Growth Response of *Calopogonium caeruleum* and *Centrosema pubescens* Ground Cover Crops toward Inoculation of *Bradyrhizobium, Aeromonas punctata,* and *Acaulospora tuberculata*

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ABSTRACT

Planting of leguminous cover crops is a standard practise in preparing land for oil palm and rubber plantations. The synergism capability of *Bradyrhizobium*, *Aeromonas punctata* (phosphate solubilizing bacteria) and *Acaulospora tuberculata* (AM fungi) to increase growth of leguminous cover crops (*Calopogonium caeruleum* and *Centrosema pubescens*) was studied in a glass house experiment using polybag (10 x 10 cm) containing acid soil with low level nutrition of Ciomas, Bogor. Research results showed that *Bradyrhizobium*, *A. punctata* and *A. tuberculata* inoculation on *C. caeruleum* signifycantly enhanced plant height, and number of leaves. However, the treatment did not increase biomass and N, P, and K uptake of plant. Number of nodule were increase when the plant was inoculated with *A. tuberculata* alone or in combination with *Bradyrhizobium* and *A. punctata*. *Centrosema pubescens* gave good response when inoculated with *A. tuberculata*. However, dual inoculation of the two bacteria *Bradyrhizobium* and *A. punctata* with *A. tuberculata* signifycantly enhance plant height, plant biomass, N, P, and K plant uptake.

Key words: *Calopogonium caeruleum, Centrosema pubescens, Bradyrhizobium, Aeromonas punctata, Acaulospora tuberculata,* leguminous cover crops.

ABSTRAK

Penanaman tanaman kacang-kacangan penutup tanah merupakan standar dalam penyiapan lahan pada pengusahaan kelapa sawit dan karet. Kemampuan sinergisme Bradyrhizobium (bakteri penambat N_2), *Aeromonas punctata* (bakteri pelarut fosfat), dan *Acaulospora tuberculata* (cendawan mikoriza arbuskula) untuk meningkatkan pertumbuhan tanaman kacang-kacangan penutup tanah (*Calopogonium caeruleum* dan *Centrosema pubescens*) dipelajari dalam percobaan rumah kaca menggunakan polibag berukuran 10 x 10 cm berisi tanah Ciomas Bogor yang bereaksi masam dan miskin hara. Hasil penelitian menunjukkan bahwa inokulasi *Bradyrhizobium, A. Punctata,* dan *A. tuberculata* pada *C. caeruleum* nyata meningkatkan pertumbuhan tinggi tanaman dan jumlah daun. Namun, perlakuan ini tidak meningkatkan biomasa dan serapan N, P, dan K tanaman. Jumlah bintil akar meningkat pada tanaman yang diinokulasi *A. tuberculata* sendiri atau dalam kombinasinya dengan *Bradyrhizobium* dan *A. punctata*. *Centrosema pubescens* menunjukkan respon yang baik bila diinokulasi dengan *A. tuberculata*. Bagaimanapun juga inokulasi dua bakteri, yaitu *Bradyrhizobium* and *A. punctata* yang disertai *A. tuberculata* nyata meningkatkan tinggi tanaman, biomasa, serapan N, P, dan K tanaman.

Kata kunci: *Calopogonium caeruleum, Centrosema pubescens, Bradyrhizobium, Aeromonas punctata, Acaulospora tuberculata,* tanaman penutup tanah perkebunan.

INTRODUCTION

Planting of leguminous ground cover crops (LCC) is a standard practice in preparing land for oil palm and rubber plantations. This activities have several beneficial affect to soil and water quality, promotion pest suppression, inhibited erotion and nutrient cycling efficiency. The total areal of oil palm and rubber 1-5 years planted with LCC were 484.272 ha and 98.640 ha respectively (Evizal and Prasmatiwi 2005). Ground cover crops could be leguminous, cerealia or Brasica (Snapp *et al*. 2005). Between those ground cover crops, LCC have beneficial effects. However, legume covers crop are slow grower and expensive to established but produce best high quality biomass. It was resulted from its ability to make symbiosis with *Bradyrhizobium* that could fix atmospheric N_2. The ability to fix atmospheric N_2 could reduce requirement of N fertilizer. Fixation of N_2 in LCC located in nodule. Therefore to get higher benefit of planting cover crops the leguminous crops should be able to form effective nodule. Field observation in many plantations showed that LCC produced less effective nodules (Suharyanto *et al*. 2005). These result showed that the LCC should be inoculated with *Rhizobium* to increase *Rhizobium* population and nodule formation.

Symbiose of *Rhizobium* with plant require high energy for metabolism and N fixation. Toro *et al*. (1998), reported that under low N fertilizer input, the availability P is ussualy the major limiting factor of N_2 fixation. Therefore, the requirement of P of leguminous plant is higher compared with others. The ability of plant to uptake P should be higher. Previously study showed that the ability of plant to uptake of P could be enhance by increasing the symbiosis of plant with AM fungi. Andrade *et al.* (1998) reported that in the absence of AM fungi infection supplementary P fertilization is generally necessary for the maintenance of N_2 fixation rates.

Staehelin *et al.* (2001) said that colonization processs of *Rhizobium* and AM fungi with plant have some similarities eventhough the interaction of *Rhizobium* and AM fungi with plant is different. It is known that phenolic compound affect as signal in inducing *Rhizobium* and AM fungi symbiosis (Albrecht *et al.* 1999). In addition, there are several inducible host proteins in *Rhizobium* and AM fungi interactions with plant (Albrecht *et al*. 1999, Staehelin *et al.* 2001). Staehelin *et al.* (2001) showed that both AM fungi and *Rhizobium* colonization regulated by ENOD2, ENOD40, ENOD5, ENOD12, VFLb29 genes.

Synergism study of *Rhizobium* and AM fungi with leguminous plant previously reported. The result showed that in leguminous plant synergism between *Rhizobium*, AM fungi and leguminous plant form tripartite interactions resulted higher P uptake, N fixation, and biomass production especially by reducing N and P fertilization, higher photosynthetic rates per unit leaf area (Jia *et al*. 2004). Synergistic interactions among the component of the tripartite symbiotic association increased plant productivity. Information about synergism between *Bradyrhizobium* (N_2 fixing bacteria), *A. punctata* (phosphate solubilizing bacteria) and *A. tuberculata* (AM fungi) of indigenous microorganism from Indonesia with LCC have not yet been reported. The aim of this experiment is to study the response of LCC *C. caeruleum* dan *C. pubescens* toward inoculation with *Bradyrhizobium, A. punctata,* and *A. tuberculata* in increasing plant biomass and nutrient uptake.

MATERIALS AND METHODS

Microorganisms. *Bradyrhizobium, A. punctata,* and *A. tuberculata* used in this study were microbial collection of Biotechnology Research Institute for Estate Crops. *Bradyrhizobium* was isolated from the rhizosphere of *C. caeruleum* under rubber plantation in North Sumatera, *A. punctata* collected from rubber rhizosphere while *A. tuberculata* was isolated from oil palm rhizosphere in Banten. *Bradyrhizobium* and *A. punctata* inoculant were formulated with zeolite and peat (20 : 80 b/b) in population of 2-5 $x10^8$ cell/g. *A. tuberculata* inoculant was prepared by pot culture using *Pueraria phaseoloides* as host in soil : sand (2 : 1 w/w) fertilized with Johnson nutrient solution. *A. tuberculata* inoculant consisting of the spore, hyphae, and infected root.

Planting material. *C. caeruleum* and *C. pubescens* of 14 days old were inoculated with *Bradyrhizobium* and *A. punctata* by dipping the seed in 50 g inoculant in 100 ml aquadest for 10 minutes. Inoculation of *A. tuberculata* was carried out by powdering the inoculant (100 spores/plant) 10 cm under the root of the plant. *C. caeruleum* and *C. pubescens* seed were germinated using zeolite as media. After germinated and shoot emergence, one seedling of uniform sizes was selected as plant material. The plant were grown and watering using tap water. Observation of growth, leave number, wide leave, plant biomass, nodule number, and percentage of AM fungi infection, and N, P, and K uptake were carried out three months after inoculation.

Biomass measurement. The plant were separated into fresh shoot and root and oven dried at 65^{o}C for three days. A root subsample for each treatment was taken before oven drying for estimation of AM fungi infection of the root tissue. The fresh mass of the sub sample was recorded so that the dry mass of the subsample could be added to the total root dry mass.

N and P analysis. After the determination of dry mass, the tissue were milled and analysed for total N (Kjedahl), P (spectrofotometer), and K (AAS).

AMF detection and quantification. Percentage of AM fungi colonization was evaluated from random sub-sample of approximately 25 root-seqments per plant. Roots were cleared and stained and stored in acid glycerol according to the procedure of Koske and Gemma (1989).

Experimental design. The study was conducted in glasshouse. Four treatments assessed were *Bradyrhizobium + A. punctata, A. tuberculata, Bradyrhizobium + A. punctata* and *A. tuberculata* inoculation, and without inoculation (control). Soil used in this study was unsterilized Ciomas acid soil. Experiment design used in this study was completely randomized design to assesed four treatments and each treatment was replicated three times.

RESULT AND DISCUSSION

Plant Growth

Statistical analysis showed that both *Bradyrhizobium + A. punctata, A. tuberculata*, and *Bradyrhizobium + A. punctata + A. tuberculata* significantly increase *C. caeruleum* plant height and leave number (Table 1). In addition, *Bradyrhizobium + A. punctata* and *A. tuberculata* inoculation were able to increase wide leave compared to control, and inoculation of those microorganism significantly increase wide leave.

Research of Jia *et al.* (2004) showed that plant inoculated with *Rhizobium* and AM fungi have photosyntetic rate higher per leave unit area. *Bradyrhizobium + A. punctata* inoculation did not increase nodule number but *A. tuberculata* inoculation increase significantly nodule number, and similar result was found in *A. tuberculata + Bradyrhizobium + A. punctata* inoculation. Staehelin *et al.* (2001) reported that both nodule formation and AM fungi infection especially in leguminous plant was regulated by same gene (ENOD40). Inoculation of AM fungi might be induce nodule formation in this crop. Similar result was found in observation of AM fungi infection. AM fungi infection of plant inoculated with *Bradyrhizobium + A. punctata, A. tuberculata* and *Bradyrhizobium + A. punctata + A. tuberculata* significantly higher compared to control.

Response of *C. pubescens* to *Bradyrhizobium + A. punctata, A. tuberculata*, or *Bradyrhizobium + A. punctata + A. tuberculata* inoculation was differ compared to *C. caeruleum* (Table 1). In this ground cover crops, *Bradyrhizobium + A. punctata* inoculation did not increase plant height significantly, in contrast to *A. tuberculata* inoculation. However, the highest of plant was yielded by plant inoculated with *Bradyrhizobium + A. punctata + A. tuberculata* inoculation. Similar trend was found in wide leave parameter. Moreover, *A. tuberculata* inoculation significantly increase the number of leave and also *Bradyrhizobium + A. punctata + A. tuberculata* inoculation. However, there was no differences in nodule number between plant inoculated with *Bradyrhizobium + A. punctata,* and *A. tuberculata* or *Bradyrhizobium + A. punctata + A. tuberculata.* Inoculation of *A. tuberculata* increase signifycantly AM fungi infection and similar result was found in *A. tuberculata + Bradyrhizobium + A. punctata* inoculation treatment.

Table 1. Effect of *Bradyrhizobium + A. punctata* and or *A. tuberculata* inoculation on growth of *C. caeruleum* and *C. pubescens* after three months olds.

Treatments	Plant height (cm)	Leave numbers	Wide leave (mm^2)	Nodule numbers	Percentage of AM fungi infection
C. caeruleum					
Control	16.6 b*	2.7 b	48.1 b	37.0 b	9.3 b
Bradyrhizobium + A. punctata	110.0 a	11.7 a	322.4 ab	15.7 b	56.0 a
A. tuberculata	101.0 a	9.7 a	251.9 ab	86.7 a	73.3 a
Bradyrhizobium + A. punctata + A. tuberculata	126.0 a	14.7 a	422.2 a	82.7 a	82.7 a
C. pubescens					
Control	20.5 c	3.7 b	14.5 c	0.3 a	26.7 b
Bradyrhizobium + A. punctata	35.0 c	7.3 b	51.9 c	4.7 a	53.3 ab
A. tuberculata	93.3 b	14.7 a	213.9 b	0.7 a	70.0 a
Bradyrhizobium + A. punctata + A. tuberculata	147.7 a	18.3 a	336.1 a	9.3 a	76.7 a

Number in each column followed by the same letter (s) are not significantly different according to Duncan Multiple Range Test (DMRT) P<0.05.

Observation of nodule formation and AM fungi infection both in *C. caeruleum* and *C. pubescens* revealed that there was AM fungi infection in control. In seem likely that there were indigeneous Rhizobium and AM fungi that both of them could interact with *C. caeruleum* and *C. pubescens.* However, nodule formed and infection by indigenous AM fungi by indigenous Rhizobium and AM fungi were not. This showed by higher growth parameter by *Bradyrhizobium + A. punctata, A. tuberculata,* and those of *Bradyrhizobium + A. punctata + A. tuberculata* inoculation compared to control. According to this result, inoculation was important to get beneficial effect of *Bradyrhizobium + A. punctata* and *A. tuberculata* symbioses.

Plant Fresh Weight

Fresh weight of shoot, root, and plant was higher in *Bradyrhizobium + A. punctata, A. tuberculata,* and *Bradyrhizobium + A. punctata + A. tuberculata* treatment (Table 2). But, the increasing of those fresh weight was not significant different compared to control. This result showed that *C. caeruleum* was not quite responsive to inoculation. However, there was no differences between interaction of plant with *Bradyrhizobium + A. punctata, A. tuberculata,* and *Bradyrhizobium + A. punctata + A. tuberculata.* It supposed that the effect of *Rhizobium* or *A. tuberculata* or both of them was similar. It seem that there was association between *Bradyrhizobium, A. punctata*, and A. *tuberculata.*

In *C. pubescens*, the effect of *Bradyrhizobium + A. punctata* inoculation was not significant to the shoot, root, and plant dry weight. However, *A. tuberculata, Bradyrhizobium + A. punctata + A. tuberculata* inoculation increase significantly shoot, root, and plant fresh weight. It is interesting to note that inoculation of *A. tuberculata* alone give the highest root dry weight while inoculation of *Bradyrhizobium + A. punctata + A. tuberculata* give the highest shoot fresh weight. In addition, the plant fresh weight of those treatment was not signifycantly different. These result was might be resulted from nodule formation functioning in increasing N content of plant. The increasing of N of plant decrease root growth while higher N content increase shoot growth.

Plant Dry Weight

Table 3 showed the effect of treatment to plant dry weight parameter. Statistical analysis showed that *Bradyrhizobium + A. punctata, A. tuberculata*, and *Bradyrhizobium + A. punctata + A. tuberculata* inoculation in *C. caeruleum* was not significantly increase shoot dry weight. Similar result was found in root and plant dry weight. However, shoot, root, and plant dry weight was higher compared with control.

Bradyrhizobium + A. punctata inoculation in *C. pubescens* was not significantly enhance shoot, root, plant dry weight. While *A. tuberculata* inoculation, significantly increase shoot, root, plant dry weight. *A. tuberculata* inoculation was more affect root dry weight compared to shoot dry weight. However, plant dry weight was not significantly different between *A. tuberculata* inoculation and *A. tuberculata + Bradyrhizobium.* It seem that the effect of *A. tuberculata* on root dry weight was higher compared to shoot dry weight.

Table 2. Effect of *Bradyrhizobium + A. punctata* and or *A. tuberculata* inoculation on fresh weight of *C. caeruleum* and *C. pubescens* after three months old.

Treatments	Shoot fresh weight (g)	Root fresh weight (g)	Plant fresh weight (g)
C. caeruleum			
Control	0,8 a	0,8 a	1,6 a
Bradyrhizobium + A. punctata	5,9 a	4,2 a	10,1 a
A. tuberculata	4,6 a	3,6 a	8,1 a
Bradyrhizobium + A. punctata +A. tuberculata	7,0 a	4,6 a	11,6 a
C. pubescens			
Control	0,4 c	0,4 c	0,8 b
Bradyrhizobium + A. punctata	0,8 c	0,6 c	1,4 b
A. tuberculata	4,3 b	5,3 a	9,6 a
Bradyrhizobium + A. punctata + A. tuberculata	6,1 a	2,9 b	9,0 a

Number in each column followed by the same letter (s) are not significantly different according to Duncan Multiple Range Test (DMRT) P<0.05.

Nutrient Uptake Plant

Bradyrhizobium + A. punctata, A. tuberculata, and *Bradyrhizobium + A. punctata, A. tuberculata* inoculation in *C. caeruleum* could increase nutrient plant uptake especially N, P, and K (Table 4). However, the increase of this nutrient uptake was not significant. The increasing of plant N, P, and K uptake were might be related to the increasing of leave number. Increasing of leave number will increase transpiration that increase N plant uptake. While P plant uptake was related to the ability of AM fungi symbiosis with plant both directly through externally hyphae or indirectly through better root system (Table 2 and 3).

In *C. pubescens*, inoculation of *Bradyrhizobium + A. punctata* resulted N uptake similar with control. But, inoculation of *A. tuberculata,* and *Bradyrhizobium + A. punctata + A. tuberculata* significantly increase plant N uptake. Similar result was found in P upake of plant. In K uptake of *C. pubescens*, *Bradyrhizobium + A. punctata* inoculation was not significantly increase plant K uptake but *A. tuberculata* and *Bradyrhizobium + A. punctata + A. tuberculata* significantly increase plant K uptake. Comparing with *A. tuberculata*, *A. tuberculata* inoculation along with *Bradyrhizobium + A. punctata* resulted higher K plant uptake.

In *C. pubescens*, *Bradyrhizobium + A. punctata* inoculation generally was not affect growth and nutrient uptake of plant. This might be resulted from compatibility of *Bradyrhizobium + A. punctata* inoculated in *C. pubescens*.

It was shown that the response of *C. caeruleum* and *C. pubescens* to *Bradyrhizobium + A. punctata*, *A. tuberculata* and *Bradyrhizobium + A. punctata + A. tuberculata* inoculation were different. It seem that *C. pubescens* was more responsive to *A. tuberculata*, and both *A. tuberculata + Bradyrhizobium + A. punctata* inoculation compared to *C. caeruleum*. However, the result of this study should be confirm by field study in several location.

Table 3. Effect of *Bradyrhizobium + A. punctata* and or *A. tuberculata* inoculation on dry weight of *C. caeruleum* and *C. pubescens* after three months olds.

Treatments	Shoot dry weight (g)	Root dry weight (g)	Plant dry weight (g)
C. caeruleum			
Control	0.20 a	0.06 a	0.26 a
Bradyrhizobium + A. punctata	1.31 a	0.32 a	1.63 a
A. tuberculata	1.16 a	0.32 a	1.48 a
Bradyrhizobium + A. punctata + A. tuberculata	1.79 a	0.39 a	2.19 a
C. pubescens			
Control	0.08 b	0.03 c	0.11 b
Bradyrhizobium + A. punctata	0.18 b	0.04 c	0.21 b
A. tuberculata	1.07 a	0.43 a	1.51 a
Bradyrhizobium + A. punctata + A. tuberculata	1.28 a	0.23 b	1.52 a

Number in each column followed by the same letter (s) are not significantly different according to Duncan Multiple Range Test (DMRT) P<0.05.

Table 4. Effect of *Bradyrhizobium + A. punctata* and or *A. tuberculata* inoculation on N, P, and K uptake of *C. caeruleum* and *C. pubescens* after three months old.

Treatments	Nutrient uptake (g/plant)		
	N	P	K
C. caeruleum			
Control	0.87 a	0.04 a	0.26 a
Bradyrhizobium + A. punctata	4.87 a	0.48 a	1.88 a
A. tuberculata	3.05 a	0.57 a	1.13 a
Bradyrhizobium + A. punctata + A. tuberculata	5.67 a	0.45 a	2.88 a
C. pubescens			
Control	0.29 b	0.03 b	0.08 c
Bradyrhizobium + A. punctata	0.65 b	0.06 b	0.03 c
A. tuberculata	3.83 a	0.38 a	1.31 b
Bradyrhizobium + A. punctata + A. tuberculata	3.98 a	0.40 a	2.04 a

Number in each column followed by the same letter (s) are not significantly different according to Duncan Multiple Range Test (DMRT) P<0.05.

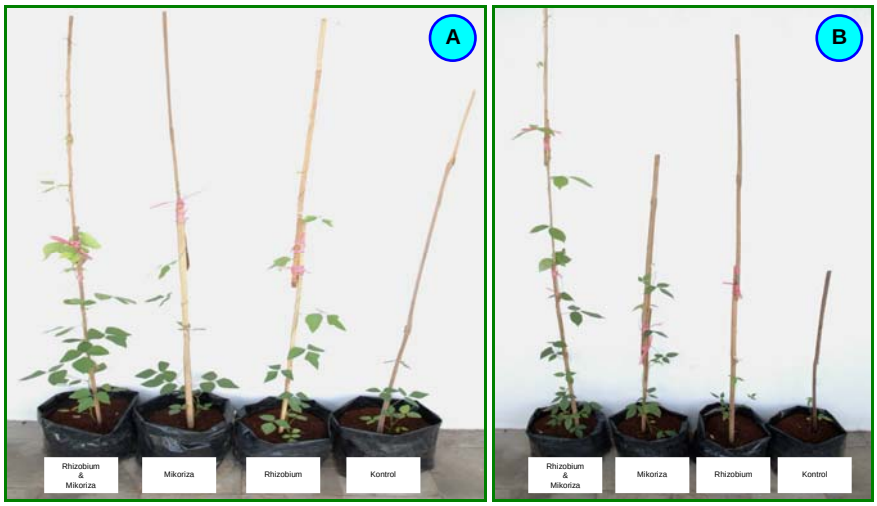

Figure 1. Growth reponse of *C. caeruleum* (A) and *C. pubescens* (B) inoculated with (a) *Bradyrhizobium* + *A. punctata* + *A. tuberculata*, (b) *A. tuberculata*, (c) *Bradyrhizobium* + *A. punctata*, (d) control after three months old.

CONCLUSIONS

The height and number of leaves of *C. caeruleum* were significantly higher in *Bradyrhizobium*, *A. punctata* and *A. tuberculata* inoculation treatment. However, the treatment did not increase biomass and N, P, and K uptake of plant. *Centrosema pubescens* gave good response when inoculated with *A. tuberculata*. However, dual inoculation of the two bacteria *Bradyrhizobium* and *A. punctata* with *A. tuberculata* significantly enhance plant height, plant biomass, N, P, and K plant uptake.

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