THE JOURNAL OF TROPICAL LIFE SCIENCE
OPEN ACCESS Freely available online
VOL. 2, NO. 2, pp. 53 – 57, May, 2012

Pseudomonas fluorescens and Pseudomonas putidato to Promote Growth of *Jatropha curcas* Seedling Root

Sri Sumarsih[1]* and Darban Haryanto[1]

[1] Department of Agrotechnology Faculty of Agriculture UPN "Veteran" Yogyakarta

ABSTRACT

Pseudomonas fluorescens and *P. putida* are Plant Growth Promoting Rhizobacteria (PGPR) that can produce growth hormone. The objective of this study is to know the effects of those two combined species of PGPR on seedling root growth of *Jatropha curcas*. The condition of the seedling root determines the success of dry land cultivation. The root which has wider coverage, is larger in number, and is bigger in diameter makes seedling more resistant to stress in dry land environment. In the experiment, two kinds of plant materials are used for seedling, the *Jatropha* seed and stem material, which are treated in a mixed culture of PGPR. For the *Jatropha* seed, this mixed culture of PGPR is given at the same time of cultivating the sprout on the seedling medium. For the stem cutting, the PGPR is poured in together during the first watering of the seedling cultivation medium. In the fourth week, the observed growth parameters are root length, root diameter, primary and secondary lateral root numbers, Root Length Density (RLD), Frequency of Lateral Root (FLR), and Specific Root Length (SRL). These data are analyzed using analysis of variant with DMRT test at 0.05 level of significance. The result of this study shows that PGPR tend to reduce FLR values on the seedling root made from seeds. On the seedling root made from stem cutting, PGPR increase the root length, primary and secondary lateral root numbers, root diameter, FLR and SRL values as well.

Keywords:*Pseudomonas, PGPR, root, Jatropha*

INTRODUCTION

Jatropha oil can be made into biodiesel. Biodiesel is a renewable resource. *Jatropha* has been chosen because it is not a food resource and it can grow on infertile land [1]. *Jatropha* planting can improve the condition of critical land, which covers up to 25 million hectares of land in Indonesia. The planting of *Jatropha* can be considered successful if the seedling is able to grow on infertile land. One aspect that can be used as a parameter of the growth of seedling on land is the widespread range of root, the great quantity of root, and the large diameter of the root. If the root can adapt itself to the condition of the cultivation land, the seedling will be resistant to the danger of marginal land environment.

The existing technique of making *Jatropha* seedling is by planting the available seed and by using stem cutting or micro-cutting. Generally, the medium of seedling production is fertile soil mixed with manure. Root seedling grows naturally in a medium that has good humidity. When the seedling is moved to infertile land, if it is not conditioned physicochemically or biologically, then the growing of the seedling will slow down or even fail. Thus, a good root is the key success of seedling. To encourage the growth of root, plant growth promoting rhizobacteria (PGPR) technique will be applied. In addition, seedling production medium will be acclimatized for planting on infertile land.

PGPR are known to influence plant growth by various direct or indirect mechanisms. PGPR have been reported to directly enhance plant growth by varieties of mechanisms: fixation of atmospheric nitrogen that is transferred to the plant, production of siderophores that chelate iron and makes it available to the plant root, solubilization of minerals such as phosphorus, and synthesis of phytohormones. PGPR that synthesize growth hormone are called auxins and cytokinins or that identified to interfere with plant ethylene synthesis [2]. An example of efficient PGPR strains with multiple activities is *Pseudomonas spp.*, which has been isolated from different rhizospheric soil of chick pea, that can

*Corresponding address:
Sri Sumarsih
Department of Agrotechnology, Faculty of Agriculture,
UPN "Veteran" Yogyakarta
Telp/Fax 0274 486692
Email: Sumarsih_03@yahoo.com

produce plant growth promoting traits like indoleacetic acid (IAA), ammonia (NH3), siderophore and catalase [3]. The dominant species of PGPR consist of two bacteria i.e. *Pseudomonas putida* and *P. fluorescens* [4]. *P. fluorescens* plays role on organic material decomposition and increases soil phosphate availability [5]. The increasing phosphate availability in rhizosfer by rhizobacteria can increase the size and dry weight of a plant [6].

The application of PGPR in the field where there is heterogeneity of abiotic and biotic factors and, competition with indigenous organisms as well, is clearly more difficult. Knowledge of these factors can aid in determination of optimal concentration, timing and placement of inoculant, and of soil and crop management strategies to enhance survival and proliferation of the inoculant [2]. The increasing soil salinity changes rhizobacteria composition [4]. *P. fluorescens* is dominant in soil which has low salinity, their population is reduced by increasing soil salinity, while *P. putida* is dominant in higher salinity [7, 8]. Inhibition of growth by cation and anion shows that cation is more inhibited on rhizobacteria growth than anion is. The most inhibiting cation is $Ca^{2+}$and then followed in order by Mg^{2+}, Na^{+}, and K^{+}, respectively. *P. putida* is more tolerant to Ca^{2+} in comparison with *P. fluorescens* [9].

The objective of the study is to find out the best PGPR concentration to affect *Jatropha* root growth. The target is to get seedling that has resistance to physicochemical condition of infertile land.

MATERIAL AND METHODS

Biological Material

Plant material used in the research was *Jatropha* seeds ripe in the tree. The material for stem cutting of *Jatropha* was cut into 20 cm [1]. Seed and stem of *Jatropha curcas* was taken from the coastal sand in Bantul, Yogyakarta. The material of PGPR was made from two bacteria, *Pseudomonas fluorescens* and *P. putida*. The strain of *P. fluorescens* and *P. putida* used in this study was obtained from Soil Biology Laboratory, Faculty of Agriculture, UPN Veteran Yogyakarta.

Bacterial culture

Bacteria were being multiplied inside liquid culture. Production medium for *P. fluorescens* and *P. putida* was nutrient broth medium for bacteria (nutrient broth 8 g/l aquadest) at pH 7. The medium was sterilized at 121^0C for 15 minutes. The fermentation in PGPR production was conducted in single culture at room temperature for 2 days with 75 rpm shaking speed, and then the two bacterial cultures were mixed. The material for PGPR was liquid cultivation from the mixing of *P. fluorescens* and *P. putida*, at the volume ratio of 1:1. The mixed culture was diluted by sterile aquadest to make the concentration of PGPR 10^4cell/ml and 10^8cell/ml, as well as the treatment of PGPR.

Experimental design

The experiment was performed in a green house by using Completely Randomized Design (CRD). Concentration of PGPR consisted of 3 level (P0: without PGPR, as control, P1: PGPR 10^4cell/ml, and P2: PGPR 10^8cell/ml). Every treatment unit consisted of 5 seeds. Every treatment was repeated for 3 times. The data were analyzed by the analysis of variant with Duncan Multiple Range Test (DMRT) at 0.05 significant level.

Growth conditions

In the experiment, two kinds of plant materials used for seedling, *Jatropha* seed and stem material were treated in the mixed culture of PGPR. *Jatropha* seeds were germinated for three days on tissue paper poured with aquadest. For the *Jatropha* seeds, the mixed culture of PGPR was given at the same time of cultivating the sprout on the seedling medium. For the stem cutting, the PGPR was poured in together on the first watering of the cultivation medium of the seedling. Seedling medium consisted of sand and compost at the volume ratio of 1:1. Seedling was grown in mini rhizotron (made from transparent mica in 24 cm height and 12.5 cm diameter), filled with seedling medium in 20 cm height. Minirhizotrons were put on wood board at 30^0 angle.

Root growth parameters

After 4 weeks, the growth of the root was observed from the root that was visible on minirhizotron. Root length was measured on minirhizotron [10]. Three plant samples from each treatment were taken from minirhizotron and soaked in tap water. Then the root were washed carefully with tap water and weighed. Primary and secondary roots were separately measured in terms of the root length, root diameter, root volume, root quantity, root wet and dry weight. Root density and root distribution were calculated in the value of RLD

(*Root Length Density*), FLR (*Frequency of Lateral Root*) and SRL (*Specific Root Length*). RLD is calculated from the total root length per volume unit of root soil area. In this study, soil volume is based on soil volume inside minirhizotron used for planting seedling [11]. FLR refers to lateral root quantity per cm primary root [10]. SRL refers to root length in meters per gram root weight [12].

RESULTS AND DISCUSSION

Length, quantity, and diameter of seedling root

The growth of seedling root made from the seed after PGPR treatment showed that PGPR concentration treatment did not affect root length, primary and secondary root quantity, and root diameter significantly. The growth of seedling root made from the stem cutting after PGPR treatment was significant (Table 1). For parameters of root length, primary and secondary root quantity, and root diameter within 4 weeks observation on concentration of PGPR 108cell/ml was significant with concentration of PGPR 104sel/ml and control (without PGPR).

The growth of seedling root made from the seed shows that PGPR tended to reduce root length compared to the control group. In contrast with the seed, for the seedling root made from stem cutting, it was found out that PGPR treatment increased root length on 10^8cell/ml application. The average of root length in this treatment was 12.58 cm (See table 1). *Pseudomonas spp*. could produce indole acetic acid (IAA), excreted to rhizosfer [3]. Root development, after seed germination, was induced with signals organized by IAA [13]. Auxin makes cell excrete proton. This causes acidification of apoplastic making cell wall become thin and cell elongation occur [14].

Primary and secondary root quantity and root diameter on seedling made from seed, after PGPR treatment, was not different from the control group (without PGPR). *Pseudomonas* can produce IAA. If PGPR treatment is the same as auxin incorporation, this result will be opposite with the one reported that incorporation of auxin 0.01-0.1 µM on seedling medium increased lateral per mm primary root [13].

In contrast with the seedling from stem cutting, concentration of 10^8 cell/ml PGPR application significantly increased root quantity (primary and secondary) and root diameter. The highest quantity of primary root is 9.31/plant and secondary root is 91.06/plant (Table 1). There were different responses to PGPR between seed and stem cutting. On stem cutting, woody plant always has high response to auxin like IAA, although it depends on the concentration and soaking time [15].

Density of seedling root after PGPR treatment

Root density can be measured by using *Root Length Density* (RLD). RLD is calculated from total root length per volume unit of root soil area. PGPR treatment affects the value of RLD. Root density decreases from 1.61 cm/cm^3 to 1.35 cm/cm^3 as the result of PGPR treatment with concentration of 10^8cell/ml. The decrease of RLD shows the decrease of root range inside the land. The complete result about RLD value after treatment is listed on table 2.

PGPR treatment did not affect RLD value of seedling root from stem cutting if compared to the control group without PGPR treatment. PGPR treatment increased the value of RLD but not significant with control. However it is not significant, RLD value on the seedling from stem cutting tend to increase about 0.56-0.65 cm/cm^3 (Table 2). The role of PGPR for promoting plant growth is different among several strains, only a specific strain that can increase the root length. There are demonstrated seed inoculation with PGPR that consists of *Bacillus pumilus* (IM-3), alone or in combination with other strains. *Bacillus pumilus* (IM-3) enhanced shoot length, dry shoot mass, dry root mass, dry total plant mass, leaf area, and chlorophyll content. However *Bacillus polymyxa* (KRU-22) supported maximum root length of *Jatropha curcas* seedling [16].

Distribution of seedling root after PGPR treatment

FLR (*Frequency of Lateral Root*) value describes the root distribution inside the soil. The experiment result shows that PGPR treatment affects FLR value in four-week seedling. For seedling makes from seed, PGPR treatment with concentration of 10^4cell/ml decreased FLR value significantly compared to control, but PGPR treatment with concentration of 10^8cell/ml is not significant if compared to 10^4cell/ml (Table 2).

Many lateral roots that grow from primary root will determine root distribution inside the soil. This can be measured by using FLR parameter, that is the quantity of lateral root per cm primary root. For *Jatropha* seedling that comes from stem cutting, PGPR treatment with concentration 10^8cell/ml significantly increased the FLR value. Greater FLR value shows better root distribution in root area. Thus, PGPR

Table 1. Average of root length, primary and secondary root quantity, root diameter of seedling

PGPR Concentration	Root length (cm)		Root quantity/plant				Root diameter (mm)	
			primary		secondary			
	S	SC	S	SC	S	SC	S	SC
Control (without PGPR)	15.89a	12.14ab	4.08a	8.09ab	184.25a	73.33ab	0.92a	1.07b
PGPR 10^4cell/ml	14.86a	10.92b	3.97a	6.39b	117.84a	50.11b	0.75a	1.09b
PGPR 10^8cell/ml	15.13a	12.58a	4.11a	9.31a	161.50a	91.06a	0.92a	1.17a

Notification: S: Seedling made from seed, SC: Seedling made from stem cutting
The same alphabet in the column shows not significant according to DMRT 0.05.

Table 2.Root Length Density (RLD), Frequency of Lateral Root (FLR), and Specific Root Length (SLR)

	Seedling made from seed			Seedling made from stem cutting		
PGPR Concentration	RLD (cm/cm^3)	FLR (cm^{-1})	SLR (m/g)	RLD (cm/cm^3)	FLR (cm^{-1})	SLR (m/g)
Control (without PGPR)	1.61a	1.05a	4543.23a	0.56a	0.42ab	1300.74b
PGPR 10^4cell/ml	1.71a	0.75ab	2843.00a	0.65a	0.25b	838.41b
PGPR 10^8cell/ml	1.35a	0.92b	4004.28a	0.59a	0.53a	2155.82a

Notification: The same alphabet in the column shows not significant according to DMRT 0.05

treatment affected the growth of lateral root in 4 weeks.

In this study, PGPR 10^8 cell/ml concentration increased significantly the value of FRL on the seedling from stem cutting, but it decreased on the seedling from seed. The wooden plant cutting is typically responsive to auxine treatment, though it depends on the concentration and period of submersion [15]. The forming of lateral root on seed which has sprout is determined by signal that is arranged by IAA. The absorption response of exogenous IAA by sprout seed will increase followed by the decrease of pH auxine solution, and optimum on pH 5.5-6.0 [14]. IAA exogen can increase quantity of lateral root, with optimum concentration 11 µM [17].

SRL (Specific Root Length)

SRL is the parameter for measuring the root size besides using root diameter parameter. The measuring of root diameter for showing the root size is often not precise because the root shape from the base to the peak is not same. Thus, the measuring of root size needs to use this SRL parameter. In fact, SRL (*Specific Root Length*) of seedling root from seed, PGPR treatment does not affect SRL value. SRL average of *Jatropha* seedling root grown from seed is in range of 2843.00 – 4543.23 m/g of root (see table 2). The greater SRL value shows smaller root size.

SRL (*Specific Root Length*) of seedling root from stem cutting, PGPR treatment with concentration 10^8cell/ml causes the increase of SRL value significantly compared to the control group. The highest average SRL of *Jatropha* seedling root that is grown from stem is 2155.82 m/g root and it happens on PGPR treatment with concentration of 10^8cell/ml.

The effect of PGPR to root weight and root volume tends to augment but it is not significant (see table 1). The increase of root quantity and root length in the same weight and volume shows that the size of root becomes smaller. Based on *Specific Root Length* (SRL), that is meter root length per gram root weight, the SRL of seedling root made from stem cutting increases significantly on weeks 4. The highest SRL value of seedling root is from stem cutting (2155.82 m/g), however, it is still lower than SRL of seedling root made from seed (2843-4543m/g). It shows that seedling root made from stem cutting is bigger than the one from the seed. Root shape of seedling from seed is bigger on the base and becomes smaller on the peak, especially at primary root, but the root from stem cutting is smaller [1]. There are reasons to choose seed to make seedling. In fact, application of PGPR 10^8 cell/ml can increase root size of stem cutting.

CONCLUSION

PGPR treatment on seedling that comes from the seed tends to decrease FLR values. PGPR treatment on seedling that comes from the stem cutting can increase root length, primary and secondary lateral root quantity, root diameter, FLR and SRL values. The best concentration of PGPR is 108 cell/ml.

ACKNOWLEDGMENTS

The authors acknowledge the Institute of Research and Community Service of University

of Pembangunan Nasional Veteran, Yogyakarta Indonesia for funding this study.

REFERENCES

1. Nurcholis M and Sumarsih S (2007) Jarak Pagar dan Pembuatan Biodisel. PT. Penerbit Kanisius. Yogyakarta.
2. Nelson L M (2004) Plant growth promoting rhizobacteria (PGPR): Prospects for new inoculants. Online. Crop Management doi:10.1094/CM-2004-0301-05-RV.
3. Joseph B, Ranjan Patra R and Lawrence R (2007) Characterization of plant growth promoting rhizobacteria associated with chickpea (Cicerarietinum L.). International Journal of Plant Production. 1(2).
4. Matsuguchi T and Sakai M (1995) Influence of soil salinity on the populations and composition of fluorescent pseudomonad in plant rhizosphere. Soil Sci Plant Nutr. 41(3): 497-504.
5. Sumarsih S (1998) The effect of organic matter and organic acid producing-Pseudomonas sp. to Al, Fe, and P status in Latosol. Kopertis Wilayah V Yogyakarta. Unpublished
6. Jiang HY and Sato K (1994) Interrelationships between bacterial populations on the root surface of wheat and growth of plant. Soil Sci Plant Nutr. 40(4): 683-690.
7. Sakai M, H Futamata, Y Urashima and T Matsuguchi (1995) Effect of cations on the growth of fluorescent pseudomonad isolates from spinach roots grown in soil with different salinity levels. Soil Sci Plant Nutr. 41(3): 605-611.
8. Sakai M, H Ozawa, H Futamata, JS Kim and T Matsuguchi (1998) Comparison of root colonization by LR- and HR-isolates of fluorescent pseudomonads in soil with high salinity. Soil Sci Plant Nutr. 44(1): 129-134.
9. Sakai M, H Ozawa, H Futamata and T Matsuguchi (1996) Effect of calcium ion on spinach root colonization by fluorescent pseudomonads through chemotaxis. Soil Sci Plant Nutr. 42(2): 323-332.
10. Bell DL and Sultan SE (1999) Dynamic Phenoypic Plasticity for Root Growth in Polygonium: a Comparative Study. American Journal of Botany. 86:807-819.
11. Wynn TM, Mostaghimi S, Burger JA, Harpold AA, Henderson MB and Henry LA (2004) Variation in Root Density along Stream Banks. Journal Environment Quality 33: 2030-2039.
12. Blakely LM, Blakely RM, Colowit PM and Elliott RM (1988) Experimental Studies on Lateral Root Formation in Radish Seedling Roots. II. Analysis of the Dose-Response to Exogenous Auxin. Plant Physiology. 87: 414-419.
13. Marchant A, Bhalerao R, Casimiro I, Eklof J, Casero PJ, Bennet M and Sandberg G (2002). AUX1 Promotes Lateral Root Formation by Facilitating Indole Acetic Acid Distribution between Sink and Source Tissues in the Arabidopsis Seedling. The Plant Cell. 14: 589-597.
14. Cleland RE (1992) Auxin-induced growth of Avena coleoptiles involves two mechanisms with different pH optima. Plant Physiology. 99: 1556-1561.
15. Jarvis B (1988) Endogenous Control of Adventitious Rooting in Non-Woody Cutting in Jackson MB (1988) New Root Formation in Plant and Cutting. Martinus Nijhoff. Boston. p146.
16. Desai S, Narayanaiah Ch, Kranti Kumari Ch, Reddy MS, Gnanamanickam SS, Rajeswara Rao G and Venkateswarlu B (2007) Seed inoculation with Bacillus spp. improves seedling vigour in oil-seed plant Jatrophacurcas L. Biol Fertil Soils. 44:229–234.
17. Blakely LM, Blakely RM and Galloway CM (1986) Effects Dimethyl Sulfoxide and pH on indoleacetic Acid-Induced Lateral Root Formation in the Radish Seedling Root. Plant Physiology. 80: 790-791.