THE EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) FROM SABAH TEA PLANTATION ON THE GROWTH PERFORMANCE OF *Amaranthus gangeticus* L.

IYLIA HUZAIFAH BINTI ZAMRI

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[IYLIA HUZAIFAH BINTI ZAMRI]
BR10110031
17Th JANUARY 2014
1. Mr. Clament Chin Fui Seung
   SUPERVISOR

2. Assoc. Prof. Dr. Harpal S. Saini
   EXAMINER 1

3. Madam Devina David
   EXAMINER 2

4. Assoc. Prof. Dr. Sitti Raehanah binti Muhamad Shaleh
   DEAN of School of Sustainable Agriculture
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ABSTRACT

This study was carried out to test the effect of plant growth promoting rhizobacteria (PGPR) isolated from tea roots on the growth performance of red spinach, *Amaranthus gangeticus* L.. The mode of action of the PGPR that have been focused were the ability in synthesizing particular compounds of plant growth regulator (auxin) and facilitating nutrient uptake such as phosphorus and nitrogen. A total of 17 selected strains were tested in laboratory for its capability in producing auxin and/or exerting phosphate solubilizing activity. The auxin (IAA) concentration was determined by using spectrophotometer at 535 nm. Although the result showed no significant difference between the isolates, the IAA produced varied based on the PGPR strains. Phosphate solubilizing and nitrogen fixing ability were qualitatively determined with PVK's and N-free medium respectively. Almost all isolates were capable in fixing atmospheric nitrogen, while only six isolates has ability in phosphate solubilizing. Isolate S3 was the only bacteria that showed positive reaction on antifungal activity against red root disease and basal stem rot of oil palm. In the pod trial, 20 treatments including two control sets of red spinach were measured based on the plant growth parameters such as plant height, leaf area, total dry weight, soil pH and electrical conductivity (EC). The red spinach treated with selected PGPR strains showed significant difference over control on the mean of plant height, leaf area and total dry weight. As for soil pH and EC, the results diversified between the inoculated soil and non-inoculated soil, since numerous factors may contribute to such findings, especially in the field condition. In short, isolate S10 was observed as the best among the isolated PGPR strains from Sabah Tea roots, due to the fact that it has all beneficial traits in improving plant growth performance of red spinach, *Amaranthus gangeticus* L..
KESAN RHIZOBAKTERIA PENGGALAK PERTUMBUHAN POKOK (RPPP) DIPENCILKAN DARIPADA LADANG SABAH TEA KE ATAS PRESTASI PERTUMBUHAN Amaranthus gangeticus L.

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CHAPTER 1

INTRODUCTION

1.1 Brief Background

Bacteria are one of an essential component in soil and simply bound together since both have strong interaction with each other. Since the discovery of microorganism several decades ago, many research reported that soil bacteria greatly affect the soil fertility as well as the condition of plant growth through their role in several biochemical transformation and mineralization activities (Gaddeya et al., 2012). Soil bacteria can be further divided into various classes which had been in contributing mainly for plants is called plant growth promoting rhizobacteria (PGPR). Basically, this kind of bacteria found in the area surrounding the plant tissues either growing in, on or around the roots (Vessey, 2003). According to Figueiredo et al. (2010), the contribution of PGPR in implementing sustainable agriculture practices receives a huge acceptance all over the world.

The term PGPR arise after much finding of beneficial effect existed between the bacteria living around the rhizosphere of plant and the soil. The roles of PGPR that commonly react in three distinct ways via inducing colonization of roots and stimulation growth of various plants, in such a manner of making more nutrients available for the
uptake, besides the ability to synthesize specific compounds for the usage of plant, and also effectiveness in suppressing the pathogen attacks on plants (Hayat et al., 2010)

Tea plant, *Camellia sinensis* is regarded as a commercial crop in Malaysia considering its economic value in the market demand and production since the beverages has become a tradition drink. Apparently, tea plants adapt well in subtropical and tropical region countries all over the world (Sedaghathoor et al., 2009) including Sabah, a Borneo island which is suitable for cultivating tea plants in a rainforest area with its rich soil. Sabah Tea plantation mainly grown its tea tree without the application of chemical pesticide, instead they control the pest by using biological and natural method. Therefore, the roots play a major role in absorbing the nutrient from the soil surrounding it or simply known as rhizosphere as to ensure an optimum growth performance of the tea trees.

Auxin or the natural auxin namely as indole-3-acetic acid (IAA) is categorized in the active hormone of plant which produces by soil bacteria as secondary metabolites. The bacteria which practically inhabit the surrounding of the rhizosphere has somewhat developed a symbiosis relationship where eventually encourage the stimulation of root growth and its development. Additionally, the synthesizing of auxin occurs due to the presence of the precursor called tryptophan that undergoes different types of pathways (Woodward and Bartel, 2005). This phytohormone indulge with roots as it promotes the growth of root hairs and lateral roots by multiplying the amount since root is crucial part for plants. Lateral root help the plants to absorb nutrient which available on the surface parts of the soils, whereas tap root has more advantage as it can penetrate deep into the soil.

Phosphate-solubilizing bacteria literally enhance the availability of phosphorus (P) in soil as one of the macronutrient for plant since it is strongly held by the soil particles, making it hardly available for plant uptake. Phosphorus exists in soils in two forms either as an organic or inorganic which mostly presence due to the application of chemical fertilizers. Problem concerning nutrient deficiency particularly for phosphorus occurred long time ago and it is believed that this serious issue in agriculture arises due to the limited P in the form of plant uptake. Research conducted stated about the depleted
amount of P in soil solution as well as the nutrient is found to be bound together in a way with soil minerals or organic materials. A natural mineralization process which been carried out by microorganisms is the only pathway that can help to release organic P into the soil through organic material decomposition.

1.2 Justification

Agriculture practices have been claimed by most of the researchers around the world as the major contributor in environmental pollution mainly because of intense used and application of chemical inputs. Due to the fact, implementation of sustainable agriculture practices through assimilation of microorganisms with soils recently reported to be high-rocketed (Vessey, 2003). This so called biofertilizer which derived from biology and fertilizer word sometime defined by combining both concept of living organisms into improving the growth performance and quality of the crops. Therefore, the study of plant growth promoting rhizobacteria (PGPR) as one of the significant element in biofertilizer become the high interest scope of study due to its mechanism of action that benefit plant the most. According to Mohammadi and Sohrabi (2012), the characteristics of biofertilizer which favor the farmers in applying it are their ability in sustaining the soil fertility and in the same time help to increase the yield of productivity. However, only few study of PGPR isolated from tea roots were reported in their beneficial usage (Tennakoon, 2007), hence this study was an effort done for better understanding of PGPR effect from tea roots to the growth performance of leafy vegetable, *Amaranthus gangeticus* L..

1.3 Objectives

There are two aims of the study which are:

1. To isolate PGPR from tea roots that are capable to produce auxin and exert phosphate solubilizing activity
2. To test selected PGPR strains on the growth performance of red spinach (*Amaranthus gangeticus* L.) seedling
1.4 Hypotheses

$H_0$: 

i. There is no PGPR present in the rhizosphere of tea plant, *Camellia sinensis* that are capable in producing auxin and exert phosphate solubilizing activity

ii. There is no significant difference on the growth performance of red spinach seedling (*Amaranthus gangeticus* L.) after being treated with PGPR

$H_a$: 

i. There is PGPR present in the rhizosphere of tea plant, *Camellia sinensis* that are capable in producing auxin and exert phosphate solubilizing activity

ii. There is a significant difference on the growth performance of red spinach seedling (*Amaranthus gangeticus* L.) after being treated with PGPR
CHAPTER 2

LITERATURE REVIEW

2.1 Plant Growth Promoting Rhizobacteria (PGPR)

Rhizobium or rhizobacteria is a term derived from the combination of two words which are rhizosphere and bacteria, since this group of bacteria most abundant in the area within or surrounding the plant roots. Kloepper et al. (1991) stated that larger ecological niche including the root colonization with bacterial population that are not directly attached to the root but within the area by a process called rhizosphere colonization. The long existence of rhizobacteria has develop a symbiotic relationship with the roots especially in legume crops where rhizobia promote the nodules formation that enable nitrogen fixation process from the air for plant uptake (Tortora, 2010). In general, PGPR has numerous definitions depending on the understanding and experienced, nevertheless, PGPR is actually bacteria found around root area which wholly promote plant growth via colonization with roots.

According to Ahmad et al. (2008), PGPR comprises of various group of bacteria that associate with roots in order to improve the plant growth. In addition, some described PGPR as bacteria having a mutual relationship with plant roots benefiting each other that lead to increase plant growth, quality and also yield (Boon and Shamsuddin, 2011; Jamil et al., 2011; Saharan and Nehra, 2011; Hayat et al., 2010; Loon, 2007; McMillan, 2007; Teixera et al., 2007; Roesti et al., 2005; Husen, 2003; Rodriguez and Fraga, 1999).
Figueiredo et al. (2010) proposed that PGPR can be divided into two major classes known as extracellular (ePGPR) and intracellular (iPGPR) where both associate with roots and/or either in rhizosphere, on the rhizoplane or spaces between cells of cortex, and inside root cells.

2.1.1 PGPR isolated from tea plant

Over the past decades, researchers reported the plant growth promoting bacteria (PGPR) being isolated from various type of plants including tea, *Camellia sinensis* which considered as an essential plantation crop globally. In a recent study by Phukan et al. (2012) stated that 16 microbial strains isolated from tea plant showed plant growth promoting potential both in laboratory and field trial. The PGPR strains were also screened for its bioefficiency against black rot and blister blight disease as well as its performance in increasing leaf production of tea, since the microbes were proved for its beneficial effect on the tea plantation.

According to Chakraborty et al. (2013), a large number of the bacteria isolated from rhizosphere of tea plant showed activities which potentially promoting and improving the plant growth and also their productivity through ability in phosphate solubilization, siderophore production, antagonism to pathogens and indole-3-acetic acid (IAA) production. Plus, the PGPR proved to enhance the seedling of tea in the nursery and in the field under in vivo conditions.

Nevertheless, the beneficial use of PGPR from tea in regards that the isolated bacteria showed potential in improving and promoting plant growth is scanty in term of the further scientific information. It is observed that the PGPR majorly tested only in laboratory for its ability against phytopathogen as reported by Shobha and Kumudeni (2012) that the *Bacillus* is one of the important PGPR influencing the growth and yield of plants by suppressing the development of *Fusarium oxysporum*. 
2.1.2 Mode of actions

Numerous research and study reported that mode of action by PGPR may perhaps influence the plant growth in either two of the mechanism, direct or indirect (Erturk et al., 2010; Figueiredo et al., 2010; Shahab et al., 2009; Ahmad et al., 2008; Kloeper et al., 1991). Besides, when PGPR utilize the nutrients obtained through the host, they also secrete metabolites into rhizosphere (Loon, 2007), hence developing a symbiotic relationship.

PGPR is said to be directly improve plant growth through synthesizing of plant hormones as well as increasing nutrients availability in soil (Saharan and Nehra, 2011), whereas the indirect mechanism take place by secreting substances which help to lessen or suppress the effect of phytopathogen causing disease (Ahmad et al., 2008) or by activating the natural resistance of plant for instance, induced systemic resistance (ISR) (Linh, 2008; Loon, 2007).

Basically, the roles of PGPR which beneficial to plant acts in three different way, (i) synthesizing particular compounds of plant growth regulators (Erturk et al., 2010; Figueiredo et al., 2010; Hayat et al., 2010; Shahab et al., 2009; Ahmad et al., 2008; Linh, 2008; Vessey et al., 2003; Husen, 2003), (ii) facilitating the availability of nutrients in the soil (Figueiredo et al., 2010; Hayat et al., 2010; Dey et al., 2004; Husen, 2003; Kloeppeper et al., 1991), (iii) suppressing or preventing from deleterious effect of pathogen (Saharan and Nehra, 2011; Figueiredo et al., 2010; Hayat et al., 2010; Linh, 2008; Loon, 2007). Recent studies had proved that some particular PGPR strains may also exhibited bioremediation characteristic, since soil microorganisms has an ability in degrading various type of compounds via the transformations of elements that naturally involve in nutrients cycle (Adawiah, 2008; Zhuang et al., 2007).

PGPR assist in synthesizing the substance which essential to be utilized for the production of phytohormone, where an active one is known as indole-3-acetic acid (IAA). According to Hayat et al. (2010), the compounds produce by several PGPR genera are sufficient in synthesizing the plant hormones either through symbiotic or non-symbiotic relationship. This hormone play a pivotal role in coordination of numerous plant growth,
also involve in the behavioral processes in plant life cycle (Dey et al., 2004). On top of that, minerals and nutrients can be increase in terms of their availability in the soil to promote plant growth (Saharan and Nehra, 2011). The PGPR gradually enhance the nitrogen fixation from the atmosphere as well as emitting some substance that help to solubilize phosphate in a form for plant uptake. Nitrogen fixing agent secrete by the soil bacteria mainly regulate around leguminous crops accompanied by the role of nodule itself (Zafar et al., 2012). While the organic phosphate amount in soil usually bound to be increasing through its solubilizing effect when associate with PGPR that can also obtained naturally via phosphorus cycle in soils (Espinoza et al., 1914). In addition, PGPR is said to be an agent of biological disease control since it may lessening or even preventing the disease incidence cause by majorly soil pathogen (Figueiredo et al., 2010). Thus, PGPR has been recently reported to be exploited commercially by Jamil et al. (2011) as essential medium against pest and disease for plant. However, there are several environmental factors which can alter the viability, types, and amount of the soil bacteria strains such as pH, temperature, moisture and soil characteristic, which all of these will influence the performance of PGPR towards their host (Gaddeyya et al., 2012; Ahmad et al., 2006).

2.1.3 Key in implementing sustainable agriculture practices

People nowadays have grown a serious concern towards their surrounding which resulting a huge acceptance of environmentally friendly products in agriculture mostly for horticulture and nursery production (Erturk et al., 2010). The beneficial effect of soil microorganisms described by Gaddeyya et al. (2012) is to increase the soil fertility and at the same time improving the plant growth via biochemical transformation and mineralization process. Hayat et al. (2010) stated that the assimilation of rhizobia with rhizosphere become a major part in the ecological environment for their symbiosis interaction. Bashan et al. (1993) proposed that PGPR can be found in all rhizosphere area as they has been associated with plant roots decades ago, which conclude that there are no specific places or area inhabiting by PGPR.

Soil is the respository towards various plant nutrients making it crucial for maintaining its fertility. Sustainable agriculture practices revolve around the concept of preservation and conservation of environment by applying biological inputs in order to
produce a good quality and healthy product as to ensure food security. In details, sustainability involves multiple combinations of mostly microbial inoculants for managing plant nutrients. In India, biofertilizer is a kind of agricultural input via microbiological inoculants comprises mainly of beneficial microorganisms to provide sufficient nutrients for plant growth and/or hormone production (Brahmaprakash and Sahu, 2012).

Figueiredo et al. (2010) stated that biofertilizers are agricultural biological products having living microorganisms leading to increase in yield either through direct or indirect mechanisms. One of the major benefits when inoculated rhizobacteria into the soil is the nutrient for plant uptake was demonstrated increasing through the presence of microorganisms in the rhizosphere. However, there are some failure concerning the utilization of biofertilizers with PGPR is believed due to interspecific genetic interaction by rhizobacteria and their host.

Furthermore, application of PGPR to plants reported by Zhuang et al. (2007) is capable to be a phytoremediation that help in degrading the contaminants help to cure the environment, thus becoming a huge interest in nation worldwide. Enhance of technology enable the selection of specific contaminant-degrading bacteria among PGPR with respect to the properties of contaminants. The bacteria metabolize particular compound whilst at the same time PGPR still improve plant growth and also capable in tolerating to various kind of pollutants.

2.2 Indole-3-acetic acid (IAA)

Phytohormones considered as part of plant physiology since their role and involvement in numerous natural processes may affect the survivability and crops growth. Normally the effects of plant hormones depend fundamentally on the target tissues and the environment condition. Auxin or exist naturally as indole-3-acetic acid (IAA) is the most abundant phytohormone in plants, and it play a major role in coordinating signal in plant development.
2.2.1 Response to plant growth

Indole-3-acetic acid (IAA) is an important compound which generates the effects of auxin in plants and become a most potent native auxin for crops. The response of auxin to plant growth occurs in most of the important growing stages, for example auxin affects both cell division and cellular expansion during cellular level. As a whole, auxin promotes plant growth through the development of root structure that involves in the absorbance of nutrients and water from the soil (Shahabet et al., 2009).

The growth and development of roots relatedly dependent towards the endogenous level of IAA in plant (Erturk et al., 2010). The effect or the performance of auxin in plants is control by exogenous applications (Khalid et al., 2004), where if the concentration is too high, it may inhibit other plant systems. According to Teixeira et al. (2007), inoculation of PGPR with plants mostly bound about the root, stem and branch growth which definitely increases the yield. A study by Dey et al. (2004) about the growth promotion and yield enhancement of peanut showed that the inoculation with PGPR significantly increased the root length of peanut. Besides that, the plant biomass and nodule number also showed a significant increase when the peanut inoculated with PGPR over the control. Alas, due to the presence of other biological active substances excreted from bacteria, there is no direct dependence between IAA concentration and height root number of orchids (Tsavkelova et al., 2007).

Vaseekaran et al. (2010) reported that an increasing of root length and root surface area will be lead to increases in root weight thus becoming more reflective parameters of an increase volume of soil explored. To add to that, IAA-producing PGPR can improved the growth and length of root, consequently larger surface area for plant nutrient uptake from the soil.

2.2.2 Role in improving plant growth and development

IAA is synthesized from tryptophan or indole primarily in leaf primordial and young leaves, as well as in developing seeds. Although their production does not synthesized all over the
plant parts, but each of the cell has an ability to synthesize it provided a specific conditions.

Woodward and Bartel (2005) described that the production of IAA occur via two pathways which are Trp-dependent and Trp-independent, where both having its own precursor derived from tryptophan. Trp-dependent pathways for IAA biosynthesis proposed in four pathways known as indole-3-pyruvic acid (IPA), indole-3-acetamide (IAM), tryptamine and also indole-3-acetaldoxime (IOAx) pathway. A common IAA synthesizing pathway, IPA using Trp ammotransferase will oxidatively transaminates Trp to IPA, nonetheless the conversion of IPA to IAAld via IPA decarboxylase have not been further revised.

In recent study by Zafar et al. (2012) proved that PGPR also increase the growth and development of leguminous crop which cultivated under field and controlled environmental conditions. Moreover, a detailed study on nitrogen and phosphorus uptake in plants also showed an increasing stimulatory effect on growth and nodulation in nutrient-deficient in soil. It is believed that excretion of metabolites by PGPR greatly affect the stimulation growth directly. The data recorded from the study on shoot length, shoot fresh and dry weight reported an increased significantly in a term of percentage when applied with PGPR, same goes to the root length and root mass which conclude that PGPR have a high potential in promoting the plant growth capacities. The growth of root nodules in lentil plants also showed a significant in their development.
2.2.3 Commercial production

Auxins has been commercially produce as it promotes the initiation of adventitious roots, where widely used in horticulture to root stem cuttings. Despite that, auxin can also use to promote uniform flowering, fruit set and preventing premature fruit drop.

The importance of IAA on plant growth has been discovered long time ago leading to their production synthetically which mainly gain interest in horticultural industry as a catalyst for promoting the root growth mainly in stem cutting process. The root formation for cuttings treated with bacterial supernatants in study done by Tsavkelova et al. (2007) promoted with five- to fourteen-fold higher compared to the control samples and there is no inhibiting or suppressing effects occurred. Zafar et al. (2012) proposed that the characterization of bacteria act as a way of its behavior from local condition, hence the isolation and inoculation processes are crucial since they can affect the potential inoculant according to the area where they been found.
REFERENCES


