim of confirming the presence of the growth pellicle. After 3 days of incubation at 30°C, growth pellicle was emerged near the surface of the media, confirming the ability of the strain to grow in the media.

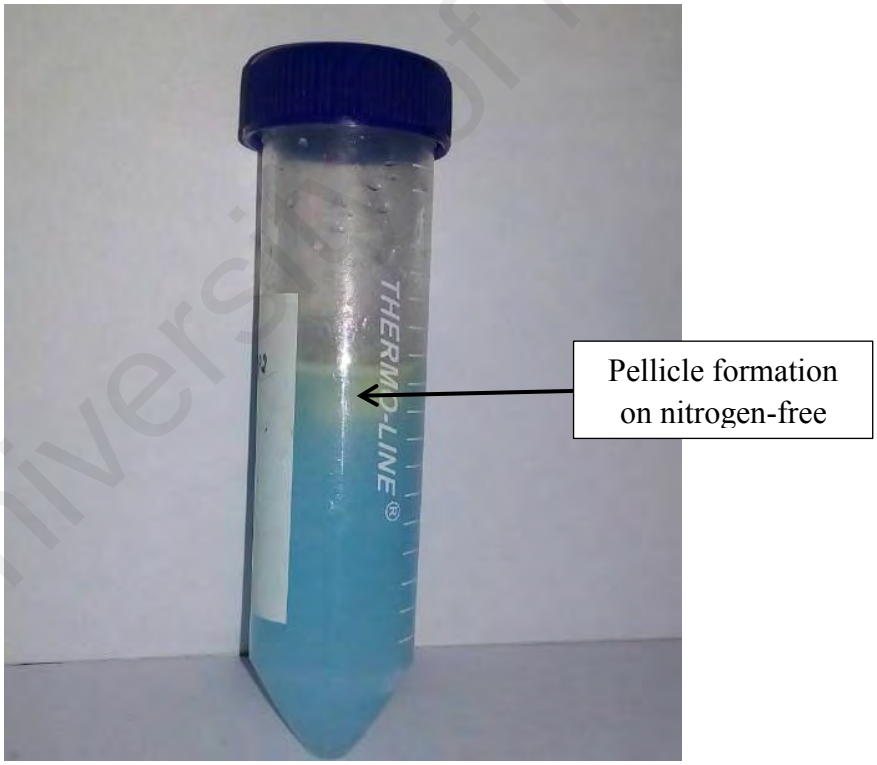


Figure 4.3: Formation of growth pellicle on semi-solid NFb media confirming the ability of the strain to grow in the nitrogen free media.

4.1.5 Growth Promotion of Oil Palm Seedling by *B. salmalaya* Strain 139SI

4.1.5.1 Vegetative Growth Measurement

a) Height and Diameter of Stem

The effects of strain 139SI inoculation on plant growth performance were also evaluated. In the presence of strain 139SI inoculation, the vegetative growth of oil palm seedling was higher compared to uninoculated palm. Figure 4.4a and 4.4b summarizes the trend for height increment of oil palm seedling inoculated with 139SI. The inoculation of 139SI on the seedling clearly gives significant effects on height increment as early as one week after inoculation. The height of stem in inoculated group recorded markedly increases by 55.4% higher than control at the end of the experiment. Plant growth promoting effects of 139SI on the seedling also can be seen on the stem diameter size. After 3 weeks, seedling inoculated with 139SI produced stem 33.3% larger than those in the control group. The differences in stem height and diameter between inoculated and uninoculated are statistically significant.

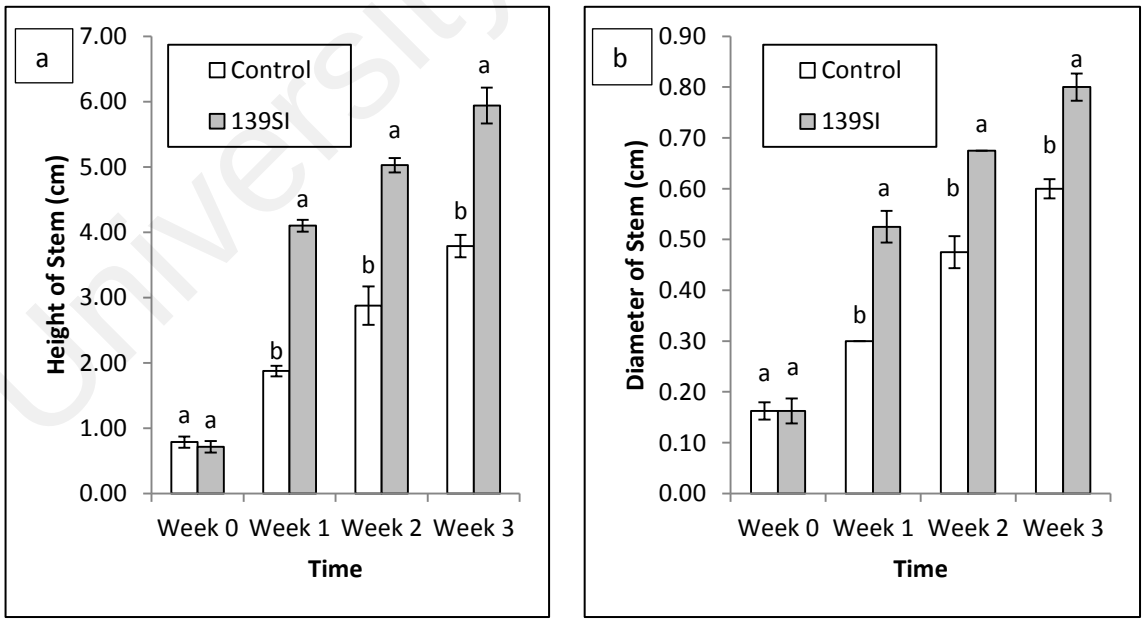


Figure 4.4: Effects of inoculation with strain 139SI on (a) height and (b) diameter of stem of oil palm seedlings

b) Root Development

Observation on the characteristics of the root was found that the seedling in inoculated and non-inoculated groups had one primary root and smaller lateral root branching horizontally from the primary root. The length of the primary root in each group was measured weekly and it was found that the 139SI-inoculated seedling produced significantly longer primary root with comparison to the non-inoculated ones (Figure 4.5a). The positive effects of 139SI inoculation on stimulating root elongation can be seen as early as the first week after inoculation. The other important characteristic that has been observed was 139SI-inoculated seedling also produced an increased number of lateral roots compared to the control (Figure 4.5b). The average number of the lateral root increase with time. Similarly, the number of lateral root was also significantly higher as early as the first week after inoculation.

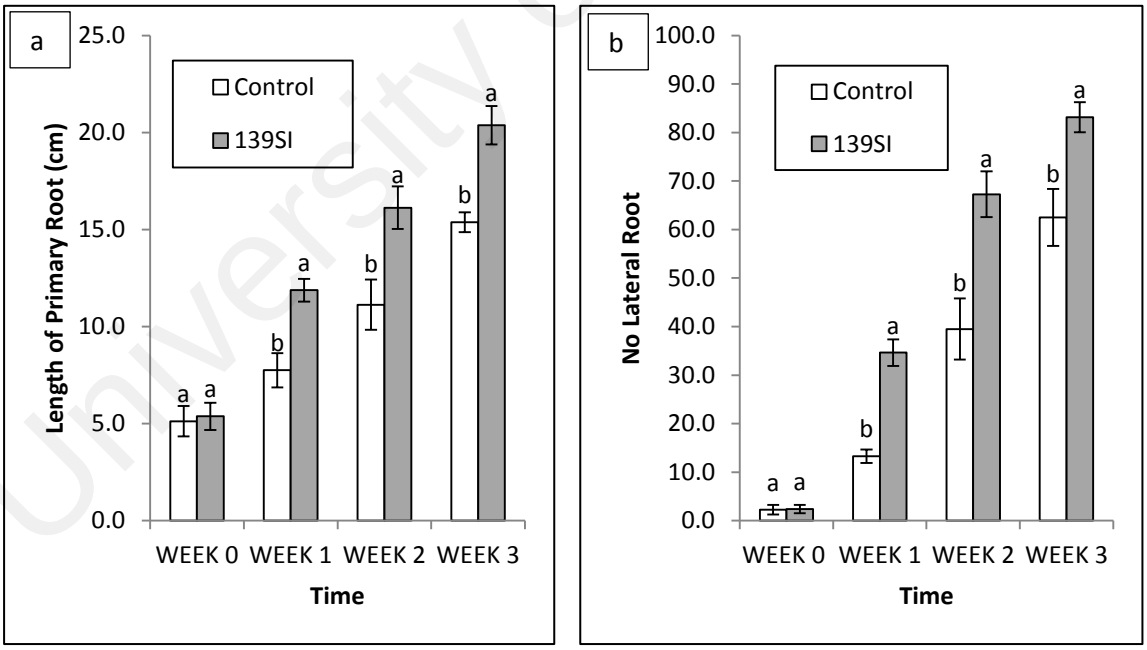


Figure 4.5: Effects of strain 139SI inoculation on root development. (a) Effect of strain inoculation on length of primary root and (b) effect of strain inoculation on the number of lateral root produced by the seedling.

c) Stem and Root Dry Weight

As expected, the significant increment on the height and diameter of stem give direct effects on dry weight reading. As shown in Figure 4.6, the stem and root of strain 139SI inoculated oil palm seedling were heavier than the control on first, second and third week after inoculation. Minor effects of strain 139SI inoculation on the stem and root dry weight were observed at the first week after treatment. Oil palm seedlings inoculated with strain 139SI start to show a greater effect for palm dry weight at the second and third week after treatment. The stem dry weight increases substantially by 31.58 % at the second and further increase to 66.67 % at the third week after treatment.

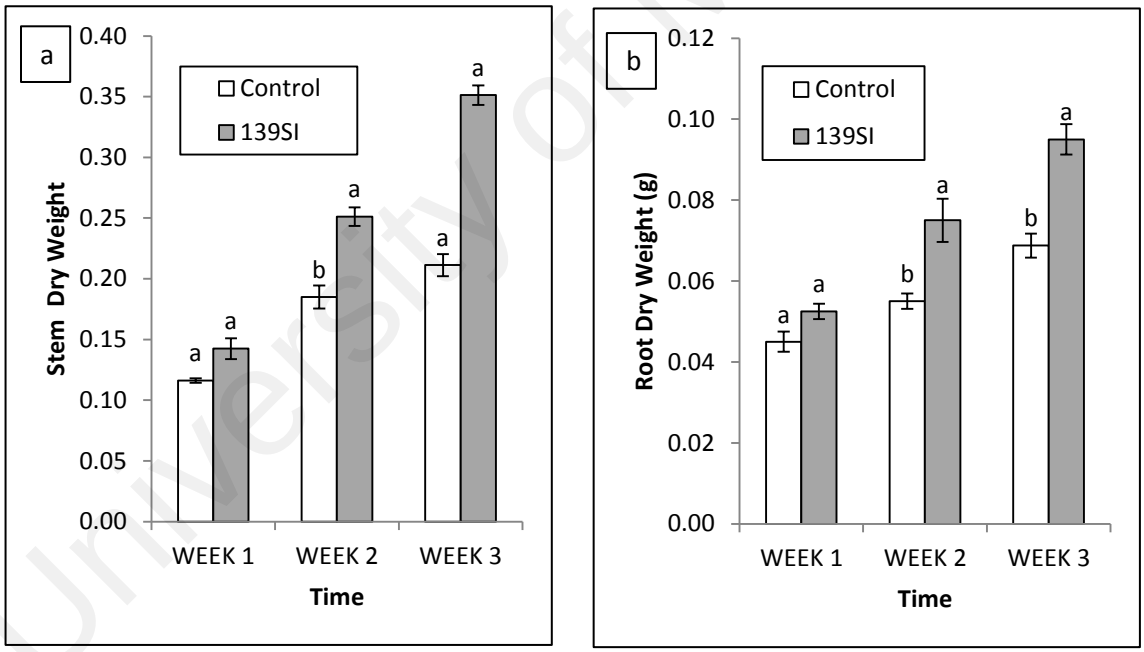


Figure 4.6: Effects of strain 139SI inoculant on oil palm seedling growth. (a) Effect of strain 139SI inoculant on stem dry weight and (b) root dry weight of inoculated and uninoculated oil palm seedling

Effects of strain 139SI inoculant on the oil palm seedling growth after one week of treatment can be observed in the Figure 4.7 below. Oil palm seedling inoculated with strain 139SI showed growth advancement compared to uninoculated control after one week of treatment. The inoculated seedling recorded higher plumule growth and produce longer main root.

Figure 4.7: Growth of oil palm seedling after one week inoculated with strain 139SI (a and b) and uninoculated control (c and d).

Oil palm seedlings growths at two week after treatment were showed in the Figure 4.8. Inoculation of strain 139SI successfully increased palm growth rate. At this stage, inoculated seedling produced a fully developed leaf and have more lateral root compared to the uninoculated control.

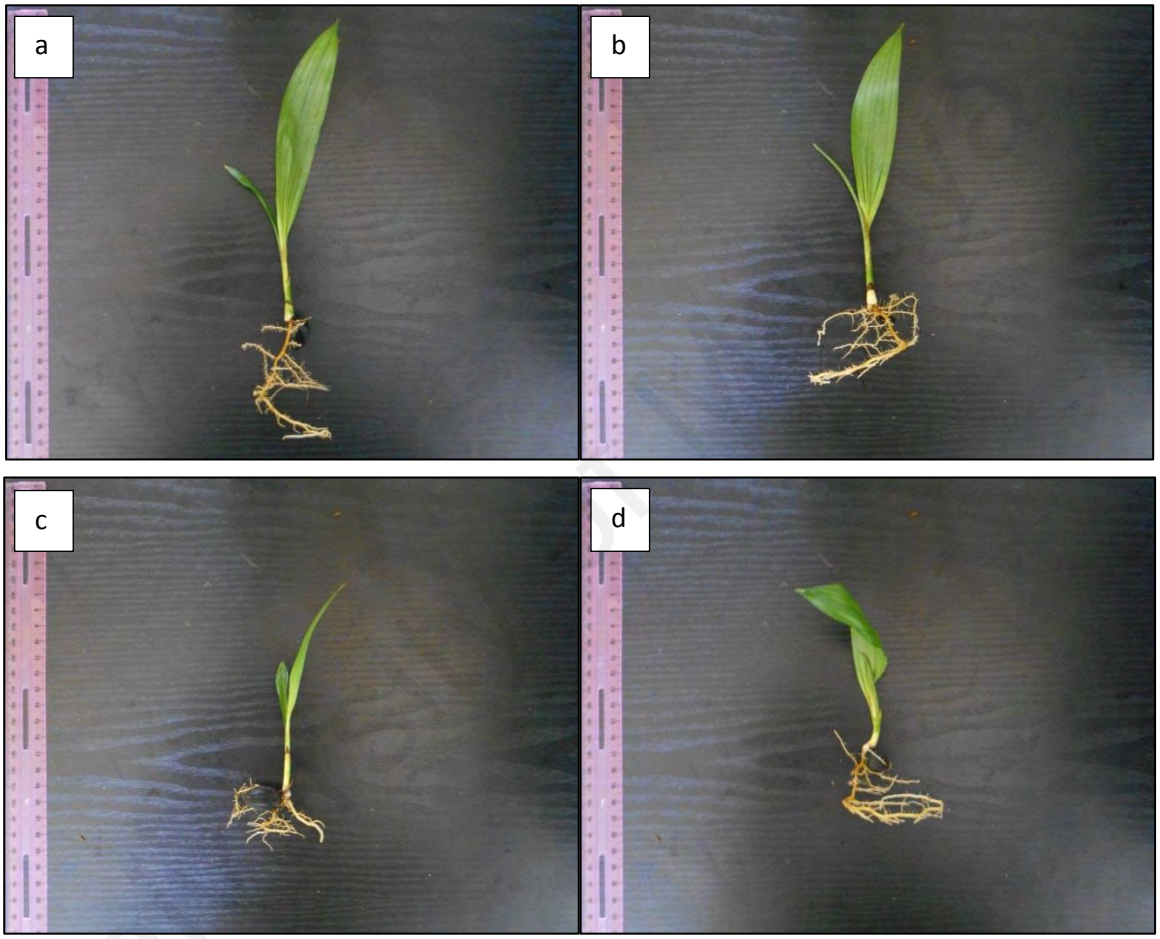


Figure 4.8: Effects of strain 139SI inoculation on oil palm seedling growth (a and b) compared to the uninoculated control (c and d) after 2 weeks of treatment.

Growth performance of oil palm seedling at third week after inoculation can be observed in the Figure 4.9. The growth rate of 139SI-inoculated seedling generally performed better than the uninoculated control. Growth parameters such as plant height, diameter size of stem and root development enhanced significantly in inoculated palm compared to the untreated control

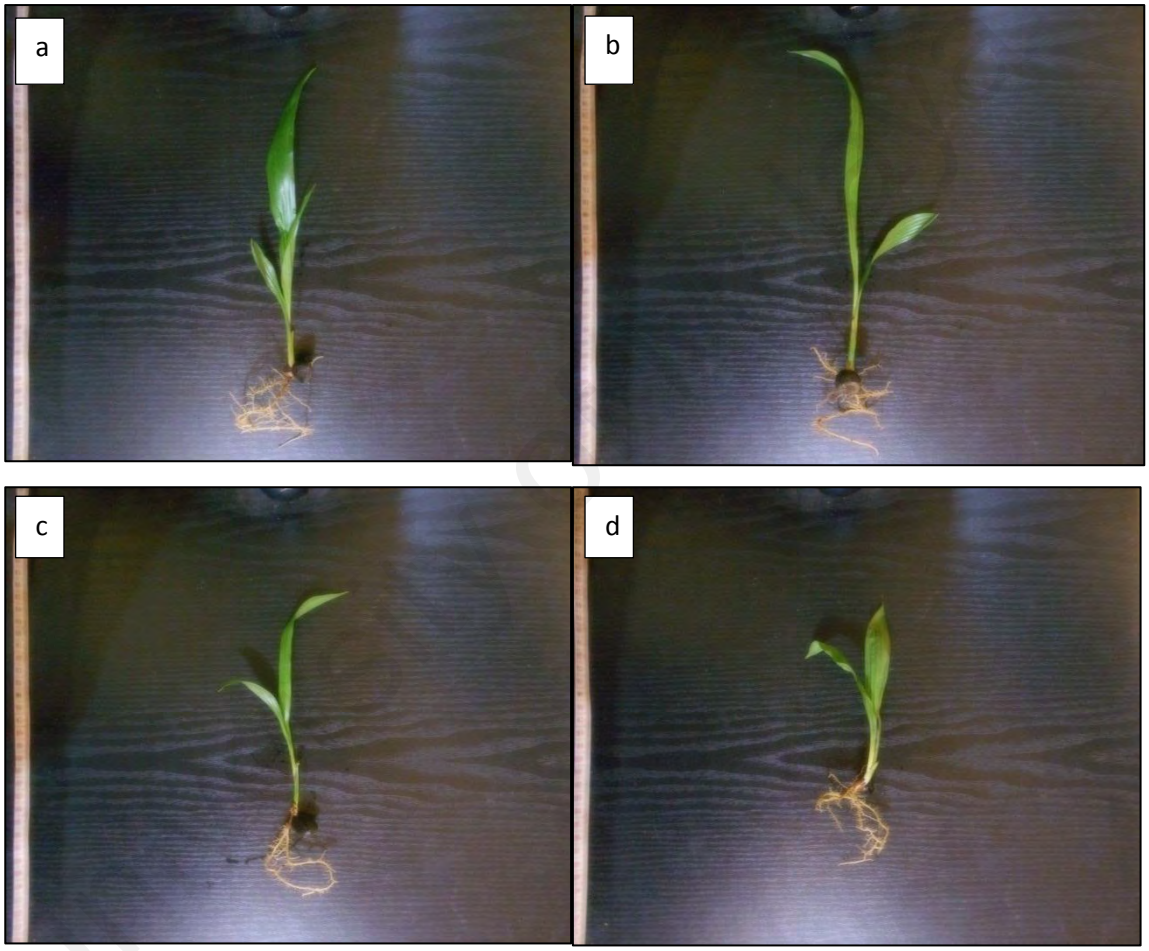


Figure 4.9: Comparison of oil palm seedling treated with strain 139SI inoculant (a and b) with the uninoculated control (c and d) after three weeks of treatment.

4.1.5.2 Bacterial Colonizing Ability

The ability of 139SI to colonize the surface of plant root was evidenced by SEM result as exhibited in Figure 4.10. The SEM examination of the surface of plant root has detected the presence of 139SI bacterial cells on plant root samples taken on first, second and third week after inoculation. The SEM analysis on plant root samples also revealed that the strain attached to the plant root surface by forming biofilm.

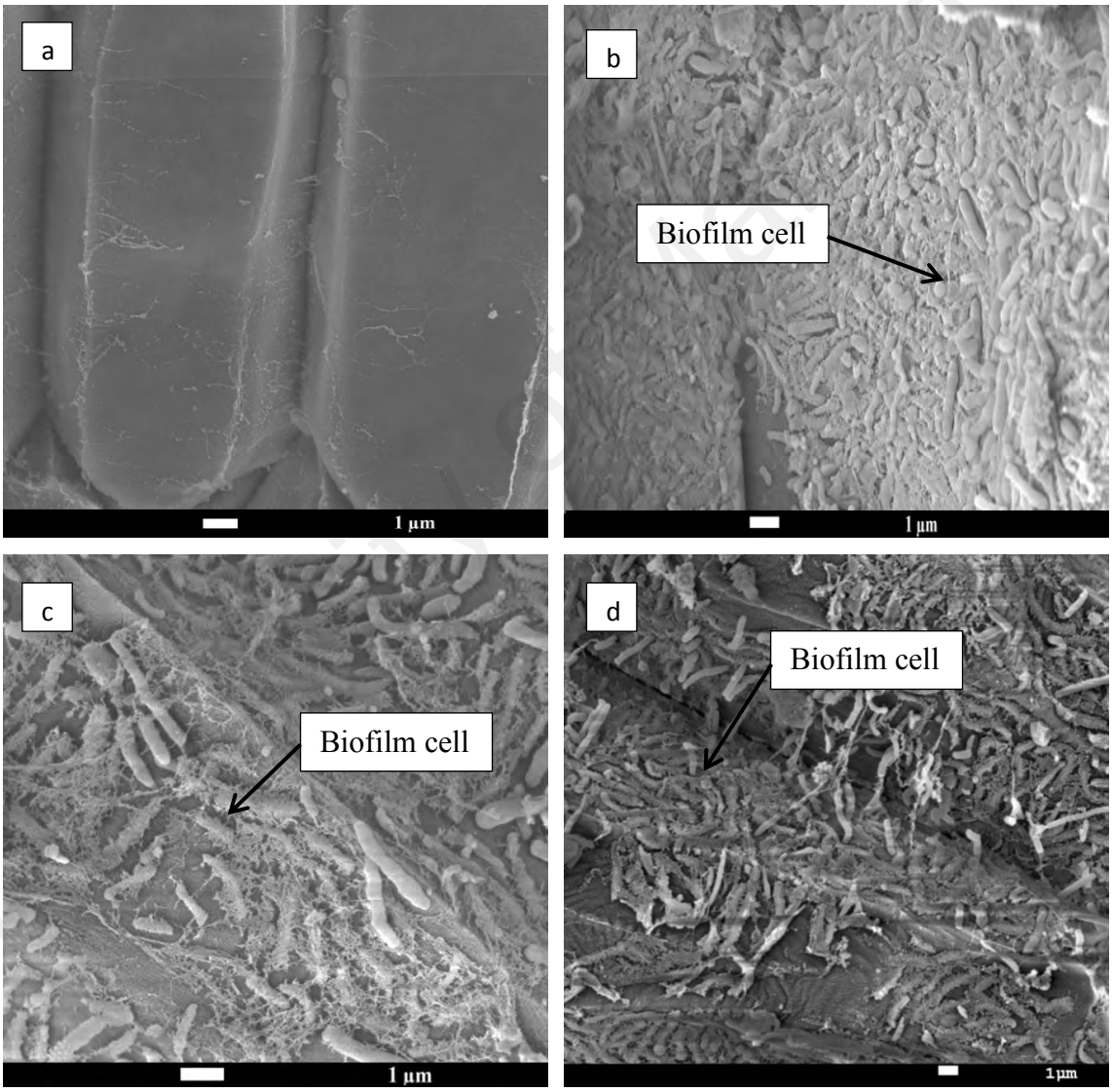


Figure 4.10: Scanning electron microscopy images of primary root colonized with strain 139SI. (a) is image of uninoculated group, (b) is SEM image of root a week after inoculation, (c) is SEM image of root two week after inoculation and (d) is SEM image of root three week after inoculation.

4.1.5.3 Strain 139SI Population Densities

The strain 139SI population densities in the rhizosphere soil, root surface and in the internal root tissues are shown in the Figure 4.11. The data obtained from the experiment clearly shows that the 139SI enable to colonize not only the surface of plant root, but it can also penetrate and lives in plant tissue. Significant differences were found between the CFU number of the strain in rhizosphere soil, root surface and in the internal root tissues. Same pattern of strain 139SI population distribution in these three different parts was observed from first week to third week after inoculation. Overall, the 139SI population densities were highest in the rhizosphere, followed on the root surface and small population was found in the internal root tissues. The results from this experiment also show that the 139SI population decrease over the time. A week after inoculation, population of strain 139SI in rhizosphere soil was found at 7.51 log CFU/g fresh weight. The population significantly decreases by 8.5% (6.87 log CFU/g fresh weight) on the second week and finally decreases about 10.0% to 6.18 log CFU/g fresh weight on third week. Meanwhile, the 139SI population on root surface was 5.23 log CFU/g fresh weight on first week before it was slightly decreased by 3.1% (5.07 log CFU/g fresh weight) and continue to significantly decrease at 4.06 log CFU/g fresh weight (19.9%). Among the rhizosphere part, population of 139SI strain in the internal root tissues was more stable. The 139SI populations in this part was recorded 2.84 log CFU/g fresh weight on first week and later decrease slightly about 7.7% to 2.53 log CFU/g fresh weight after 2 weeks of inoculation. Similarly, the population recorded a slight decrease on third week to 2.36 53 log CFU/g fresh weight (6.7%).

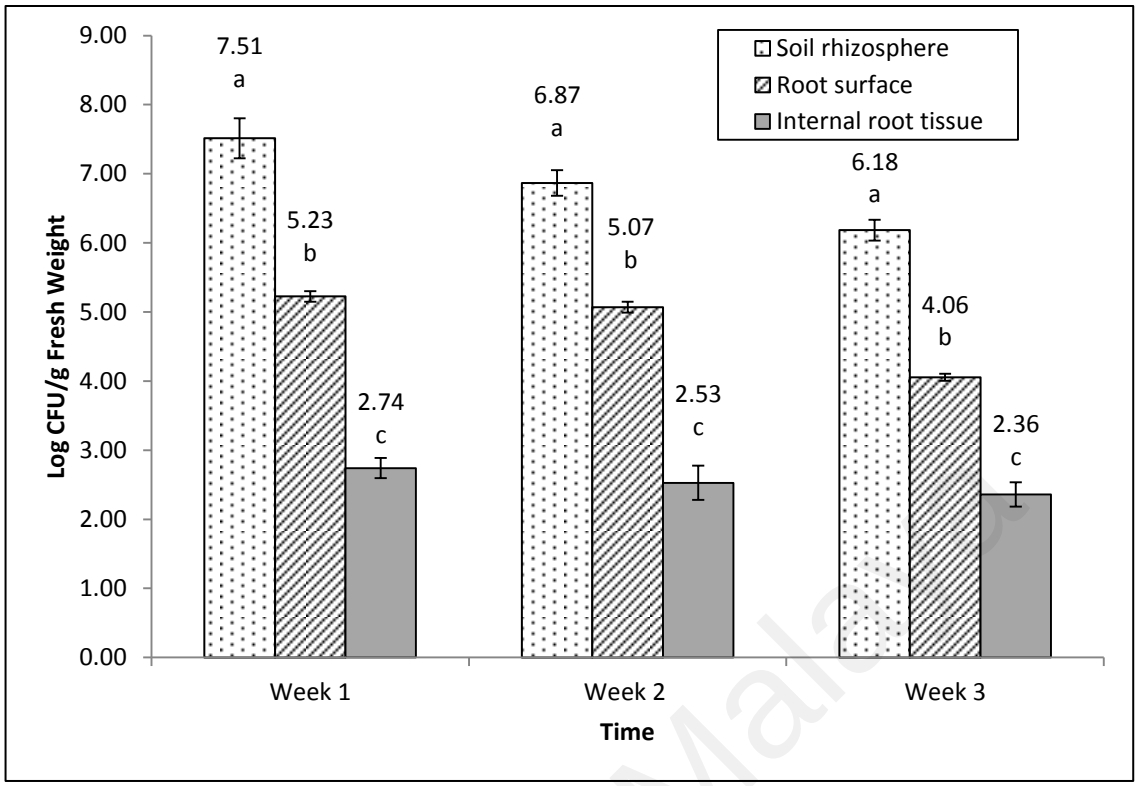


Figure 4.11: Bacterial population densities in the rhizosphere, root surface and in the internal root tissues. The individual values are depicted as mean ± standard deviation. Different letters indicate significant differences between treatments according to Tukey test ($p<0.05$).

4.1.5.4 Interaction between 139SI Population in Rhizosphere Soil and Plant Growth

Correlation analysis was carried out to establish relationship between strain 139SI populations with the growth of oil palm seedlings (Figure 4.12). In this study, the population of 139SI in rhizosphere soil was found to be positively correlated with palm growth. Significant linear correlations were found when an increase in strain 139SI population size in the rhizosphere was analyzed against stem height ($r = 0.679$) and diameter ($r = 0.739$). Similarly, a significant positive correlation also was observed on root length, amount of lateral root produced and total plant dry weight with r value of 0.900, 0.886 and 0.568 respectively.

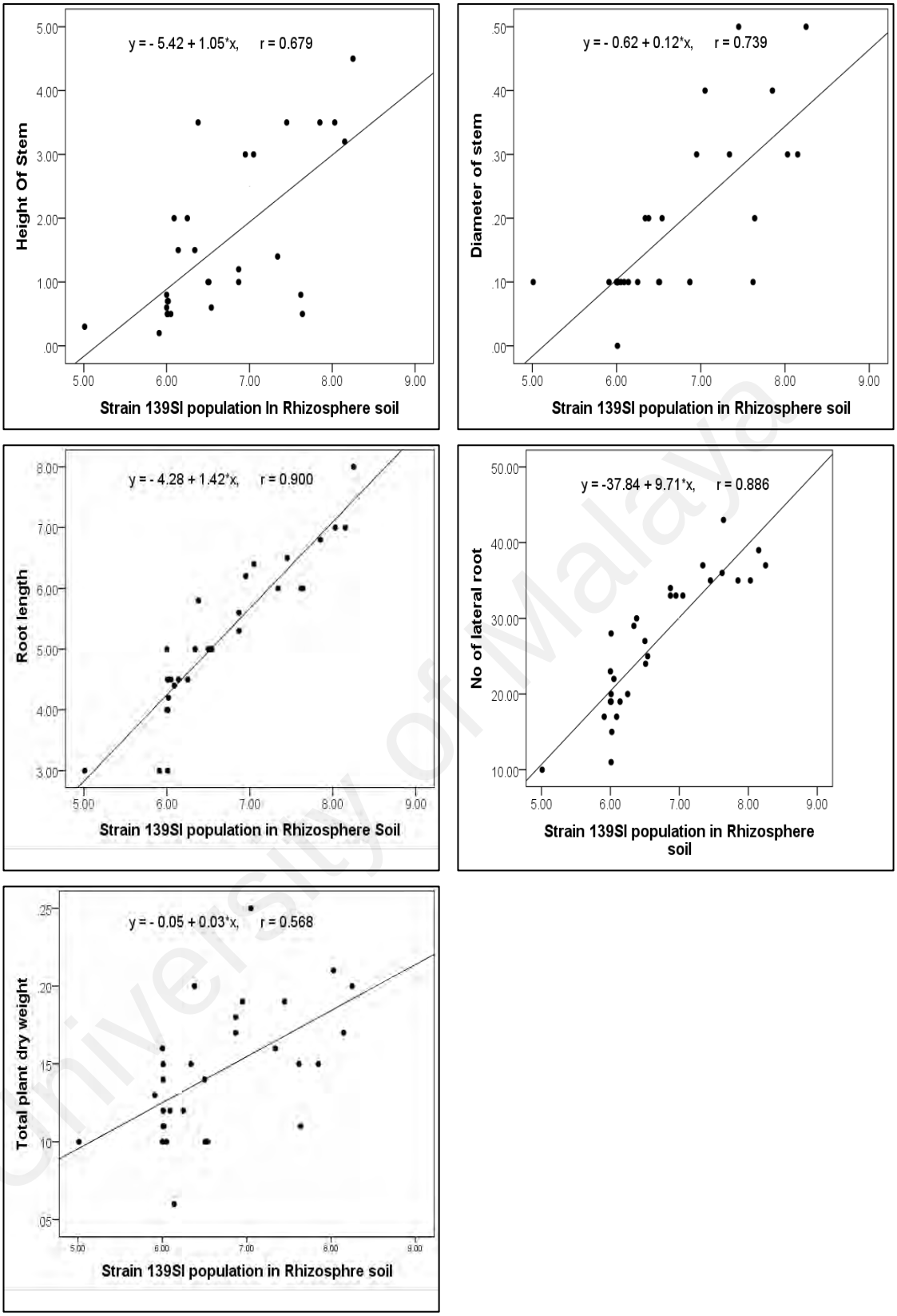


Figure 4.12: Relationships between strain 139SI populations in rhizosphere soil with the height of oil palm seedling stem, diameter of the stem, root length number of lateral root and total of palm dry weight.

4.1.5.5 Interaction between 139SI Population on Root Surface and Plant Growth

In the present study, strain 139SI was found actively colonize palm root. The strain found attached to the surface of palm root via forming biofilm. Correlation analyses revealed a high positive correlation between strain populations on the root surface with palm growth parameters (Figure 4.13). Among assessed parameters, correlation analysis between strain 139SI populations with the length of the root was resulted highest significant r value (0.844), indicating palm root was the most affected part. A significant and positive correlation was also observed on the height of the stem (r = 0.689), the diameter of the stem (r = 0.729) and the number of lateral root (r = 0.844)

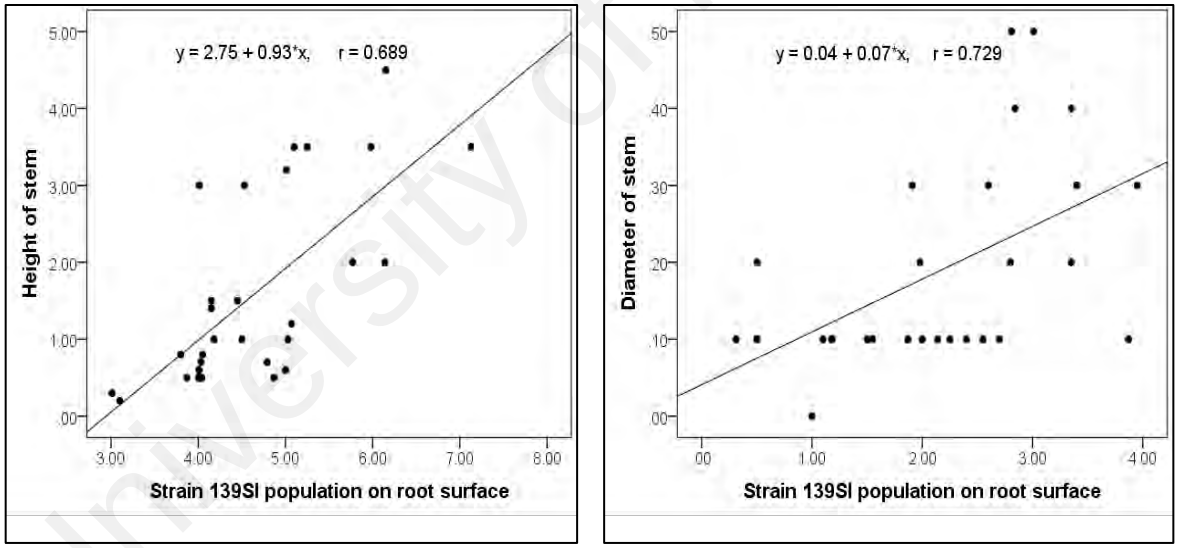
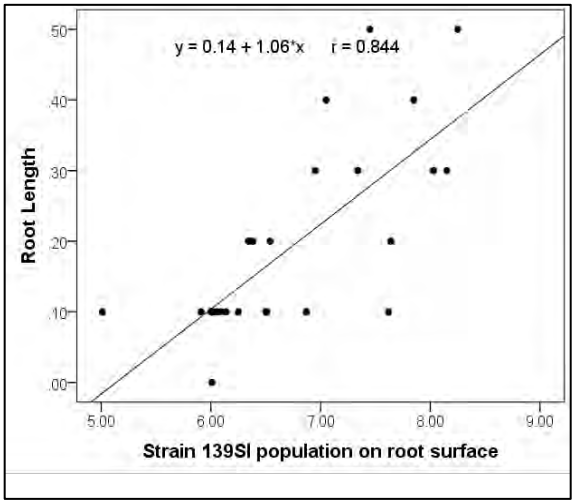
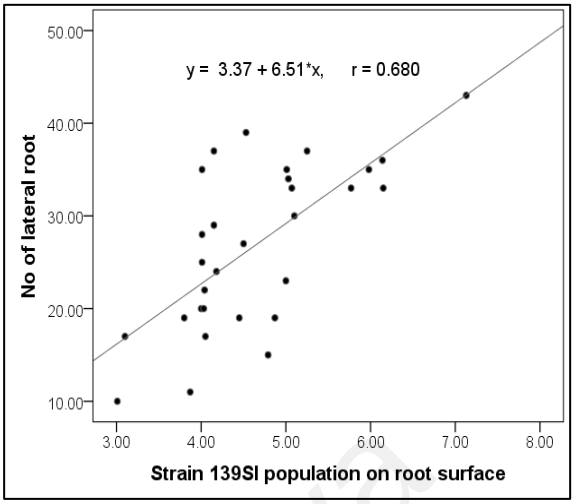


Figure 4.13: Relationships between strain 139SI populations on root surface with the height of oil palm seedling stem, diameter of the stem, root length number of lateral root and total of palm dry weight.





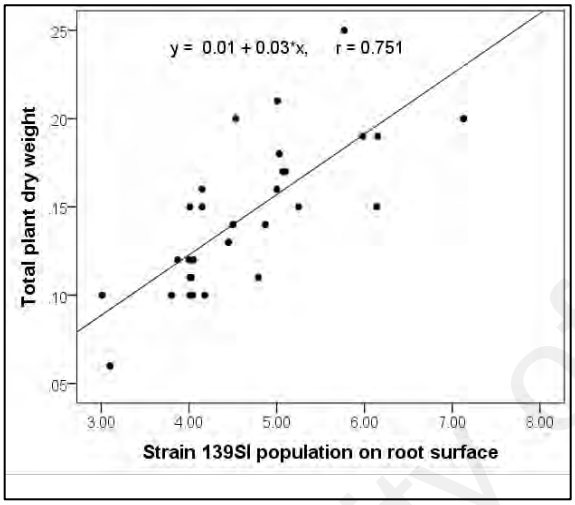


Figure 4.13, continued.

4.1.5.6 Interaction between 139SI Population in the Internal Root Tissue and Plant Growth

Based on correlation analysis, a strong relationship between the amount of strain 139SI present in the internal root tissue growth and the length of main root was observed in a significant and positive linear relationship as indicated by the high values of correlation coefficients at 0.851 (Figure 4.14). There was also a significant positive linear relationship between the strain population present in the internal root tissue with the height of the stem, the diameter of the stem and the number of lateral root.

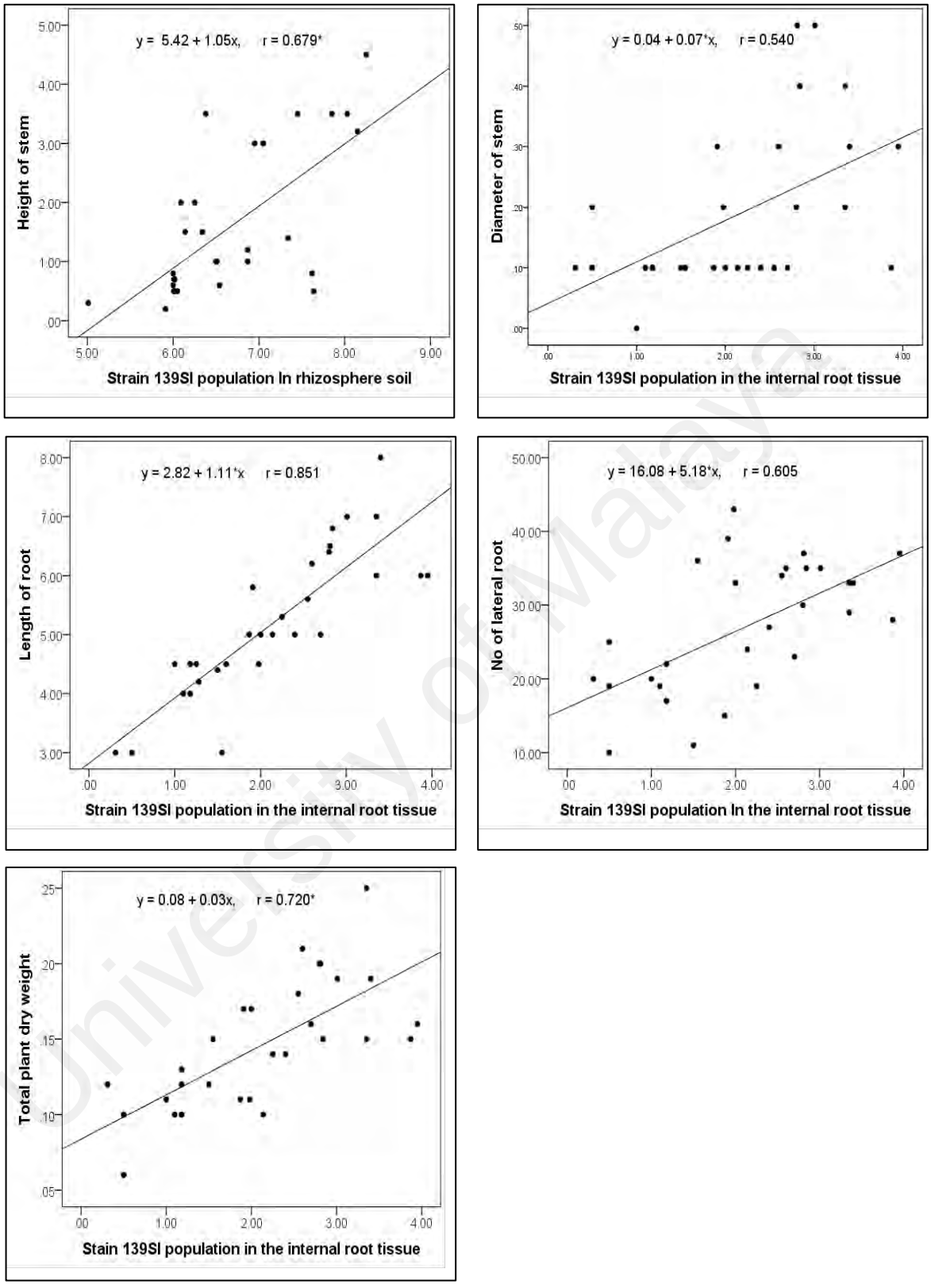


Figure 4.14: Relationships between strain 139SI populations in the internal root tissue with the height of oil palm seedling stem, diameter of the stem, root length number of lateral root and total of palm dry weight.

4.2 Synergistic Effects of *B. salmalaya* Strain 139SI Inoculation with Inorganic Fertilizer on Soil Fertility and Oil Palm Seedling Growth

4.2.1 Plant Growth

4.2.1.1 Plant Height

Vegetative growth of oil palm seedlings received different treatment were evaluated at the end of the experiment. After 4 months of treatment, growth of palms in untreated control C1 was stunted (Figure 4.15). While palms in the remaining group of treatment produced positive growth performance. Figure 4.16 illustrated the effects of 139SI inoculation with and without fertilizer on the height of stem of oil palm seedling. The effect of 139SI inoculation on the oil palm seedling growth can be seen profoundly after two months of treatment and onwards. The 139SI-inoculated seedling (T1) showed a greater growth performance than those in untreated control (C2). The results also found that, combination of 139SI + fertilizer (T2) significantly outperformed the growth increment in other treatment. After 4 months of treatment, oil palm seedling in T2 recorded statistically significant increment of stem height by 118.3%, 14.1% and 29.1% higher compared to those in C1, C2 and T1 respectively.

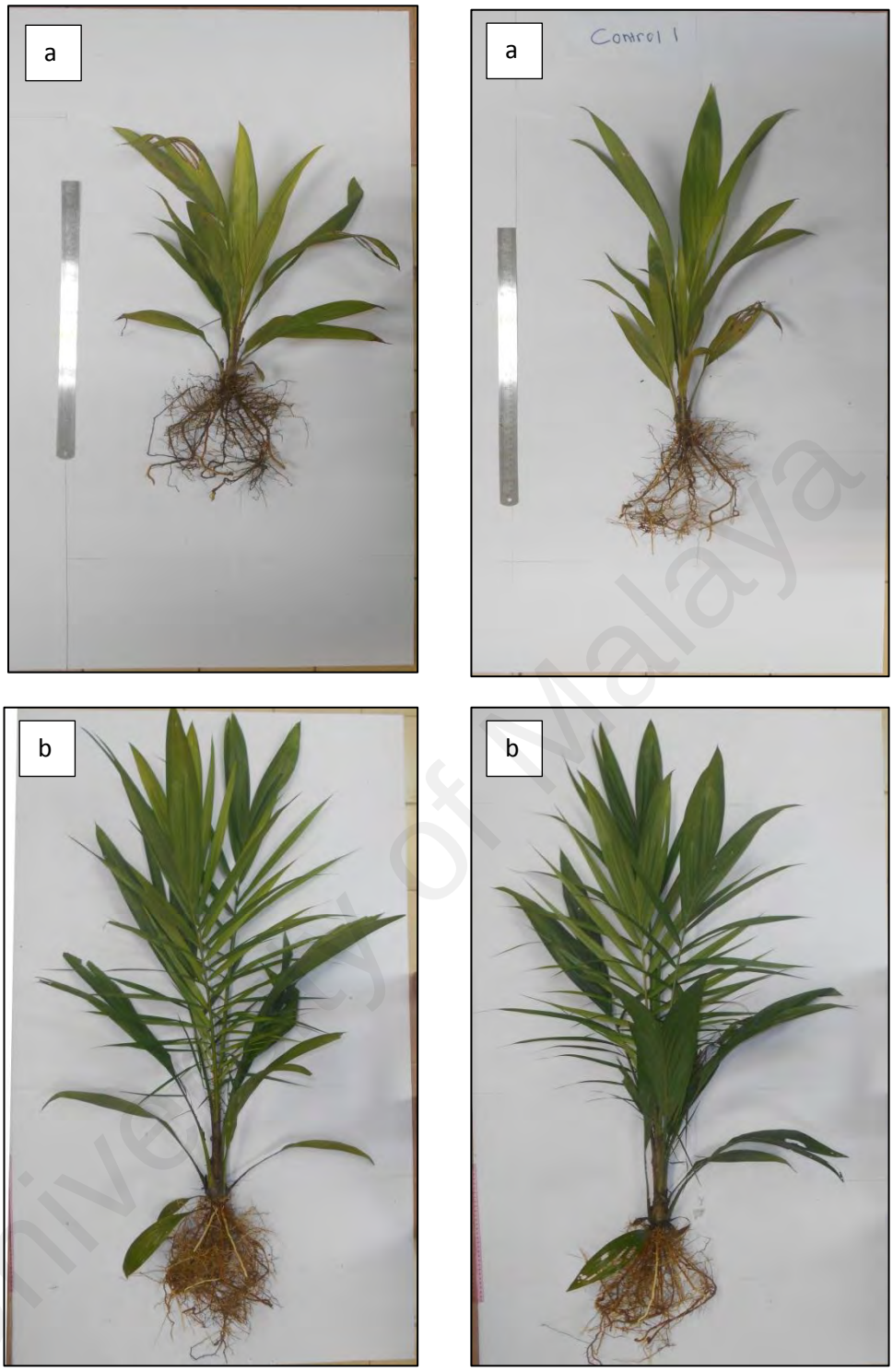


Figure 4.15: Oil palm seedling in a) untreated control (C1), b) fertilized control (C2), c) 139SI inoculation (T1) and d) 139SI inoculation + fertilizer (T2) treated group. The vegetative growth of palms in untreated control were stunted. On the other hand palm in C2, T1 and T2 showed good development of root and leaves.

Figure 4.15, continued.

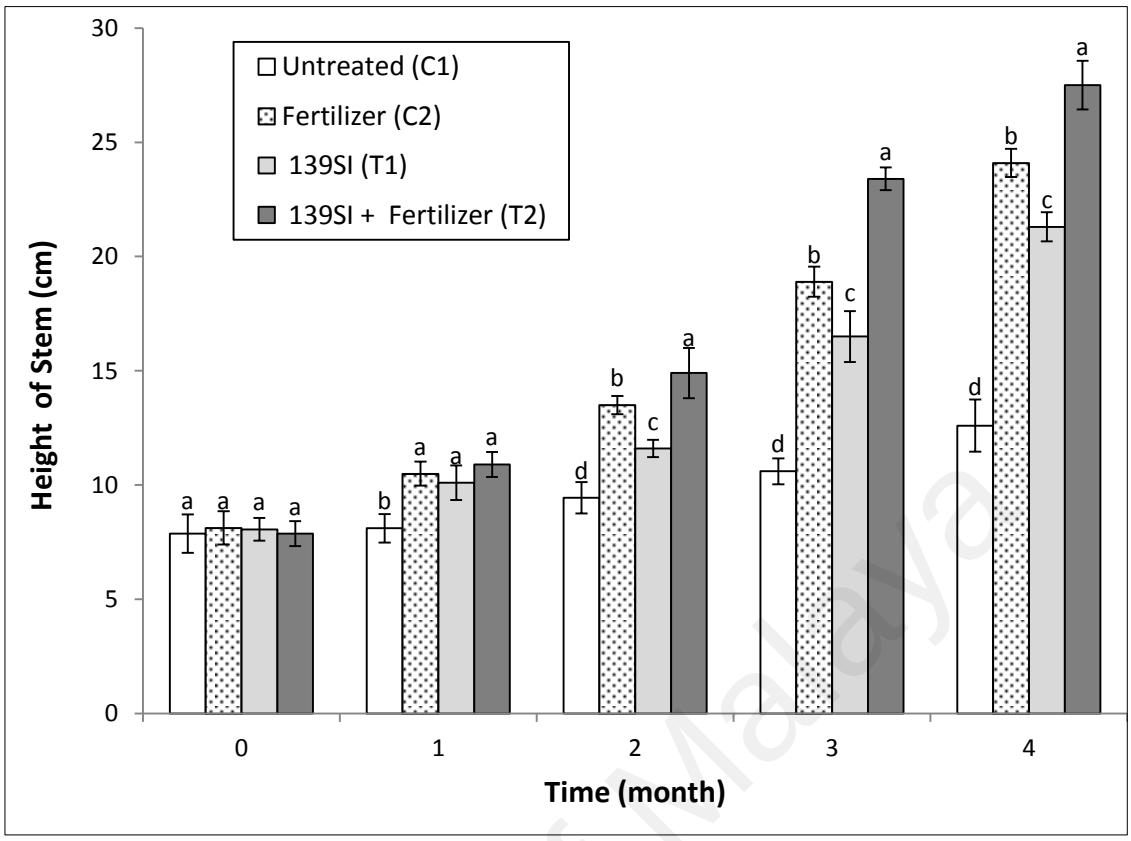


Figure 4.16: Effects of strain 139SI inoculation on oil palm seedling stem height over four months of treatment. Different letters indicate significant differences between treatments according to the Tukey test ($p<0.05$). At the end of the experiment, palm received different treatment produced significantly different height of the stem.

4.2.1.2 Diameter of Stem

The growth enhancement feature of 139SI also was depicted by the diameter increment of the stem diameter (Figure 4.17). Inoculation of strain 139SI successfully enhances the stem diameter in T1 compared to untreated C1 by 88.48%. However, the enhancement of the stem diameter in T1 was smaller compared to the fertilized control C2. The oil palm seedlings received 139SI inoculation + inorganic fertilizer (T4) produced the biggest stem diameter compared to others. The enhancement was observed more noticeable after three months of treatment. The enhancement of stem diameter in T4 significantly outperforms to those in C1, C2, and T1 by 136, 14.7, and 25.4% respectively.

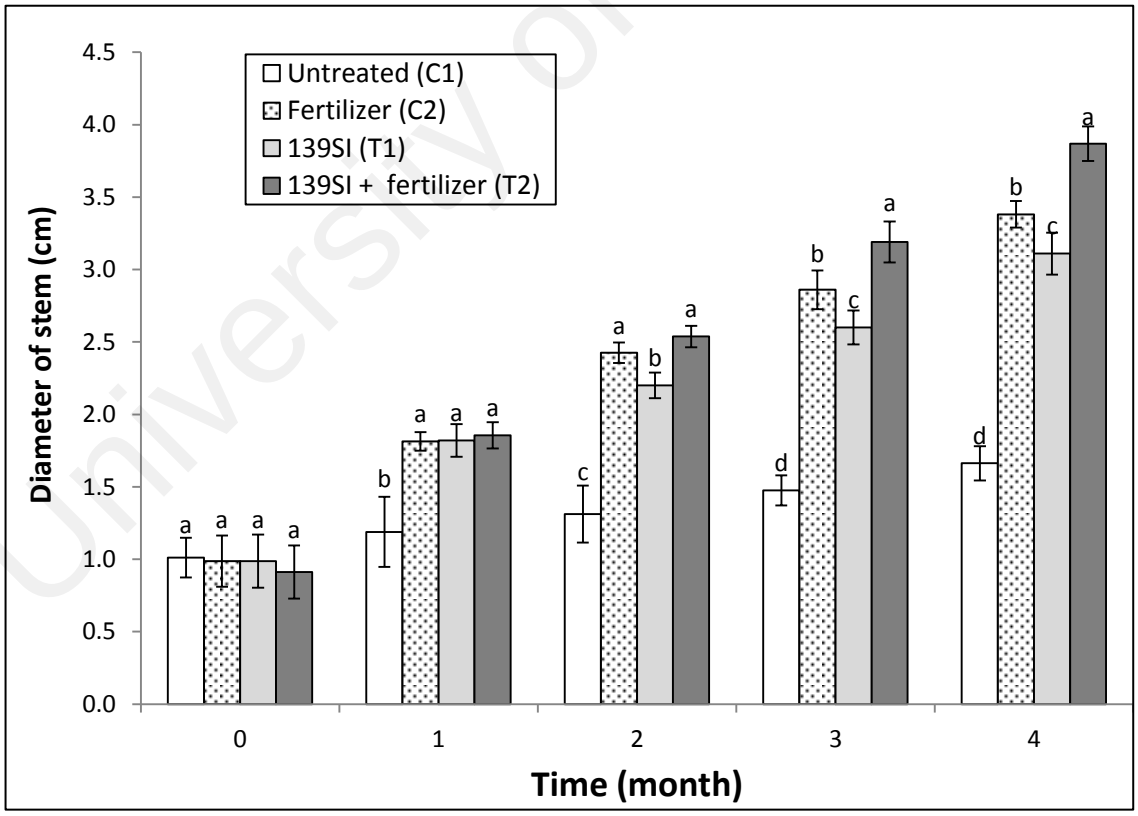


Figure 4.17: Effects of strain 139SI inoculation on oil palm seedling stem diameter over four months of treatment. Different letters indicate significant differences between treatments according to the Tukey test ($p<0.05$).

4.2.1.3 Plant Dry Weight

The results of shoot and root dry weight are presented in Table 4.2. As expected based on observations on the stem height and diameter results, shoot and root dry weight of seedling that received 139SI inoculant (T1 and T2) performed better than unfertilized control group (C1). Overall, T2 recorded the highest shoot dry weight among the group of treatment, followed by C2> T1> C1. While for root dry weight, the results display more interesting finding. At the end of the study, mean of root dry weight for seedlings of T1 were found heavier than those in C1 and C2 by 215.04% and 11.22% respectively. A higher of root dry weight recorded in T1 was contributed by a longer main root and more fibrous root produced compared to C2. A greater performance of root dry weight was resulted by seedling inoculated with 139SI and received fertilizer at the same time (T2), in which, the root dry weight recorded 285.70% and 36.17% increment compared with the corresponding untreated (C1) and fertilized (C2) control. Results from the present study also revealed that integrated inoculation of strain 139SI with fertilizer significantly increased plant total biomass over the plant received fertilizer alone.

Table 4.2: Effects of strain 139SI inoculation on plant dry weight

Group	Growth parameter	
	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})
Untreated (C1)	25.49 ± 6.40 d	8.18 ± 2.10 c
Fertilizer (C2)	92.57 ± 4.60 b	23.17 ± 4.20 b
139SI (T1)	78.70 ± 7.00 c	25.77 ± 2.00 b
139SI + fertilizer (T2)	111.50 ± 19.84 a	31.55 ± 3.80 a

The individual values are depicted as mean ± standard deviation. Different letters indicate significant differences between treatments according to Tukey test ($p<0.05$).

4.2.1.4 Number of Leaves Produced and Total Leaf Area

At the beginning of the study, the seedling in all treatment groups possesses five bifurcated leaves per palm. As the seedling grows, the palm produces at average one new leaf each month. Fully pinnate leaf was starting to appear at seven months old seedlings. However, the development of the leaf was much slower in C1 (Figure 4.18). The leaves in C1 remained in the bifurcated form with mild chlorotic symptom. In general, palm in C1 produces smaller size and fewer numbers of leaves. The oil palm seedling in C2, T1, and T2 produces an average of 10 leaves per plant (Table 4.3). As a comparison, the untreated oil palm seedling only produces an average of eight leaves. The differences in the number of leaves produced by the oil palm seedling gives significant effects to the total leaf area in each group of treatments. The total leaf area value ranged from 635.64 to 1767.69 cm2 with the highest value was recorded in T2 group followed by C2> T1 and C1. Statistical analysis of total leaf area also found that there were no significant differences between oil palm seedlings inoculated with 139SI, 139SI + fertilizer and received fertilizer alone. In other hand, the larger values of total leaf area in these three groups were found to be statistically significant compared to C1.

Table 4.3: Oil palm seedling vegetative growth after 4 months of treatment

Group	Growth parameter	
	No. of leaf	Total leaf area (cm^2)
Untreated (C1)	8.0 ± 0.43 b	635.64 ± 51.31 b
Fertilizer (C2)	10.0 ± 0.33 a	1618.54 ± 54.60 a
139SI (T1)	10.0 ± 0.33 a	1532.20 ± 57.11 a
139SI + fertilizer (T2)	10.0 ± 0.33 a	1767.69 ± 79.48 a

The individual values are depicted as mean ± standard deviation. Different letters indicate significant differences between treatments according to Tukey test ($p<0.05$).

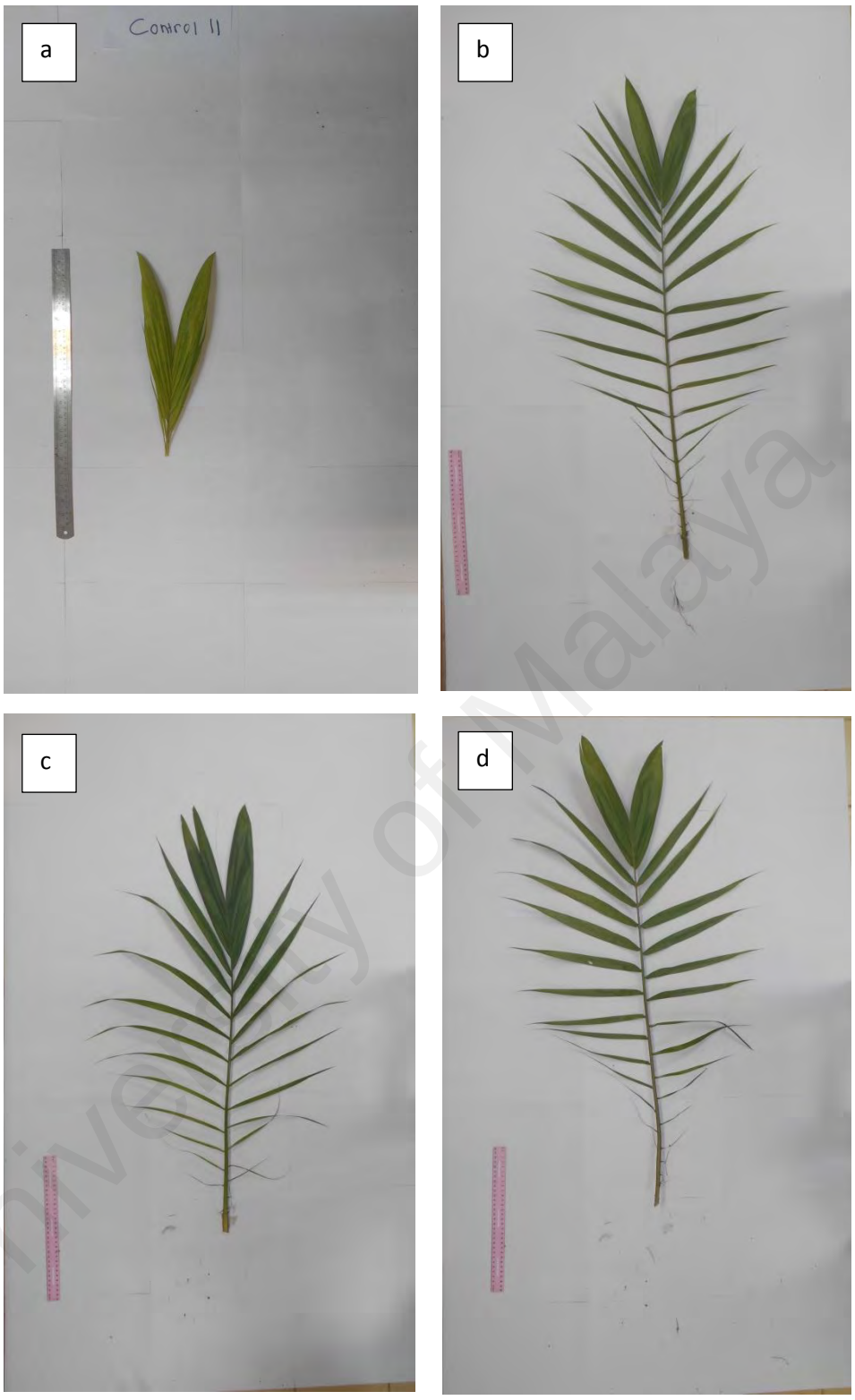


Figure 4.18: Morphology of mature leaf sample of palm seedling after 4 months of treatment. Palm oil seedlings in untreated group remain in bifurcated form with yellowish green color, a symptom of mild chlorosis (a). While palm received inorganic fertilizer (b), inoculated with 139SI (c) and inoculated with 139SI + inorganic fertilizer (d) produce significantly larger pinnate leaves and free from chlorosis symptom.

4.2.2 Chlorophyll Content

Plant chlorophyll is very important to absorb energy from light for photosynthesis process. The chlorophyll content of oil palm seedlings was recorded after four months of treatment and the results are presented in Table 4.4. The chlorosis symptom observed earlier in C1 was validated by the significantly lower chlorophyll content reading compared to the other treatment. The results of the present study were also found that inoculation of strain 139SI and application of inorganic fertilizer significantly effected leaf chlorophyll content. Inoculation of strain 139SI significantly elevates chlorophyll content in T1 by 6.11% compared to untreated C1. Meanwhile, the chlorophyll content recorded in T1 (62.86) was found comparable to those in C2 (62.90). It was also found that inoculation of strain 139SI + application of inorganic fertilizer produces the highest reading of chlorophyll content among all treatment.

4.2.3 Photosynthetic Rate

Measuring CO_2 exchange is the most common utilized technique at present in order to measure the photosynthetic rate. CO_2 exchange measurements provide a direct measure of the net rate of photosynthetic carbon assimilation through non-destructive method. The results from this study also found that the amount of chlorophyll content in leaf had a significant effect on photosynthetic rate as the value shows the rate directly proportional to the chlorophyll content (Table 4.4). The value of photosynthetic rate of the T1 group was significantly increased to 8.46 μ m^{-2} Sec^{-1}, 4.7% higher as compared to the rate recorded in C1. The photosynthetic rate recorded in T1 was also found comparable to the rate recorded in C2. Similarly, the same pattern of photosynthetic rate was observed when comparing the rate in T2 with C1 and C2.

4.2.4 Chlorophyll Fluorescence

Chlorophyll fluorescence is widely used as a probe for studying photosynthetic performance since the operating quantum efficiency of electron transport through PSII in the leaves is translated by F_V/F_M value. Chlorophyll fluorescence also directly reflected the plant health status since environmental perturbations such as extreme temperature, high intensity of light and nutrient deficiency could reduce the metabolic rate in plant and consequently, reduce the chlorophyll fluorescence value (Baker & Rosenqvist, 2004). As can be observed from the results of this study, palm seedling suffered from chlorotic and stunted growth in C1 recorded the lowest chlorophyll fluorescence value compared to other treatments (Table 4.4). An excellent reading of chlorophyll fluorescence value was recorded in oil palm seedlings treated with strain 139SI inoculation and inorganic fertilizer with T2 recorded the highest value followed by C2 and T1. The higher value of chlorophyll fluorescence reading indicates that the process of electron transport through PSII in leaves is more efficient.

Table 4.4: Photosynthetic activities of oil palm seedling under different treatment

Group	Photosynthetic parameter		
	Chlorophyll Content ($nmol/cm^2$)	Photosynthetic Rate ($\mu\ m^{-2}\ Sec^{-1}$)	Chlorophyll fluorescence
Untreated (C1)	59.24 ± 1.04 b	8.08 ± 0.27 b	0.804 ± 0.01 a
Fertilizer (C2)	62.90 ± 1.50 a	8.44 ± 0.24 a	0.817 ± 0.01 a
139SI (T1)	62.86 ± 1.08 a	8.46 ± 0.20 a	0.816 ± 0.01 a
139SI + fertilizer (T2)	63.87 ± 4.01 a	8.45 ± 0.22 a	0.819 ± 0.01 a

The individual values are depicted as mean ± standard deviation. Different letters indicate significant differences between treatments according to Tukey test ($p<0.05$).

4.2.5 Analysis of Soil Nutrient

The effects of 139SI inoculation on soil fertility were analyzed by measuring the soil pH and nutrient content in the treated soil (Table 4.5). Inoculation of 139SI significantly increase the acidity of the soil as result shows that the soil pH of T1 samples decreases to 5.14 compared to 6.73 in C1. Application of inorganic fertilizer brought the soil pH even lower compared to C1 and T1 as result shows the soil pH of T2 and C2 was 4.74 and 4.72 respectively. As a record, the soil pH before the treatment was 6.89. The results also show that inoculation of 139SI and fertilizer application significantly changed the N content in soils. Total N in soil at the beginning of treatment was measured at 0.10% and inoculation of 139SI once in every two weeks for 4 months significantly elevate the amount to 0.19%. This increment was also significantly outperformed the N content in C1 which was 0.13%. However, without fertilizer addition, inoculation of 139SI had no significant effects on other nutrient contents in soil.

Table 4.5: Nutrient content analysis of soil samples

Treatments	pH	Soil nutrient content (%)					
		N	P	K	Mg	Ca	S
Untreated (C1)	6.73 ± 0.14 c	0.13 ± 0.00 c	0.20 ± 0.00 b	0.07 ± 0.01 b	0.03 ± 0.00 a	0.39 ± 0.00 a	0.09 ± 0.01 a
Fertilizer (C2)	4.72 ± 0.05 a	0.25 ± 0.02 a	0.46 ± 0.01 a	0.26 ± 0.01 a	0.03 ± 0.00 a	0.39 ± 0.00 a	0.08 ± 0.01 a
139SI (T1)	5.14 ± 0.10 b	0.19 ± 0.00 b	0.21 ± 0.00 b	0.07 ± 0.01 b	0.03 ± 0.00 a	0.39 ± 0.03 a	0.10 ± 0.01 a
139SI + Fertilizer (T2)	4.74 ± 0.06 a	0.26 ± 0.00 a	0.43 ± 0.01 a	0.25 ± 0.02 a	0.03 ± 0.00 a	0.38 ± 0.04 a	0.08 ± 0.00 a

The individual values are depicted as mean ± standard deviation. Different letters indicate significant differences between treatments according to Tukey test ($p<0.05$)

4.2.6 Analysis of Plant Nutrient Content

4.2.6.1 Nutrient Content of Root

Inoculation of 139SI on the oil palm seedling improve the percentage of nutrient content of root samples as displayed in Table 4.6. The results show that the percentage of N, P and Mg content in in the root of T1 significantly higher compared to C1. The percentage of N, P and Mg content in root of T1 was measured at 1.11%, 0.23% and 0.17%, respectively, a significant increase by 46.15%, 53.33% and 30.77% compared to C1. However, the enhancement of these nutrients is still below the value recorded on palm received inorganic fertilizer, C2. The best improvement of percentage of nutrient content was recorded in T2, in which, the enhancement of N, P, K, Mg and Fe in this group were the highest among other. Meanwhile, the mean differences of Ca, S, and Cu among all treatment were statistically insignificant.

Table 4.6: Nutrient content analysis of plant root samples

Treatments	Nutrient concentration of root							
	N	P	K	Mg	Ca	S	Cu	Fe
	(%)							(ppm)
Untreated (C1)	0.80 ± 0.04 c	0.15 ± 0.00 c	1.07 ± 0.04 b	0.13 ± 0.00 b	0.19 ± 0.01 a	0.23 ± 0.01 a	0.05 ± 0.01 a	0.11 ± 0.01 c
Fertilizer (C2)	1.32 ± 0.02 a	0.28 ± 0.00 a	1.59 ± 0.05 a	0.17 ± 0.01 a	0.18 ± 0.01 a	0.23 ± 0.02 a	0.05 ± 0.00 a	0.14 ± 0.01b,c
139SI (T1)	1.11 ± 0.03 b	0.23 ± 0.01 b	1.09 ± 0.01 b	0.17 ± 0.01 a	0.18 ± 0.02 a	0.23 ± 0.02 a	0.05 ± 0.01 a	0.12 ± 0.01 b, c
139SI + Fertilizer (T2)	1.54 ± 0.04 a	0.32 ± 0.01 a	1.58 ± 0.04 a	0.17 ± 0.01 a	0.18 ± 0.00 a	0.24 ± 0.03 a	0.06 ± 0.01 a	0.14 ± 0.01 a,b

The individual values are depicted as mean ± standard deviation. Different letters indicate significant differences between treatments according to Tukey test ($p<0.05$).

4.2.6.2 Nutrient Content of Shoot

Analysis of nutrient content of palm upper part revealed that nutrient content in T1 palm sample was higher compared to C1 (Table 4.7). The value of N, P and K increases tremendously by 238.89%, 133.33%, and 18.70% respectively. The inoculation of 139S1 also was significantly enhanced the percentage of others nutrient in T1 samples, except for S, in which the increasing is marginal. It was also found that, the enhancement of N, Mg, Ca, Cu and Fe content in T1 palm upper part were comparable to C2. On the other hand, the percentage of P, K and S content in T1 were significantly lower than C1. The positive effects of adding 139SI inoculant to the fertilized palm can be observed more profoundly on percentage of nutrient content of shoot samples. The percentage of nutrient content of palm shoot in T2 had been always higher than C2. Among those, the percentage of N and Mg recorded a significant increase with values of 3.31% and 0.34% as compared to C2 with values of 2.73% and 0.26% respectively. The results on a percentage of nutrient content also portray the distribution of the nutrient in the palm. Most of the nutrients were found to accumulate in the shoot part compared to root.

Table 4.7: Nutrient content analysis of plant upper part samples

Treatments	Nutrient concentration of shoot							
	N	P	K	Mg	Ca	S	Cu	Fe
				(%)				(ppm)
Untreated (C1)	0.72 ± 0.03 c	0.12 ± 0.02 c	1.07 ± 0.06 c	0.13 ± 0.01 c	0.18 ± 0.00 b	0.20 ± 0.02 b	0.05 ± 0.01 b	0.36 ± 0.02 d
Fertilizer (C2)	2.73 ± 0.17 b	0.31 ± 0.02 a	1.73 ± 0.04 a	0.26 ± 0.03 b	0.72 ± 0.11 a	0.43 ± 0.01 a	0.07 ± 0.01 a	0.45 ± 0.02 b, c
139SI (T1)	2.44 ± 0.05 b	0.28 ± 0.00 b	1.27 ± 0.01 b	0.22 ± 0.02 b	0.46 ± 0.04 a	0.27 ± 0.01 b	0.08 ± 0.01 a	0.42 ± 0.02 c
139SI + Fertilizer (T2)	3.31 ± 0.01 a	0.38 ± 0.00 a	1.84 ± 0.01 a	0.34 ± 0.01 a	0.88 ± 0.01 a	0.57 ± 0.02 a	0.08 ± 0.02 a	0.47 ± 0.02 a, b

The individual values are depicted as mean ± standard deviation. Different letters indicate significant differences between treatments according to Tukey test ($p<0.05$).

4.2.7 Analysis of Plant Nutrient Uptake

In general, the growth and physiological performance of a plant are closely reflecting its nutrient uptake status. Nutrient deficiency symptoms such as stunted growth, chlorosis and lower photosynthetic activities shown by oil palm seedlings in C1 were confirmed by a significant decrease in their nutrient uptake. Inoculation of 139SI greatly affects the nutrient uptake of oil palm seedling as shown in Table 4.8. Unlike the percentage of nutrient content in the palm, the difference in mean increment of nutrient uptake in T1 was statistically significant for all nutrients as the results show that the amount of nutrient in T1 increase tremendously compared to C1. The uptake of N by palm in T1 recorded the highest increment by six fold, followed by Mg and Cu by more than three folds respectively compared to C1. However, the increment of nutrient uptake by the palm of T1 group is not as high as recorded in C2. Fertilized palm of C2 recorded significantly higher in most of nutrient analyzed, except for Mg and Cu, in which the amount of both nutrient uptakes by C2 were comparable to those of T1. Adding 139SI inoculant to the fertilized palm was resulting enhancement on nutrient uptake. The enhancement of nutrient uptake recorded in T2 significantly higher even compared to the C2.

Table 4.8: Analysis nutrient uptake of plant samples under different treatment

Treatments	Total plant biomass (g plant^{-1})	Plant nutrient uptake							
		N	P	K	Mg	Ca	S	Cu	Fe
					(g/plant)				(mg/plant)
Untreated (C1)	33.67 ± 7.15 d	0.51 ± 0.04 d	0.70 ± 0.03 d	0.72 ± 0.03 d	0.09 ± 0.00 c	0.23 ± 0.02 d	0.14 ± 0.01 d	0.03 ± 0.00 c	0.16 ± 0.01 d
Fertilizer (C2)	115.73 ± 7.15 b	4.72 ± 0.51 b	1.26 ± 0.11 b	3.81 ± 0.11 b	0.50 ± 0.10 b	0.53 ± 0.05 b	0.54 ± 0.02 b	0.14 ± 0.02 b	0.68 ± 0.03 b
139SI (T1)	104.47 ± 6.89 c	3.71 ± 0.11 c	1.05 ± 0.12 c	2.25 ± 0.02 c	0.40 ± 0.05 b	0.37 ± 0.02 c	0.46 ± 0.03 c	0.13 ± 0.02 b	0.57 ± 0.03 c
139SI + Fertilizer (T2)	143.05 ± 19.70 a	6.94 ± 0.14 a	1.66 ± 0.03 a	4.90 ± 0.12 a	0.73 ± 0.04 a	0.65 ± 0.01 a	0.75 ± 0.03 a	0.20 ± 0.03 a	0.87 ± 0.04 a

The individual values are depicted as mean ± standard deviation. Different letters indicate significant differences between treatments according to Tukey test ($p<0.05$).

4.2.8 Correlation Analysis

Correlation analysis was performed to evaluate the degree and significance of the correlation between the amounts of nutrient uptakes with the palm growth (Table 4.9). Based on correlation analysis, a significant and positive correlation between nutrient uptake and total plant dry weight was found as evidenced by the high correlation coefficient values as shown in Table 4.9. Correlation analysis also revealed that the chlorophyll content in oil palm seedling was found to be positively correlated to the amount of nutrient uptake, especially N, Mg, S and Fe with correlation coefficient values of 0.858, 0.875, 0.876, and 0.867 respectively. A significant positive correlation of nutrient uptake and photosynthetic rate was also recorded with correlation coefficients ranging from 0.744 (Mg) to 0.840 (K).

Table 4.9: Pearson's correlation coefficients for the plant growth and strain 139SI population in rhizosphere

Plant physiology	Nutrient Uptake						
	Nitrogen (N)	Phosphorus (P)	Potassium (K)	Magnesium (Mg)	Sulfur (S)	Copper (Cu)	Iron (Fe)
Stem height	0.968*	0.974*	0.955*	0.941*	0.969*	0.805*	0.979*
Stem diameter	0.979*	0.982*	0.956*	0.954*	0.978*	0.933*	0.984*
Leaf area	0.926*	0.969*	0.889*	0.916*	0.934*	0.922*	0.958*
Total plant biomass	0.929*	0.933*	0.894*	0.896*	0.937*	0.818*	0.957*
Chlorophyll content	0.858*	0.816*	0.836*	0.875*	0.876*	0.844*	0.867*
Photosynthetic rate	0.839*	0.824*	0.840*	0.744*	0.817*	0.812*	0.817*

Notes: * significant at levels of $p < 0.05$

4.3 Effects of *B. salmalaya* Strain 139SI Inoculation on Yield and Palm Oil Quality

4.3.1 Physio-chemical Characteristics of Soil Sample

The physio-chemical characteristics of soil samples from each treatment were present in the Table 4.10. Application of inorganic fertilizer significantly increased the acidity of the soil in T2 and T4 compared to untreated T1 and 139SI inoculated group T3. The pH values of soil samples from T2 were significantly lower than T1 by 8.9% and lower than T3 by 9.8 % respectively. Meanwhile, the pH values of soil samples in T4 were found 10.5 % and 11.32 % lower than those in T1 and T3 respectively.

Of all treatment, application of inorganic fertilizer was resulting in higher of total N, P and K. The percentage of total N, P and K in soil samples of T2 and T4 were significantly higher than T1 and T3. The percentage of total N in T4 was the highest, about 112.12% and 75% higher over the unfertilized T1 and T3. The percentage of P in soil samples of T4 had also significantly higher by almost one fold compared to T1 and T3. There was also an obvious positive effect of inorganic fertilizer application on the level of K in the soil. The level of K was found significantly higher in T2 and T4 compared to T1 and T3.

In contrast, application of inorganic fertilizer and 139SI inoculation did not affect the level of Ca, Mg and S in the soil as the difference in mean values of the elements in each treatment was insignificant. The level of Ca in soil was ranged between 0.22% and 0.20% in T3 and T1 respectively. The highest level of Mg was recorded in T4 and the lowest was in T1. Meanwhile, the highest level of S was in T4, followed by T2, T1 and T3.

Table 4.10: Physio-chemical characteristics of soil from different treatment

Group	Ph	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Calcium (%)	Magnesium (%)	Sulfur (%)
Untreated (T1)	7.16 ± 0.01 b	0.33 ± 0.03 b	0.16 ± 0.03 b	0.50 ± 0.05 b	0.20 ± 0.01 a	0.19 ± 0.01 a	0.09 ± 0.01a
Inorganic fertilizer (T2)	6.07 ± 0.03 a	0.64 ± 0.05 a	0.28 ± 0.02 a	0.68 ± 0.04 a	0.20 ± 0.06 a	0.20 ± 0.03 a	0.10 ± 0.02 a
Strain 139SI (T3)	6.21 ± 0.03 b	0.48 ± 0.02 b	0.15 ± 0.03 b	0.49 ± 0.04 b	0.22 ± 0.02 a	0.20 ± 0.01 a	0.08 ± 0.01 a
Strain 139SI + inorganic fertilizer (T4)	6.12 ± 0.04 a	0.70 ± 0.01 a	0.29 ± 0.02 a	0.68 ± 0.11 a	0.21 ± 0.01 a	0.23 ± 0.04 a	0.11 ± 0.02 a

The individual values are depicted as mean ± standard deviation. Different letters indicate significant differences between treatments according to Tukey test ($p<0.05$).

4.3.2 Analysis of Plant Nutrient Content

Palm nutrient status from different treatment is presented in Table 4.11. Substantial elevated of nutrient uptake by palm was observed in 139SI inoculated and fertilized group. The level of palm nutrient status was always found highest in T4 compared to T1, T2 and T3. The level of N in T4 was increased significantly by 100%, 48.28% and 28.64% than those in T1, T3 and T2 respectively. Likewise, the level of P in T4 was about 111.11%, 52% and 11.76% higher than in T1, T3 and T2. The positive effect of strain 139SI inoculation on fertilized palm can also be observed in the level of K. When compared with untreated control T1 and 139SI inoculated palm T3, the level of K was significantly increased by 68.97% and 53.13% respectively. On the other hand, the difference in an increment of K was only marginal compared to T2.

Palm inoculated with 139SI and at the same time received inorganic fertilizer also recorded a higher level of Mg, Ca and S compared to other treatment. The significantly higher level of Mg was recorded in T4, followed by T3, T2 and T1. Palm in T4 also exhibits higher level of Ca and S compared to other treatment, although the increment was not statistically significant.

Table 4.11: Nutrient content of frond samples from different treatment

Group	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Calcium (%)	Magnesium (%)	Sulfur (%)
Untreated (T1)	0.43 ± 0.04 d	0.18 ± 0.02 c	0.58 ± 0.04 b	0.20 ± 0.01 a	0.43 ± 0.01 b	0.21 ± 0.02 a
Inorganic fertilizer (T2)	0.69 ± 0.04 b	0.34 ±0.05a	0.91 ± 0.04 a	0.23 ± 0.01 a	0.49 ± 0.01 b	0.27 ± 0.02 a
Strain 139SI (T3)	0.58 ± 0.02 c	0.25 ± 0.03 b	0.64 ± 0.04 b	0.23 ± 0.02 a	0.48 ± 0.01 b	0.26 ± 0.01 a
Strain 139SI + Inorganic fertilizer (T4)	0.86 ± 0.01 a	0.38 ± 0.02 a	0.98 ± 0.11 a	0.28 ± 0.01 a	0.67 ± 0.03 a	0.31 ± 0.02 a

The individual values are depicted as mean ± standard deviation. Different letters indicate significant differences between treatments according to Tukey test ($p<0.05$).

4.3.3 Fresh Fruit Bunch (FFB) Yield

Performance of FFB yields from different treatment is described in Figure 4.19. It was noted that FFB yield was varying every month. From the result obtained, the yield was peak in September with palm from T2 recorded the highest FFB yield at 3.81 t ha^{-1} and palm from T1 recorded the lowest yield at 3.64 t ha^{-1}. In contrast, the FFB yield in February was the lowest during the trial period with the yield ranged from 2.12 t ha^{-1} in T4 to 1.37 t ha^{-1} in T1.

At the beginning of the study, the FFB yield from each treatment was ranged from 2.97 to 2.69 t ha^{-1}, and the difference in mean FFB yield among the treatment was insignificant. Application of inorganic fertilizer and inoculation of 139SI to the palm has caused the difference in mean FFB yield among the treatment group became wider. The ranking order for mean FFB yield based on weight is T4> T2 > T3 and T1. Inoculation 139SI increases the FFB yield to 11.43% higher than the untreated palm of T1. However, this increment is still lower when compared to the palm received inorganic fertilizer. The effect of 139SI inoculation was more profound on palm received inorganic fertilizer at the same time. At the end of the study, palm in T4 produced a higher FFB yield by 54.29%, 16.13%, and 38.46% compared to T1, T2 and T3 respectively. The increment of FFB yield from palm in T4 over T1 and T3 is statistically significant.

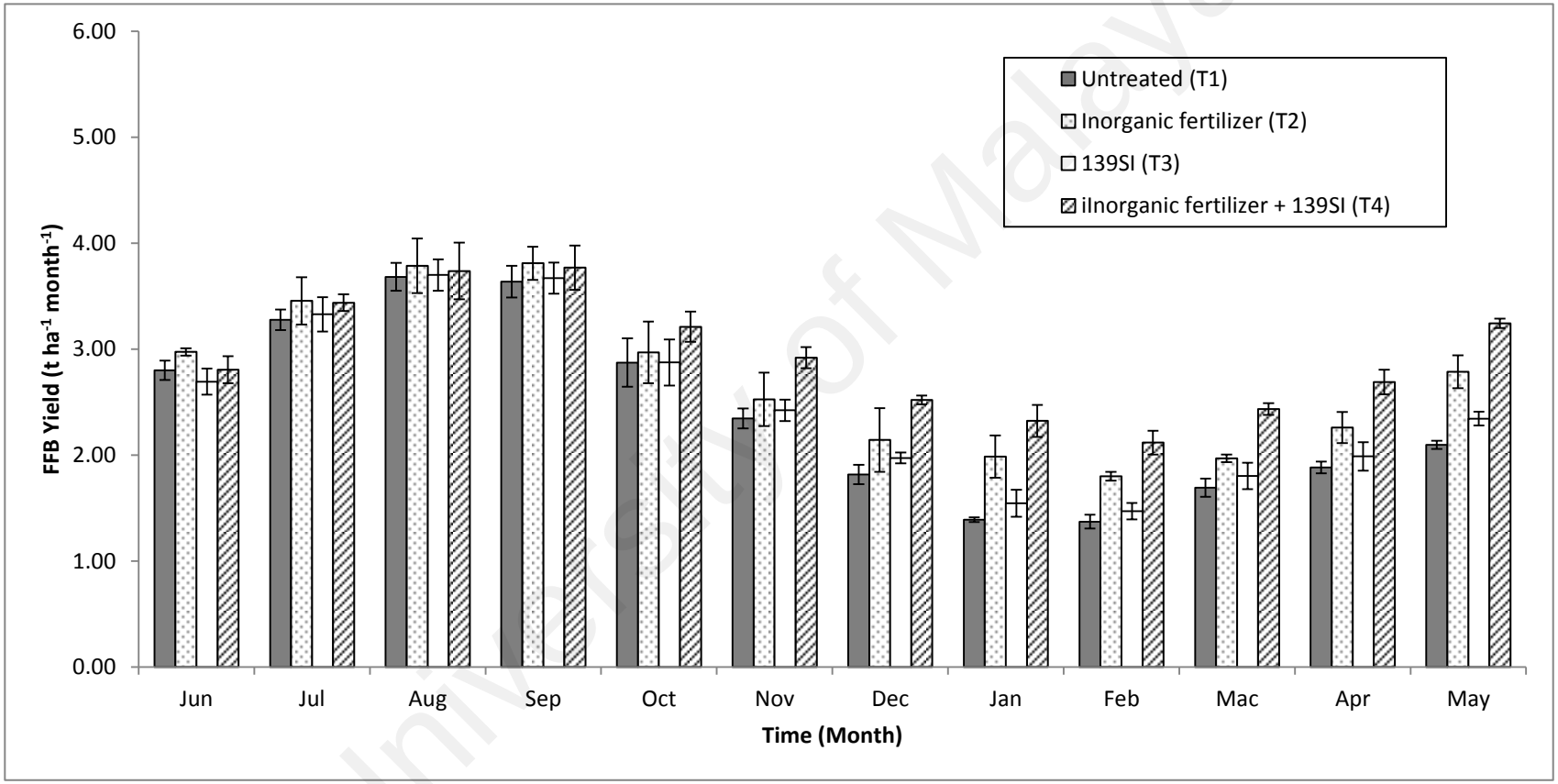


Figure 4.19: FFB yield of palm received different treatments

4.3.4 Number of Fresh Fruit Bunches Produced

In general, the number of FFB produced from different treatment is varying each month during the study period (Figure 4.20). Based on FFB analyses carried out from June 2015 to May 2016, the mean values for number of FFB produced were reaching the highest number in September 2015. At the peak season, number of FFB produced ranged from 178 to 168 bunches/ hectare in T2 and T3 respectively. Then, the number of FFB produced gradually decreases and reached the lowest in February 2016. The palm in T4 produced the highest number of FFB on February 2016 with the average of bunches produced were 97 bunches/ hectare. While the lowest was recorded in T1 with average FFB produced were only 85 bunches/ hectare.

Analyses of variance on the data found that the inoculation of 139SI and application of inorganic fertilizer had no significant effect on the number of FFB produced. This is because the mean values of number of FFB produced among the treatment every month are statistically insignificant.

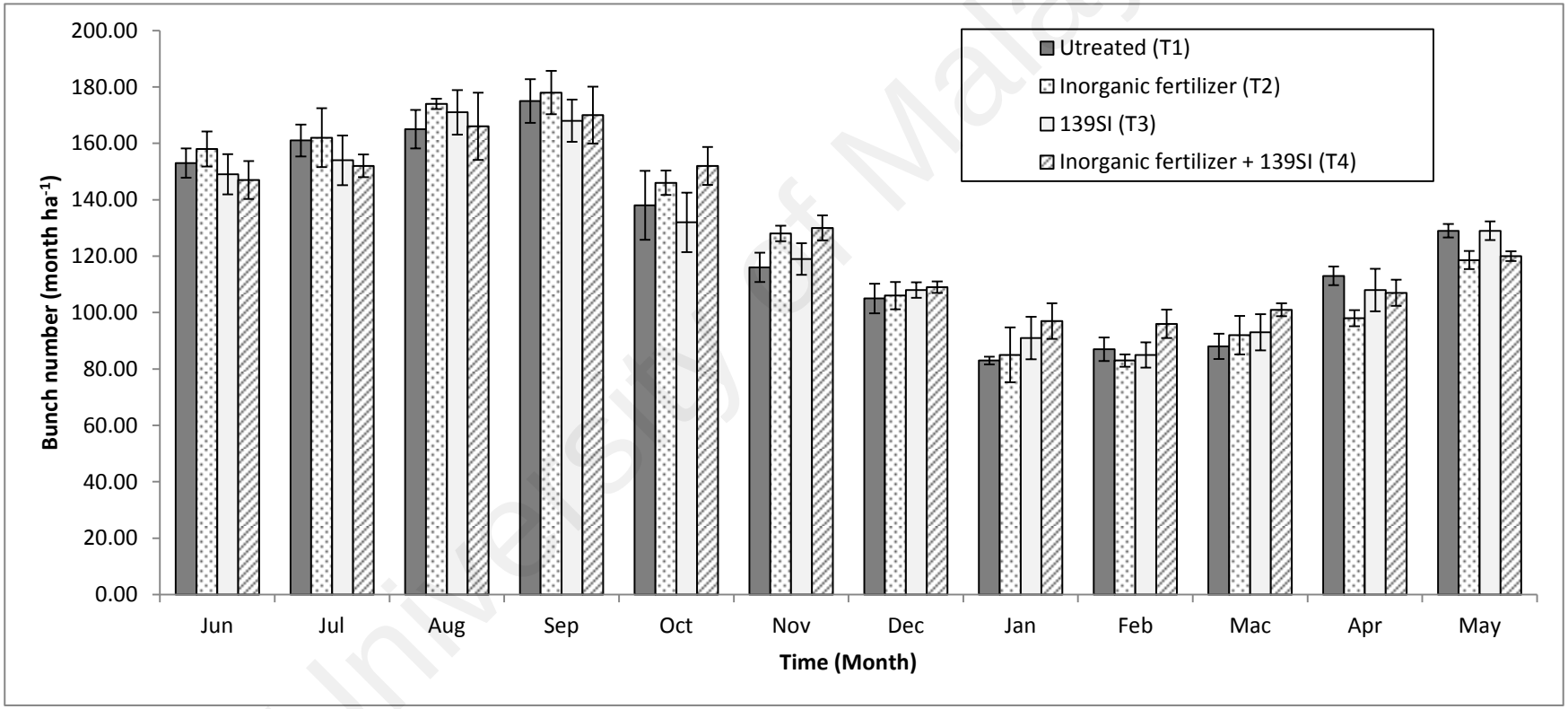


Figure 4.20: Number of bunches produced from different treatments.

The results for annual yield performances of palm received different treatment are given in Table 4.12. The total number of bunches produced from each treatment throughout the study period were ranged from 1527.00 to 1547.00 of bunches ha^{-1} yr^{-1} without much significant difference. On the contrary, results obtained show that the bunch weight much affected by the inoculation of 139SI and application of inorganic fertilizer. Of all treatment, 139SI inoculated and fertilized palm (T4) produced heaviest bunch at average 25.80 %, 6.87% and 20.09% heavier than bunches from T1, T2 and T3 respectively. The palm from T4 and T2 produced significantly heavier bunches compared to T1 and T3. Inoculation of 139SI increases the bunch weight when compared to T1. However, the increment is minimal and statistically insignificant.

The average bunch weight produced by palm from each treatment directly influenced the FFB yield. The results obtained show that giving 139SI inoculant to the palm can improve the FFB yield. Although statistically insignificant, the increment of FFB yield from palm in T3 over T1 is more than 2 t ha^{-1} yr^{-1}. However, this increment is still lower compared to the FFB yield from fertilized palm. As expected, supplying fertilizer to the palm increases the FFB yield. Interestingly, Addition 139SI inoculant to the fertilized palm enhances the effectiveness of the fertilizer as the results show that palm from T4 was the most productive, produced approximately 7.66, 5.40, and 2.74 tonnes more FFB yield than those untreated (T1), 139SI inoculated (T3) and received inorganic fertilizer only (T2).

Table 4.12: Yield performance of oil palm from different treatments

Treatment	Number of bunches (ha^{-1} yr^{-1})	Average Bunch weight (kg)	FFB yield (t ha^{-1} yr^{-1})
Untreated (T1)	1533.00 ± 31.03 a	17.68 ± 1.17 b	27.54 ± 1.18 c
Inorganic fertilizer (T2)	1538.00 ± 35.12 a	20.81 ± 1.20 a	32.46 ± 3.32 a,b
139SI (T3)	1527.00 ± 29.90 a	18.52 ± 1.10 b	29.80 ± 1.37 b,c
Inorganic fertilizer + 139SI (T4)	1547.00 ± 27.47 a	22.24 ± 1.98 a	35.20 ± 1.68 a

The individual values are depicted as mean ± standard deviation. Different letters indicate significant differences between treatments according to Tukey test ($p<0.05$).

4.3.5 Rate of Oil Extraction from FFB

As shown in Table 4.13, Inoculation of strain 139SI and application of synthetic fertilizer had no effect on the percentage of oil extraction as all groups of treatment recorded oil extraction rate around 22 %. No significant differences were found when comparing the percentage of oil extraction between treatment groups and control groups.

Table 4.13: Percentage of oil extraction rate from FFB for different treatment

Treatment	Oil extraction rate (%)
Untreated (T1)	22.15 ± 0.4 a
Inorganic fertilizer (T2)	22.23 ± 0.2 a
139SI (T3)	22.19 ± 0.1 a
139SI + inorganic fertilizer (T4)	22.08 ± 0.2 a

The individual values are depicted as mean ± standard deviation. Different letters indicate significant differences between treatments according to Tukey test ($p<0.05$).

4.3.6 Analysis of Fatty Acid Profile

Fatty acids from T1, T2, T3 and T4 palm oil samples were classified as saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). The classification is based on the number of double bonds present in the structure. The SFA consist of lauric (12-0), myristic (14-0), palmitic (16-0), margaric (17-0), stearic 18-0) and arachidic (20-0). Fatty acids that have one double bond in the fatty acid chain or MUFA included palmitoleic (16-1), oleic (18-1) and gadoleic (20-1). While linoleic (18-2) and linolenic (18-3) are categories as PUFA.

Chromatogram from the gas chromatography result of T1, T2, T3 and T4 shows that palmitic (16-0) produced the highest peak at retention time 12.186 – 12.203. The second highest peak was produced by monounsaturated fatty acid of oleic (18-1) at retention time 16.122 – 16.148 and followed by linoleic (18-2) at retention time 17.474 – 17.488. These data mean that palmitic, oleic, and linoleic are the three major fatty acid found in the samples.

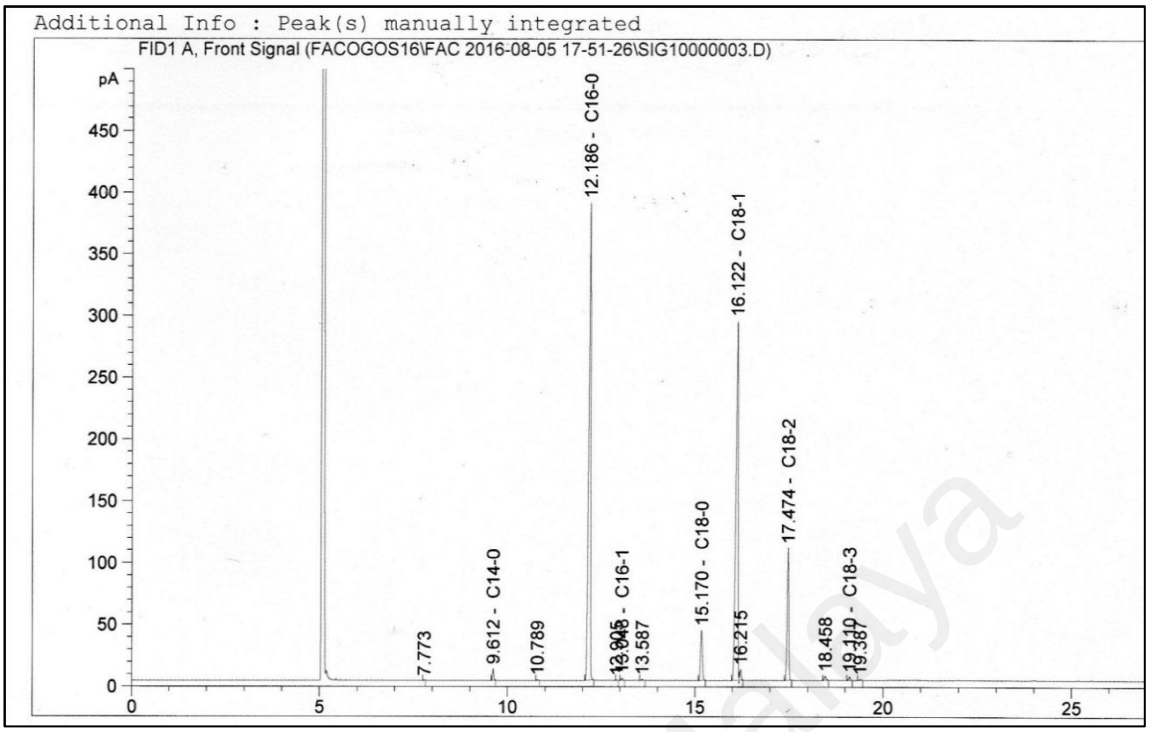


Figure 4.21: Full chromatograms of FAME from untreated (T1) samples

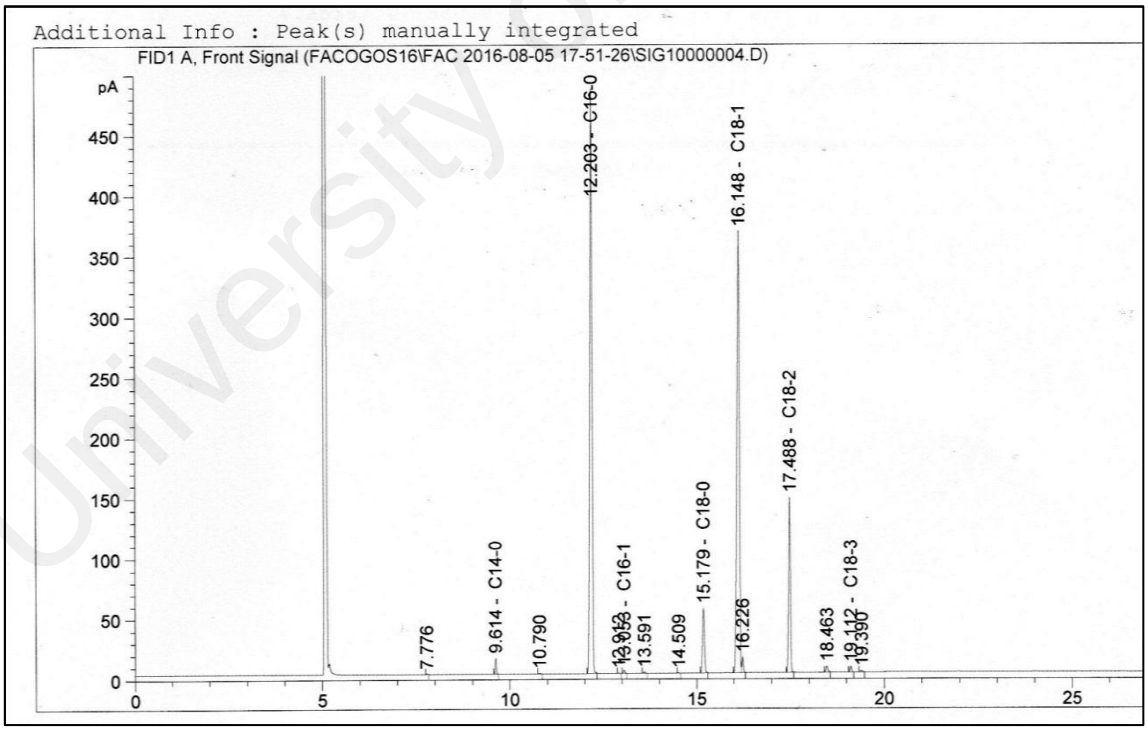


Figure 4.22: Full chromatograms of FAME from inorganic fertilized palm (T2)

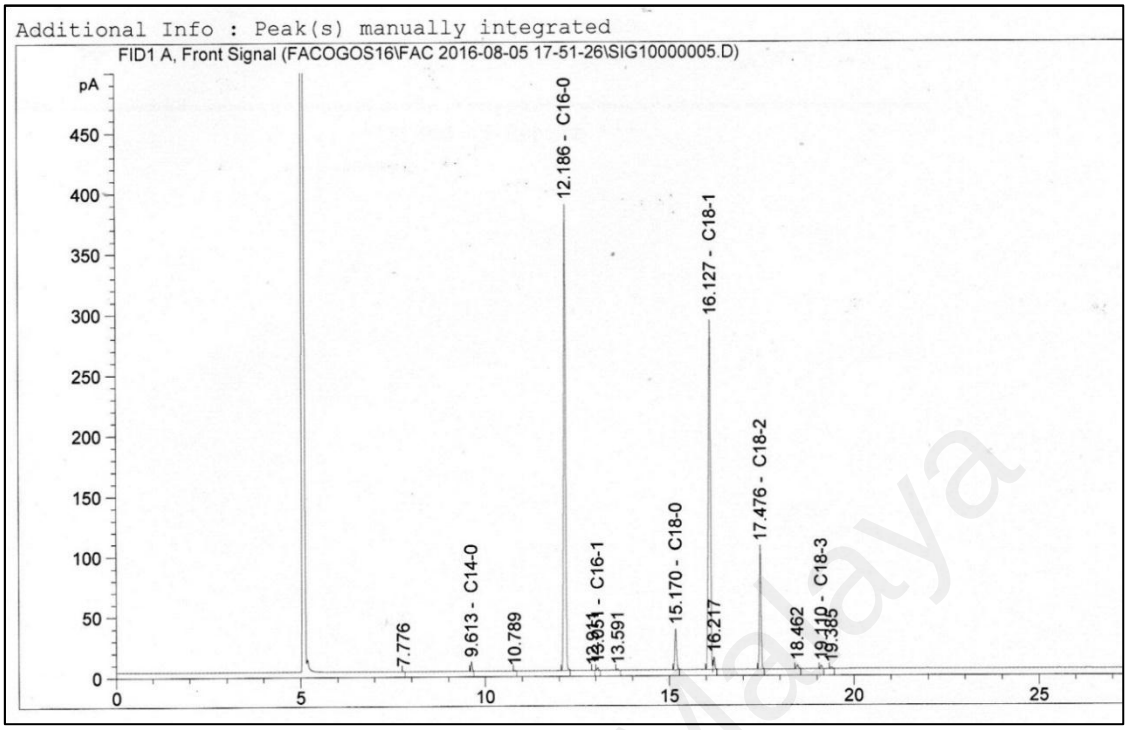


Figure 4.23: Full chromatograms of FAME from 139SI inoculated palm (T3) samples.

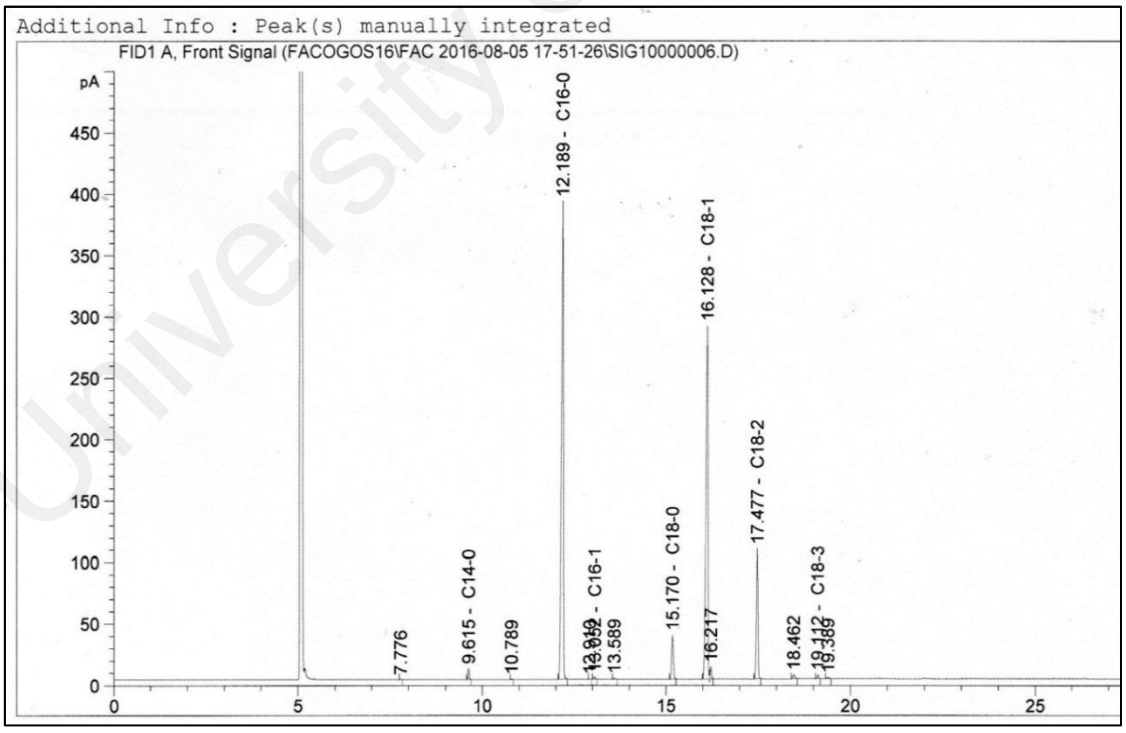


Figure 4.24: Full chromatograms of FAME from 139SI inoculated and fertilized palm (T4) samples.

The full fatty acid profile in palm oil derived from T1, T2, T3 and T4 palm mesocarp were found to display the same characteristic (Table 4.14 and Table 4.15). The SFA is the major fatty acid found in all treatment, represented more than 50% of total fatty acid content, followed by MUFA and PUFA in which represent approximately 40% and 10% of total fatty acid content respectively. Palmitic acid is the main fatty acid found and represented about 45.04%, 45.13%, 45.18% and 44.93% of the total fatty acid in T1, T2, T3 and T4 respectively. Oleic and linoleic also represent among major fatty acid found in all treatment in which oleic acid contribution of total fatty acid in T1, T2, T3 and T4 ranged from 38.72% to 38.47%. Meanwhile, linoleic account for 10.42, 10.37, 10.44 and 10.55% of total fatty acid in T1, T2, T3 and T4 respectively.

Generally, nearly equal proportion of SFA, MUFA and PUFA were observed in all treatment. The mean differences of all fatty acid percentages among treatment also small thus make the difference statistically insignificant

Table 4.14: Percentage of Saturated fatty acid (SFA), Monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) of crude palm oil from different treatment

Fatty acid	Treatments			
	Untreated (T1)	Inorganic fertilizer (T2)	139SI (T3)	Inorganic fertilizer + 139SI (T4)
Saturated fatty acid (SFA)	50.49	51.05	51.23	51.11
Monounsaturated fatty acids (MUFA)	38.79	39.05	38.79	38.82
Polyunsaturated fatty acids (PUFA)	10.72	10.67	10.73	10.85

The individual values are depicted as mean ± standard deviation. Different letters indicate significant differences between treatments according to Tukey test ($p<0.05$).

Table 4.15: Percentage of fatty acid of crude palm oil from different treatment

Fatty acid	Treatments			
	Untreated (T1)	Inorganic fertilizer (T2)	139SI (T3)	Inorganic fertilizer + 139SI (T4)
Saturated fatty acid				
Lauric acidc12-0	0.02 ± 0.00 a	0.02 ± 0.00 a	0.02 ± 0.00 a	0.03 ± 0.01 a
Myristic acid c14-0	0.68 ± 0.04 a	0.67 ± 0.07 a	0.69 ± 0.05 a	0.69 ± 0.05 a
Palmitic acid c16-0	45.24 ± 0.15 a	45.13 ± 0.18 a	45.18 ± 0.38 a	44.93 ± 0.36 a
Margaric acid c17-0	0.09 ± 0.00 a	0.09 ± 0.00 a	0.09 ± 0.00 a	0.09 ± 0.00 a
Stearic acid c18-0	4.08 ± 0.18 a	3.95 ± 0.27 a	4.09 ± 0.25 a	4.17 ± 0.25 a
Arachidic acid c20-0	0.38 ± 0.02 a	0.38 ± 0.01 a	0.35 ± 0.03 a	0.39 ± 0.01 a
Monounsaturated fatty acids (MUFA),				
Palmitoleic acid c16-1	0.20 ± 0.00 a	0.21 ± 0.00 a	0.20 ± 0.00 a	0.20 ± 0.00 a
Oleic acid c18-1	38.47 ± 0.36 a	38.72 ± 0.53 a	38.47 ± 0.52 a	38.50 ± 0.15 a
Gadoleic acid c20-1	0.12 ± 0.00 a	0.12 ± 0.01 a	0.12 ± 0.01 a	0.12 ± 0.01 a
Polyunsaturated fatty acids				
Linoleic acid c18-2	10.42 ± 0.13 a	10.37 ± 0.28 a	10.44 ± 0.14 a	10.55 ± 0.30 a
Linolenic acid c18-3	0.30 ± 0.00 a	0.30 ± 0.01 a	0.29 ± 0.00 a	0.30 ± 0.01 a

The individual values are depicted as mean ± standard deviation. Different letters indicate significant differences between treatments according to Tukey test ($p<0.05$).

CHAPTER 5: DISCUSSION

PGPR is gaining more interest in recent time as it has been recognized to provide a cleaner approach towards maintaining crop production and eventually could reduce synthetic fertilizer harmful effects. The novel strain 139SI used in this study exhibited several desirable features for plant growth promotion. Previous study also found that several *Bacillus* sp are very effective in promoting plant growth. For example, *B. amyloliquefaciens, B. thuringiensi* and *B. subtilis* enhance plant growth by biofertilization, phytostimulation and biocontrol from disease infection (Masciarelli *et al.,* 2014; Hyakumachi *et al.,* 2013; Marulanda *et al.,* 2006). One of the important PGPR trait presented by the strain 139SI, that may directly enhance the plant growth is the production of IAA. IAA is the most important auxins produced by PGPR. It acts as phytostimulation as it can directly benefit the plant root system by stimulating root development and increase the number of adventitious roots (Walia *et al.,* 2014). Obviously, the same result was recorded in our study as root development indication such as root dry weight, length of the main root and number of lateral root showed significant increase in plant inoculated with strain 139SI compare to uninoculated group. Increase in root system will enable the plant to exploit larger soil volume, thus the plant can obtain larger amounts of nutrients. This also will benefit the bacteria that inhabit in the rhizosphere with high levels of root exudates (Ribeiro *et al.,* 2014). We also found that the ability of strain 139SI to produce IAA is not depending on the availability of precursors for IAA synthesis, which is tryptophan. Although tryptophan is the most important precursor for IAA synthesis, certain PGPR are able to produce the hormone through several tryptophan-independent pathways as explained by Spaepen *et al.* (2009), and this would explain the production of IAA by strain 139SI in the absence of tryptophan. This, of course, is advantageous to the plant as tryptophan is not ubiquitous in the soil.

The other beneficial plant growth promoting feature of 139SI that can be observed was its potential to make soil more fertile by increasing the availability of nitrogen, phosphate and iron elements to the plant. Nitrogen, phosphorus and iron are among the most crucial plant nutrient required for plant growth. Despite both elements are abundantly available in nature, but they mostly exist in an unusable form to the plant (Richardson *et al.,* 2009; Guerinot & Yi, 1994). The atmospheric nitrogen can be converted into plant usable form by biological nitrogen fixation (BNF). Certain microbes also are able to transform insoluble phosphate in the soil into usable forms for plant utilization through processes of acidification, exchange reactions, chelation and production of organic acids (Sharma *et al.,* 2013; Rodríguez *et al.,* 2004). A lot of microbes have been identified to play an important role in fixing the atmospheric nitrogen into soil and solubilize rock phosphate and eventually enhance plant growth by improving the soil fertility. Results from our study demonstrated that growth stimulation of oil palm seedling inoculated with nitrogen-fixing and phosphate-solubilizing bacterium, strain 139SI. Similarly, Istina *et al.* (2015) showed that the inoculation of phosphate solubilizing and nitrogen fixing bacteria, *Burkholderia gladiolus,* have contributed to the growth of oil palm seedling. Research done by Kaur and Reddy (2014) found that inoculation of phosphate solubilizing bacteria of *Pantoea cypripedii* and *Pseudomonas plecoglossicida* enhance plant growth and increase phosphate uptake. A similar finding also was reported by Hameeda *et al.* (2008) using *Serratia marcescens* and *Pseudomonas* sp.

As mentioned earlier, we also found that strain 139SI produce siderophore. Siderophore are iron-specific chelating compounds that are produced under low iron stress to capture iron from the environment (Ngamau *et al.*, 2014). Siderophore produced by strain 139SI may directly promote oil palm seedling growth by increasing the availability of iron in the soil surrounding root. According to Hansen *et al.* (2007),

iron deficiency will result in photosynthetic rate reduction in plant because chlorophyll synthesis is inhibited in younger leaves. This eventually will inhibit growth of plants and reduces crop yield. Research done by Sharma *et al.* (2003) found that the siderophore-producing *Pseudomonas* strain GRP3 increase chlorophyll a and chlorophyll b content in *Vigna radiate*. While Nagata *et al.* (2013) also found that chlorophyll synthesis was enhanced in *Solanum lycopersicum* when the plant supplemented with pyoverdine, a siderophore synthesized by *Pseudomonas fluorescens*. Besides that, siderophore producing bacteria can act as a good biological control of plant diseases since efficient utilization of siderophore by these bacteria restricts the growth of the pathogenic microbes in the rhizosphere due to iron starvation (Raaijmakers *et al.,* 1995). The potential of PGPR to promote not only plant growth, but also protects plants from pathogens has been discovered for the first time by Kloepper *et al.* (1980). Since then, the promising potential of PGPR as biocontrol of plant disease was largely studied. For example, *Bacillus subtilis* CAS15, has a biocontrol effect on Fusarium wilt (Yu *et al.,* 2011). Van Peer *et al.* (1991) reported *Pseudomona*s strain WCS417 protection against carnation from fusarosis due to phytoalexin. While Park *et al.* (2001) reported that *Bacillus amyloliquefaciens* strain EXTN-1 protects cucumber against anthracnose disease caused by *Colletotrichum orbicularein*. However, the potential of strain 139SI as a biocontrol agent against plant disease need to be further studied.

In the pre-nursery study, we have also analyzed the ability of strain 139SI to colonize the rhizosphere and how the amount of 139SI population presence in the rhizosphere could affect plant growth. We found that there was a significant linear correlation between the amount of strain 139SI population presence in the rhizosphere and growth of oil palm seedling. This result clearly shows the influence of strain 139SI in promoting oil palm seedling growth. The ability of PGPR to colonize the plant root is

very important since a close contact of the bacteria with the plant root is needed in order for the plant to get benefit from the microbial products (Bashan, 2008). Thus, the larger population of strain 139SI in rhizosphere could provide more growth regulators and nutrient for plant. The largest population of 139SI was discovered in soil followed on the root surface and smallest population of 139SI was found in the internal root tissue. The population densities of strain 139SI in different part of rhizosphere indicate the movement of the strain in this niche. Most of strain 139SI population was found in the soil since the inoculation procedure was done by mixing the strain inoculum into the soil. Then the strain 139SI moved from rhizosphere soil to colonize plant root and finally enter the internal root tissue. The larger population of strain 139SI presence on plant root compared to the internal root tissue is similar to the phenomenon previously observed by Compant *et al.* (2005).

A key feature of efficient root colonization is attachment of bacteria to the plant root. Among factor that might be involved in plant root-bacterial attachments are plant lectins, a Ca^{2+}-binding bacterial protein called rhicadhesin and biofilm (Smit *et al.,* 1991; Rudiger & Gabius, 2001; Rodr´ıguez-Navarro *et al.,* 2007). The results of the present study found that strain 139SI attachment to the plant root occur in the form of biofilm. The ability of this strain to form biofilm is in line with our previous results (Ismail & Dadrasnia., 2015). Biofilm is populations of microorganisms in which the cells stick to each other and adhered to environmental surfaces in which sufficient moisture is present. These adherent cells usually embedded in self-produced polymeric matrix (Costerton *et al.,* 1995). A similar result was reported by Timmusk *et al.* (2005), where they found that *Paenibacillus polymyxa* colonized and forming biofilm on the root tip of *Arabidopsis thaliana*. According to Rodr´ıguez-Navarro *et al.* (2007), sufficient moisture and nutrients provided by the plant in the rhizosphere create an environment that fulfills the requisites for biofilm formation. Biofilm is one of the life

strategies for bacteria in natural environments. By adhering to plant roots, the bacteria will be able to exploit various compounds in root exudates such as sugar, amino acid, organic acid and vitamin for their survival (Dakora & Phillips, 2002). This would explain the ability of the strain to maintain its high population in rhizosphere even after 3 weeks of inoculation.

Generally, PGPR enhances plant growth by increasing the nutrient availability for the plant, acts as phytostimulation by producing growth hormone, or serves as biocontrol agents by reducing the adverse effects of various pathogens on plant growth and development (Ahemad & Kibret, 2014; Hayat *et al.,* 2010). In recent year, inoculant preparation consists of several PGPRs or rhizobia received a lot of attention. This is to enhance the plant growth-promoting effect of the inoculant. However, this technique could create competition for the same niches in the rhizoplane and sometimes mask the growth-promoting features of some PGPR as reported by García *et al.* (2004). Thus, it is a great advantage to use a single strain of PGPR that have several plant growth-promoting features rather than using several strains of PGPR in an inoculation.

Results of nursery experiment found that integrating the application of strain 139SI inoculant with inorganic fertilizer on oil palm seedlings produced profound synergistic effects on various growth parameters especially biomass of the palm and nutrient uptake. This interesting feature was noticed based on the amplifying effects of strain 139SI inoculant on palm growth with the presence of inorganic fertilizer. A significant enhancement of palm vegetative growth, as well as enrichment of palm nutrient concentration, were observed in the group treatment involving palm received a combination of strain 139SI inoculant and fertilizer over the palm that received fertilizer only. The results obtained from this nursery trial are also in line with our previous pre-nursery study. Previously, we found that the strain 139SI exhibited several promising

plant growth-enhancement characteristics such as involved in biological nitrogen fixation (BNF), act as phosphate solubilizer, produce IAA, and siderophore.

Obviously, inoculation of strain 139SI induced massive root growth of oil palm seedlings as observed in T3 and T4. The enhanced of root development of the 139SI inoculated palms were manifested by the increase of their roots dry weight compared to the fertilized palm. This strongly suggested the influence of phytohormone IAA produced by the strain on root growth. There were a lot of reports from the previous study that showed IAA-producing PGPR helps plant to develop deeper and more widespread root that will enable the plant to obtain more nutrients and eventually enhances plant growth since the plant root can exploit larger volume of soil (Ribeiro *et al.,* 2014; Calvo *et al.,* 2017). Improved nutrients acquisition due to the large root growth-promoting effects of the inoculant was also observed in the present study, clearly demonstrated by oil palm seedlings of T1 and T2 (Table 4.2). Furthermore, the strong relationship between root growths with nutrient uptake was indicated by the positively significant of Pearson correlation test. This would also explain the higher concentration of nutrient content that was recorded in the inoculated palm compared to the untreated control even though no fertilizer was supplied on both treatments.

Besides that, the addition of ammonium sulfate fertilizer induced soil acidification by 2 pH units in C2 and T2 (Table 4.5). Rhizosphere acidification by the ammonium-based fertilizer was anticipated due to hydrogen ion (H^+) formation during nitrification of the ammonium in fertilizers, which in turn increased the acidity of the soil. Ammonium-based fertilizers also induce acidification of rhizosphere soil due to proton excretion by root cells. Hydrogen ion is secreted during ammonium absorption by plant roots to maintain charge balance across the membranes of the plant cell walls (Zaccheo *et al.,* 2006). Slightly decrease in rhizosphere soil pH can strongly increase the solubility of nutrients and consequently,

improve plant nutrient uptake (Dakora & Phillips., 2002) as also observed in the nursery stage of the present study. Thus, the combination of the rhizosphere acidifying potential resulting from the inorganic fertilizer application, which could further increase the availability of sparingly soluble nutrients such as P and other micronutrients, together with the highly significant stimulation of root growth mediated by the inoculant would induce synergistic effects on nutrient acquisition. Earlier observations reported by Liu *et al.* (2013) and Arif *et al.* (2016) showed that addition of non-endophytic associative PGPR inoculant significantly increased fertilizer use efficiency. The respective researchers were also reported the enhancement of parameters that represent root development, such as increased of root dry weight or root length, indicating the improvement of root system development help better absorption of nutrients from fertilizer.

Interestingly, strain 139SI inoculation was also induced acidification of rhizosphere soil by 1.5 pH unit as observed in T1 (Figure 4.5). This is usually characteristic for application of phosphate solubilizing bacteria, particularly in neutral and alkaline soils. Phosphate solubilizing bacteria reduce the pH of rhizosphere soil through the production of low molecular weight organic acids such as gluconic and keto gluconic acids to dissolve soil P as thoroughly discussed by Goldstein (1995) and Deubel *et al.* (2000). The substantial increase of P uptake of inoculated palm over the untreated as observed in the present study suggest the dual effects of IAA phytostimulation on root growth coupled with phosphate solubilization, facilitate P acquisitions by the plant. Meanwhile, according to Miransari (2013), only a small portion of P from fertilizer is available for the plant because the remaining part would precipitate and become unavailable. The use of phosphate solubilizing PGPR with chemical fertilizer was found to be effective to improve the availability of P in the soil since such PGPR are able to transform the insoluble P into soluble forms (Oufdou *et al.,*

2016). Thus, integrating PGPR with inorganic fertilizer could be a potential strategy in nutrient management system to increase crop production and at the same time, the sustainable practice in agriculture could be achieved.

Improving soil fertility by inoculating beneficial microbes, such as the strain 139SI used in this study is environmentally yet economically practice to reduce synthetic N fertilizer application. In the present study, the increased of N concentration of T1 rhizosphere soil can be hypothesized due to the ability of the strain 139SI to fix the atmospheric N_2. Enhanced of N concentration in the vicinity of plant roots due to PGPR inoculation was also reported previously (Prasanna *et al.,* 2016). In other reports, by using ^{15}N isotope dilution technique, Kuan *et al.* (2016) suggest that non-symbiotic PGPR can provide crops with significant quantities of N derived from the atmosphere. However, on the contrary, the study was done by Biswas *et al.* (2000) indicated that the increase of N uptake due to inoculation of certain strains of rhizobia most likely was through mechanisms that involve changes in growth physiology or root morphology rather than BNF. This is due to the high energy required in BNF process as well as the relatively low metabolic activity of free-living organisms that must compete for root exudates in rhizosphere leads to limiting the ability of non-symbiotic bacteria to fix significant quantities of N for plant use (Martínez-Viveros *et al.,* 2010). This was supported by the study done by Okon and Lanbandera-Gonzalez (1994), which discovered contribution of N derived from BNF in the plant tissues of sorghum, maize, and wheat inoculated with *Azospirillum* was insignificant. Thus, in face of the well-documented difficulties in transferring observations of BNF properties of rhizobacteria on artificial media to real rhizosphere conditions, the increased of N uptake recorded in the inoculated palm of T1 and T2 is inferred was contributed most likely due to the enhancement of root morphology induced by IAA phytostimulation. The ability of

strain 139SI to increase the percentage of N derived from the atmosphere in plant tissue need to be further confirmed by using ^{15}N isotope technique.

At the end of treatment, a mild chlorotic symptom was also observed on untreated palm's leaves grown in the nursery. Further analysis has confirmed that lower chlorophyll content was recorded in untreated palm compared to the other treatments. It was also noted that the chlorophyll levels affected photosynthetic process directly as the result showed a higher photosynthetic rate was recorded in the well-nourished palm over the palm suffered from malnutrition. The increase in chlorophyll levels of palm's leaves was postulated as the result of the enhancement of nutrient uptake from soil. This was strengthened by the outcome of correlation analysis that found the chlorophyll levels and photosynthetic parameters were significantly correlated with nutrient uptakes especially N, Mg and Fe. As deeply discussed in the previous report, these elements are crucial for chlorophyll synthesis and photosynthesis process. The N and Mg are part of the important element in the chlorophyll molecular structure, thus, explaining the positive correlation between N and Mg with chlorophyll content reading. Cechin and Fumis (2004) and Vos *et al.* (2005) also suggested that leaf expansion and photosynthetic activities are positively dependent on N uptake. Besides that, Mg ion is involved in the ribulose bisphosphate carboxylase activation, an enzyme that catalyzes carbon fixation process in the dark phase of photosynthesis (Andersson, 2008).

Meanwhile, the positive correlation between chlorophyll content of palm's leaves and iron uptake is agreed with those of previous reports, in which low of Fe uptake will induce rapid reduction in the content of photosynthetic pigments (Donnini *et al*., 2013; Osório *et al*., 2014; Gama *et al*., 2016). Furthermore, Ferritin, which stores and releases Fe, makes up about 75 % of the content of chloroplasts, thus make Fe is essential for the chlorophyll synthesis (Marschner, 1995). In their reports, Vansuyt *et al*. (2007) and Nagata *et al*. (2013) showed that plant supplemented with

siderophore extracted from PGPR can increase Fe content of the plant and enhance chlorophyll synthesis. In other report, Radzki *et al*. (2013) demonstrated that siderophore-producing bacteria, *Chryseobacterium* C138 treatment was found effective in providing accessible form of Fe to iron-starved tomato plant, eventually resulting in significantly increased chlorophyll levels and iron content. Thus, the higher Fe content of 139SI-inoculated palm suggests that the siderophore produced by the strain 139SI as well as IAA phytostimulation induction on root growth may assists the Fe uptake.

Considering the high amount of inorganic fertilizer needed for maintaining high yield production in oil palm plantations and the promising potential of strain 139SI in maximizing the fertilizer used efficiency, it is worth pursuing this study to determine the applicability of this PGPR in field trials. The results obtained from a one year of field trial in this research had provided sufficient evidence for the first time the positive effect of PGPR inoculation on oil palm FFB yield, especially when integrating with inorganic fertilizer, which was manifested by palm in the T4 treatment (Table 4.12). The increasing of FFB yield clearly could be attributed by the enhancement of soil fertility and eventually improving the nutrient uptake of the palm. It is apparent from the analysis of nutrient content in soil, the N level was higher in 139SI inoculated T3 and T4 treatment (Table 4.10). This data were consistent with our previous pre-nursery and nursery trial finding indicating the ability of strain 139SI to fix N_2. There were a lot of researchers reported PGPR enhance plant growth and yield, as well as enriching nutrients in plants via increased mineral uptake and assimilation (Adesemoye *et al*., 2009; Souza *et al*., 2015; Berger *et al*., 2017).

Apart from enhancing N in soil, strain 139SI also play an important role in enhancing nutrient availability to plants by solubilizing P from soil. Most of large reserves of P in soils is not soluble, which cannot be absorbed by plants and consequently limiting the plant growth and yield (Pérez-Montaño *et al*., 2014).

Furthermore, only a small percentage of P from fertilizer application is available for the use of plant because the remaining part would undergo processes such as desolubilization and precipitation (Zabihi *et al*., 2011). Thus, increasing the population of phosphate solubilizing bacteria through inoculation is a great advantage in order to turn insoluble P to plant usable form. The effectiveness of using 139SI to enhance P availability in this study was proven with the increasing of P uptake by palm. In a similar way, PGPR such as *Azospirillum, Bacillus, Burkholderia, Pseudomonas,* and *Rhizobium* are reported able to enhance P uptake by means of solubilizing P through acidification, enzymatically or chelation (Sudhakar *et al*., 2000; Mehnaz & Lazarovits, 2006; Hameeda *et al*., 2008; Richardson *et al*., 2009; Pereira & Castro, 2014).

In addition, previous research also found that inoculation of PGPR could enhance plant uptake of several other nutrients such as K, Mg, Ca, S, Cu, Mn and Zn (Karlidag *et al*., 2007; Meyer, 2007; Rana *et al*., 2012; Goteti *et al*., 2013). Usually, slightly decrease in soil pH improves solubilization of these nutrients. This uptake usually occurs during acidification of the soil rhizosphere via organic acid production by PGPR or via stimulation of proton pump ATPase (Pérez-Montaño *et al.,* 2014; Miransari & Smith, 2007; Mantelin & Touraine, 2004). Enhancement in macronutrient uptakes are also known to trigger changes in the mineral uptake rate (Gastal & Saugier, 1989; Touraine *et al*., 1994). Besides that, the direct effect of IAA produced by PGPR on root development also contributes in macro and micro nutrient uptake enhancement. This would explain the slightly fall in soil pH and the enhancement of micronutrient uptake observed in T3 and T4 treatment (Table 4.10).

It was also noted that 139SI inoculation and fertilizer application effect mainly on FFB weight rather on the number of bunches produced from palm. This is based on the results of the field trial that shows the higher FFB yield in T2, T3 and T4 over the T1 were greatly influenced by the higher average of bunch weight (Table 4.12). The

fluctuation in FFB yield every month is due to variation in number of bunches produced. According to Chow (1988) the seasonal variation in number of bunches produced is largely influenced by climate, especially rainfall. This would explain the insignificant effect of 139SI inoculation and fertilizer application on numbers of bunches produced by palm. The pattern of FFB yield from this study is also parallel with the average of FFB yield ha^{-1} reported by Malaysia Oil Palm Board (MPOB, 2015; MPOB, 2016). Based on 2015 and 2016 report, the FFB yield was highest in August – September 2015 and lowest in January – February 2016. The same insignificant effect of 139SI inoculation and fertilizer application was also observed in the oil extraction rate. However, the performance of the oil extraction rate of palm from this study is slightly higher to the average of oil extraction rate performance data record by MPOB which was 20.23% (MPOB, 2016). The difference of the oil extraction rate obtained from this study with the recorded by MPOB is due different technique employed to extract the palm oil.

Analysis of fatty acid composition is important because it could be used to evaluate the nutritional quality of palm oil. However, to date, there is no information regarding the effect of palm nutrient uptake on the nutritional quality of palm oil. Previous research reported that the fatty acid content in palm oil changes over the ripening period and at optimal harvesting stage or 22 weeks after anthesis, SFA recorded the highest composition of fatty acid followed by MUFA and PUFA (Prada *et al.,* 2011). Generally, the data obtained from the present research were consistent with previous reports for crude palm oil fatty acid composition. In their research, Bafor and Osagie (1986), Sambanthamurthi *et al.* (2000), Edem (2002) and Prada *et al.* (2011) reported that SFA represented the biggest proportion of fatty acid in palm oil, i.e. around 40 – 50% of total fatty acid, whereas MUFA and PUFA represented 35 – 40% and 10 – 15% of total fatty acid respectively.

Eventhough the results from this field trial gave valuable insight on the integrating PGPR application with chemical fertilizer, more data is required from field trials to confirm the potential of this technique. Furthermore, since agronomic practice starting from planting of seedling to the later mature phase is crucial to determine FFB yield, a complete study on PGPR application during this period will provide more information on the effect of this procedure on yield produced. The insignificant results of number of bunch produced, the oil extraction rate and the quality of palm oil produced from PGPR inoculated plam indicated that a longer period of treatment is required to validate the results of the present study.

Overall, the main purposes of this study were to evaluate the effects of PGPR application on soil fertility, oil palm nutrient uptake, growth and physiology, as well as FFB yield harvested. The results of the present study are not only revealed the effects of PGPR application on different stages of palm growth, but also provide a clearer perspective on the great potential of its use for improving oil palm yields. Based on the findings from pre-nursery, nursery and field experiment, it can be suggested that the application of PGPR in the agronomic practice of oil palm industry can exert positive effects for production of high quality seedling, yield enhancement and for more sustainable agronomic practice of oil palm industry.

High quality of planting material is indispensable for the seedling to survive in various condition of planting site. Therefore, productions of good quality of oil palm seedlings are very important. The oil palm breeding process starts with the selection of good parental breed to produce progeny with the desirable characteristic. Next at the nursery stage, the seedling will undergo culling process to remove all runts, abnormal and unproductive seedlings. At this stage, inoculation of PGPR could assist the establishment of palm-PGPR interaction in the rhizosphere. Since certain PGPR able to colonize and attach to the roots as shown by strain 139SI, the PGPR could still remain

in the rhizosphere during transplanting process. The early stage of transplanting to the site is critical in view of the seedling need to adapt to the new environment and well established of palm-PGPR interaction could reduce the stress. This fact was evidenced by a lot of report from previous studies that found PGPR can play important role in adaptation of agriculture crops to various biotic and abiotic stresses (Grover *et al.,* 2011).

The potential of PGPR in improving crop yields by augmenting nutrient availability, enhancing plant growth and providing protection to plants from diseases and pests provide an alternative approach in maintaining the fertility of soil. However, application of PGPR in oil palm agronomic practice is still scarce. This is contrary to the current trend in which these beneficial microbes are increasingly being used for more sustainable agriculture practice. Based on the results of the present study, we found that the best approach to apply PGPR in oil palm agronomic practice is by integrating its use with inorganic fertilizer. Integrating PGPR application with inorganic fertilizer significantly increase FFB yield by enhancing plant nutrient uptake, hence at the same time, optimizing the efficiency of the fertilizer used.

In addition, optimization the efficiency of the fertilizer used by integrating inorganic fertilizer application with PGPR could offer another approach that can be further explored that is integrating PGPR with a reduced rate of inorganic fertilizer. Several studies have showed that reduced rates of inorganic fertilizer coupled with PGPR inoculants still could produce plant growth, yield, and nutrient uptake levels equivalent to those with full rates of the fertilizer (Adesemoye *et al.,* 2009). In that way, nutrient management cost can be reduced without compromising crop yield.

Maintaining soil fertility is one of the major challenges faced in oil palm industry, as oil palm is well known for having a high fertilizer input demand to sustain high yields. A huge amount of inorganic fertilizer application is popular option to tackle this problem; however, minimizing the application of inorganic fertilizer and concurrently optimizing the efficiency of the fertilizer used are among the issues highlighted by The Roundtable on Sustainable Palm Oil (RSPO) committee to achieve more sustainable of oil palm industry.

Integrating of inorganic fertilizers application with PGPR hold great promise in achieving more sustainable oil palm's agronomic practice by looking at PGPR's potential in optimizing the efficiency of the fertilizers used as shown in this study. The use of PGPR has been proven to be an environmentally sound option to increase crop yields since their use in agriculture can favor a reduction in agro-chemical use. A reduction in the amount of inorganic fertilizers used to replenish soil nutrient will directly result in decreased environmental pollution. Furthermore, the enhancement in the efficiency of the fertilizer used in integrated PGPR-inorganic fertilizer agronomic practice could reduce greenhouse gas emissions from fertilizers and nutrient loss due to leaching that can cause water pollution.

CHAPTER 6: CONCLUSION

Based on the results of pre-nursery, nursery and field experiment, it can be concluded that all objectives outlined in this research were successfully addressed. The results of pre-nursery study suggest that the novel strain of *B. salmalaya* strain 139SI has promising features as plant growth promoting rhizobacteria. The early screening on the plant growth-promoting features of the *B. salmalaya* strain 139SI showed that the strain was positive for indole acetic acid (IAA) and siderophores production. The strain was also involved in biological nitrogen fixation (BNF) and able to solubilize phosphate, thus, indicates that the strain promotes palm growth via phytostimulation and increase the availability of nutrients to the plants. Analysis of the plant-microbe interaction between *B. salmalaya* strain 139SI with plant root in rhizosphere niche by analyzing the distribution and root colonization pattern of strain 139SI showed that the strain colonized and attached to the root surface by forming a biofilm. The strain 139SI was also identified as endophytic bacteria as it shows the ability to colonize plant rhizosphere and penetrate into the plant internal root tissue. Inoculation of this strain at the early stage of the seedling growth could enhances the growth performance of the seedling, thus, could increase survival rates once transplanted to the field.

The plant growth promoting features of strain 139SI were further confirmed by the growth enhancement of oil palm seedling inoculated with this strain in the nursery experiment. The results of nursery experiment also successfully demonstrated the strong synergistic interactions between ammonium-based fertilizer with strain 139SI inoculant in terms of nutrient uptake and plant growth. Addition of 139SI inoculation to the fertilized palm produces the best results for plant growth and significantly enhanced nutrient uptake. A slight reduction in pH of rhizosphere soil resulting from the ammonium-based fertilizer application together with the highly significant stimulation of root growth mediated by the inoculant has induced synergistic effects on nutrient

acquisition. The increased of nutrient acquisition of inoculated palm is also inferred was contributed by the enhancement of nutrient availability due to the action of siderophore produced by the strain as well as phosphate solubilization. In addition, analysis of soil nutrient content found that inoculation of strain 139SI increases total N content in the soil. Ultimately, enhanced in palm nutrients uptake has directly increased the photosynthetic activity.

Overall, the present study revealed the potential of integrating the usage of inorganic fertilizer with strain 139SI to optimize nutrient uptake by palm. This is evidenced by the results of the experiment at nursery and field stage that have shown the beneficial effects of strain 139SI inoculant on the improvement of palm nutrient uptake and eventually leads to an enhancement of oil palm fresh fruit bunch (FFB) yield. A one year of field experiment found that inoculation of *B. salmalaya* strain 139SI produced higher palm FFB yield over the untreated. Field experiment also revealed that combination of strain139SI inoculant and inorganic fertilizer produced the best FFB yield performance. Integrating the 139SI inoculant with inorganic fertilizer resulted in more substantial FFB yield than palm received recommended inorganic fertilizer rate. Enhancement of N level in soil samples from field site and nutrient uptake was also recorded in strain 139SI inoculated palm. While the number of bunches produced by palm, the oil extraction rate, and fatty acid profile shows comparable reading among all treatments.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

A) Scientific Peer-Reviewed Journal Articles

1. Azri, M. H., Ismail, S., & Abdullah, R. (2018). An endophytic *Bacillus* strain promotes growth of oil palm seedling by fine root biofilm formation. *Rhizhosphere, 5*, 1 -7.

2. Azri, M. H., Ismail, S., and Abdullah, R. (2018). Effects of *Bacillus salmalaya* strain 139SI inoculation on yield and nutrients uptake of oil palm. *International Journal of Agriculture and Biology*, *20*(3), 499 – 506.

B) Scientific Conference Proceedings

1. Azri, M. H., Ismail, S., and Abdullah, R. (2016). Promotion of oil palm seedling (*Elaeis guineensis* Jacq.) growth by *Bacillus salmalaya* strain 139SI (Oral Presentation). *International Postgraduate Research Awards Seminar (InPRAS)*, University of Malaya, Kuala Lumpur, Malaysia.

2. Azri, M. H., Ismail, S., and Abdullah, R. (2016). Effects of *Bacillus salmalaya* strain 139SI inoculation on yield and palm oil quality (Oral Presentation). *Malaysian Society for Microbiology Postgraduate Seminar (MSMPS2016).* Universiti Kebangsaan Malaysia, Bangi.

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An endophytic *Bacillus* strain promotes growth of oil palm seedling by fine root biofilm formation

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ABSTRACT

Plant-microbe interaction is one of the most important determining factors that could influence plant health and soil fertility. In this research, plant-microbe interaction between *Bacillus salmalaya* strain 139SI and oil palm (*Elaeis guineensis* Jacq.) was initiated by inoculating *B. salmalaya* strain 139SI at the early stage of oil palm seedling growth. Colonization of the strain 139SI on oil palm seedling roots and its mechanisms of plant growth promotion were evaluated and characterized. Analysis of strain 139SI colonization showed that the strain colonizes and attached to the root surface by forming biofilm. The strain 139SI was identified as endophytic bacteria as it shows the ability to colonize plant rhizosphere and penetrate into the plant internal root tissue. The results also indicate that the strain was positive for indole acetic acid (IAA), nitrogen fixation, phosphate solubilization, and siderophores production. The plant growth promoting features of strain 139SI were further confirmed by growth enhancement of oil palm seedling inoculated with this strain. The overall findings of this study suggest that associations of this novel strain could enhance growth quality of oil palm seedlings, hence, enable better adaptation of the seedlings to the environmental conditions of the planting site.

1. Introduction

Agriculture area cultivated with oil palm (*Elaeis guineensis* Jacq.) in Malaysia has expanded tremendously in recent years. First introduced as an ornamental tree in early 1870s, oil palm has since undergone significant leaps in production and planted areas to become the most important industrial crop. Palm oil production has risen by more than 2 million tonnes in the past 10 years from 15.8 million tonnes in 2007 to 17.3 million tonnes in 2016. The total area planted with oil palm had increased from 4.3 million hectares in 2007 to 5.7 million hectares in 2016, an increase of 32.6% (MPOB, 2017). Various factors have contributed to spurring oil palm industry occupying its present position, including the introduction of high-quality planting material through genetic improvements and also the implementation of good agronomic practices (Wahid et al., 2005).

Breeding and selection of high-quality planting material for oil palm plantation industry are not only focusing on achieving high fresh fruit bunch yield, but the quality of the oil produced and desirable vegetative characters such as reduced rates of trunk extension and long bunch stalks are also taken into account (Basri et al., 2003 0in press). Strong demands for high-quality planting material has initiated the efforts to propagate the oil palm through tissue culture. Vegetative propagation of oil palm by tissue culture allows rapid multiplication of uniform planting materials with desired characteristics (Sogeke, 1998).

Besides that, inoculation of plant growth-promoting rhizobacteria (PGPR) at the early stage of oil palm seedling growth is one of the promising alternative strategies as a measure to produce high-quality planting material. Inoculation of PGPR to oil palm seedling at the early stage of growth would enable early associative interactions between the bacteria and the seedling. These early associations would enhance adaptation of the seedlings to the environmental conditions, hence, increase the survival rate (Sturz and Nowak 2000; Azlin et al. 2007). Previous studies have shown that inoculation of PGPR at the early stage of plant growth could significantly enhance survival rates of the host plants (Pandey et al. 2000). Thus, in this study, the potential of a new strain of soil bacteria, *B. salmalaya* strain 139SI, as plant growth promoting bacteria were evaluated. Subsequently, the effects of strain 139SI inoculation at the early stage of oil palm seedling growth and the colonization of the strain on palm root were also determined.

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Full Length Article

Effects of *Bacillus salmalaya* Strain 139SI Inoculation on Yield and Nutrients Uptake of Oil Palm

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Abstract

Integrating the application of inorganic fertilizer with bacterial inoculants could enhance plant nutrient uptake and increase crop yield. However, their effects on oil palm plantation industry are still less studied. Thus, this study was designed to evaluate the potential of *Bacillus salmalaya* strain 139SI inoculant on the enhancement of soil fertility, nutrient uptake, yield and eventually, the quality of oil produced. The results demonstrate that inoculation of *B. salmalaya* strain 139SI produced higher palm fresh fruit bunch (FFB) yield over the untreated. Integrating the 139SI inoculant with inorganic fertilizer resulted in more substantial FFB yield than palm received recommended inorganic fertilizer rate. Enhancement of N level in soil and nutrient uptake was also recorded in strain 139SI inoculated palm. While the number of bunches produced by palm, the oil extraction rate and fatty acid profile shows comparable reading among all treatments. Thus, the results suggest that the application of *B. salmalaya* strain 139SI inoculant and inorganic fertilizer is effective for improving soil fertility, nutrient uptake and yield of oil palm. © 2018 Friends Science Publishers

Keywords: Plant growth promoting rhizobacteria; Soil fertility; Crop yield; Fatty acid analysis

Introduction

Palm oil, the oil derived from the mesocarp of a tropical crop of *Elaeis guineensis* Jacq, is of one the most consumed vegetable oil in the world. Oil palm was introduced in Malaysia in early 1870's as an ornamental plant, since then, it has undergone expansion and modernization to become the most important commodity crop in Malaysia with total area cultivated with oil palm in 2016 reached 5.74 million hectares (MPOB, 2017). Many factors involved in spurring the oil palm industry, of which, agronomic practice plays one of the most important roles. This is because oil palm growth and yield are recognized for highly responsive to fertilizer input. A high demand for nutrients, especially from fertilizer input is not surprising, in view of its high dry matter production (Wahid *et al*., 2005).

Based on previous studies, a hectare of oil palm plantation is estimated to require approximately between 100 to 200 kg/year of nitrogen (N), 50 to 100 kg/year of phosphate (P), and 200 to 300 kg/year of potassium (K) for optimum yield production (Wahid *et al*., 2005; Bakar *et al*., 2011; Lee *et al*., 2014). As a comparison, a hectare of wheat required nutrient inputs of N, P, and K at 100 to 120, 60 to 75 and 50 to 60 kg/year, respectively (Khalid *et al*., 2004; Rana *et al*., 2012). While rice needed 100 to 180 kg/year of N, 50 to 100 kg/year of P and 60 to 120 kg/year of K for every hectare (Sagarika *et al*., 2015; Xu *et al*., 2015; Hoseinzade *et al*., 2016). These huge amounts of fertilizer are needed by oil palm to replace the nutrients that are removed continuously through the harvested fresh fruit bunch (FFB). However, nutrient leaching, precipitation, erosion, volatilization and denitrification could cause low fertilizer use efficiency since the major portion of the applied inorganic fertilizers is not available to the plants (Powell *et al*., 2010). Furthermore, over-fertilization may result in undesired economic and environmental problems, including underground water contamination due to nitrate leaching into waterways, increased gaseous emissions of ammonia and nitrous oxide to the atmosphere and soil degradation that could cause a decline in crop yields (Diacono and Montemurro, 2010; Zaman *et al*., 2015).

As attempts to address this problem, the best agronomic practices that are efficient, sustainable and less harmful to the environment have to be developed. One of the best options is integrating the application of inorganic fertilizer with microbial inoculant such as plant growth-promoting rhizobacteria to optimize the use of fertilizer and minimize nutrient losses. As reported by previous research, integrated nutrient management with inorganic fertilizers and microbial inoculant can improve crop productivity as well as soil fertility (Tiyagi *et al*., 2015; Hoseinzade *et al*., 2016; Thilagar *et al*., 2016). The beneficial effects of these

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