

Potential use of *Claroideoglosum etunicatum* to enrich signal grass (*Brachiaria decumbens* Stapf.) for silvopasture preparation

Potensi penggunaan *Claroideoglosum etunicatum* untuk pengkayaan rumput bede (*Brachiaria decumbens* Stapf.) untuk persiapan silvopastura

Risa ROSITA^{1)2)*}, Rahayu WIDYASTUTI¹⁾, Irdika MANSUR^{1)2)**} & Sarah Asih FAULINA³⁾

¹⁾ Bogor Agricultural University, Darmaga Campus of IPB, Bogor 16680, Indonesia

²⁾ SEAMEO BIOTROP, Jl. Raya Tajur Km 6. Bogor 16134, Indonesia

³⁾ Forest Products Research and Development Center, Jl. Gunung Batu No 5, Bogor 16610, Indonesia

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Abstrak

Peningkatan sistem Silvopastura sebagai upaya pengelolaan sumber daya lahan pasca tambang terus dilakukan melalui pencarian rumput yang berkualitas. Salah satu spesies rumput yang terseleksi adalah rumput bede (*Brachiaria decumbens* Stapf.). Penelitian ini bertujuan untuk mempersiapkan rumput bede melalui inokulasi FMA *Claroideoglosum etunicatum*, sebagai upaya untuk meningkatkan pertumbuhannya sebelum diaplikasikan pada tanah pasca tambang. Tahap penelitian yang dilakukan adalah kultur spora *C. etunicatum* menggunakan rumput bede sebagai inang dan tahap selanjutnya pemindahan rumput bede yang telah terkolonisasi *C. etunicatum* ke pot dengan media zeolit steril. Pada kultur spora, perlakuan yang diuji adalah tanpa inokulasi (dosis 0 spora) dan dengan inokulasi (dosis 20 spora) yang diulang sebanyak 12 kali. Inkubasi pada kultur spora adalah 4 minggu dan selanjutnya di pot dengan medium zeolit steril diinkubasi 8 minggu. Data penelitian dianalisis menggunakan uji normalitas Shapiro-Wilk, uji *t* Independen Sampel, dan korelasi Pearson. Hasil pengamatan menunjukkan bahwa inokulasi *C. etunicatum* pada rumput bede berpengaruh nyata terhadap peningkatan tinggi tanaman, diameter batang, jumlah daun, jumlah anakan, bobot basah tajuk dan akar, dan bobot kering tajuk ($p < 0,05$). Pengamatan mikroskopis menunjukkan bahwa pada akar rumput bede yang diberi perlakuan terjadi kolonisasi FMA sebesar $55\% \pm 0,06\%$ dengan spora berjumlah $252 \pm 9,82$ per 10 g zeolit sedangkan pada tanaman yang tidak diinokulasi tidak terjadi infeksi FMA. Diharapkan dengan tersedianya bibit rumput bede terkolonisasi FMA *C. etunicatum* maka pertumbuhannya akan lebih baik di lahan pasca tambang untuk sistem Silvopastura.

[Kata kunci: inokulasi FMA, jumlah spora, zeolit]

Abstract

Silvopasture system improvement in managing post-mining land resources has been done by searching for a quality grass. One of the selected grass species is signal grass (*Brachiaria decumbens* Stapf.). This research aimed to prepare signal grass through the inoculation of AMF *Claroideoglosum etunicatum*, as an effort to enrich its growth before being applied to post-mining soil. Research stages included the AMF inoculation on signal grass through spore culture and then transferred the colonized grass to the pot using sterile zeolite as a growth medium. The treatment on the first stage was without and with AMF inoculation (dose of 20 spores) on signal grass which was repeated for 12 times. Incubation in a spore culture was 4 weeks while incubation in a pot containing sterile zeolite medium was 8 weeks. Research data were analyzed using the Shapiro-Wilk's normality test, Independent Sample T-test, and Pearson's correlation test. Observation results showed that the inoculation of *C. etunicatum* on signal grass was significantly impact on the increase of plant height, stem diameter, number of leaves, number of tillers, shoot and root fresh weight, and shoot dry weight ($p < 0.05$). Microscopic observation showed that there was AMF colonization on treated signal grass roots in the amount of $55 \pm 0.06\%$ with number of spores was 252 ± 9.82 per 10 g zeolites, while AMF infection was not found in uninoculated signal grass. It is expected that by providing signal grass inoculated with AMF *C. etunicatum* would support its growth in post-mining land for Silvopasture system.

[Keywords: AMF inoculation, number of spores, zeolite]

*) Email: risa@biotrop.org

**) Correspondent author: irdikam@gmail.com

Introduction

One of the alternatives for post-mining land resources management is Silvopasture system, a combination of forestry and animal husbandry activities. This system is carried out by planting grass or green forage as a cover crop without damaging the forest stand. The function of cover crops is to suppress the weed growth, maintain soil moisture, provide large quantities of dry matter and plant nutrients in improving the soil fertility (Araujo *et al.*, 2015).

Signal grass (*Brachiaria decumbens* Stapf.) is one of the best forages that have a good quality for ruminants which is original from Uganda, Africa. At present, the grass is already widespread through South America, Australia, Indonesia, Vanuatu and Malaysia due to its adaptation to a wide range of soil types and environments (Low, 2015). Signal grass is a highly productive tropical grass. This grass is a long-lived grass, growing by forming thick beds, and spreading through its stolons. Fanindi (2016) reported that signal grass could develop growth widely in marginal areas, especially land acid that shows high adaptability.

Grass growth is influenced by abiotic factors such as climate, light, and water. It is also influenced by biotic factors such as rhizosphere microbes. Beneficial rhizosphere microbes can be utilized as an alternative environment-friendly method to restore the degraded land and increase agricultural productivity. One of the beneficial rhizosphere microbes is Arbuscular Mycorrhizal Fungi (AMF). Arbuscular Mycorrhizal Fungi increase plant growth and resist heavy metals and environmental stress (Begum *et al.*, 2019). Arbuscular Mycorrhizal Fungi and plants root form obligate symbioses in which AMF provide 80% of inorganic nutrients for plants that live on the land (Begum *et al.*, 2019), while AMF obtain up to 20% of monosaccharides from the fixation of CO₂ by plants. The utilization of mycorrhiza has the potential to improve the structure of post-mining soil, increase the nutrient absorption of plants, and provide essential compounds that needed by plants (Wulandari & Rosita, 2016). Cavagnaro *et al.* (2014) reported that the inoculation of AMF could increase 50% of root length in tropical grass.

Claroideoglossum etunicatum is the synonym of *Glomus etunicatum* (Schüßler & Walker, 2010). It is one type of AMF that improves plant resistance responses against biotic stress and promotes healthy growth of oil palm (Khodavandi & Alizadeh, 2015). Elviwirda *et al.* (2016) stated that the application of inoculum *C. etunicatum* (30 g) and 100% of the field capacity which is combined with *B. decumbens* on podzolic soil produced the highest shoots fresh

weight. Setyaningsih *et al.* (2018) stated that the application of *C. etunicatum* significantly increased roots colonization on *Typha* grass (*Typha angustifolia*). Inoculation of AMF *C. etunicatum* provided the best growth of *T. angustifolia* grass that planted in mixed soil-tailing media, and it could increase the length and biomass of grass. Therefore, *C. etunicatum* can be a potential AMF inoculant candidate to colonize the grass root, such as signal grass.

Studies about AMF inoculation in the grass at marginal land had been reported. However, In Indonesia studies about the utilization of *C. etunicatum* in signal grass for post-mining land have never been reported. This research aimed to study the effect of AMF *C. etunicatum* inoculation to signal grass before being used for the post-mining land area. In this research, we used a different dose of the spore and inoculation method.

Materials and Methods

Inoculation of AMF in spore culture

Ten grams of *C. etunicatum* inoculum in zeolite carrier, collection of the Landscape Biosystem, and Management Laboratory SEAMEO BIOTROP (code number BLM_MGL1), was poured with water sufficiently. Spores of *C. etunicatum* that attached to zeolites were released by agitating and pressing the inoculum by hand. Furthermore, the suspension was poured into stratified sieving, which has a filter size of 425, 212, 106, and 63 µm and then stirred using hand under running water. The sediment that captured in the bottom of three filters (hole sizes 212 µm, 106 µm, and 63 µm) was transferred to the Whatman filter pore size 20 µm. The precipitate was rinsed using water from a spray bottle and after that the spores were transferred to a petri dish. The AMF spores were inoculated to bed grass on spore culture as reported by Nusantara *et al.* (2012) and incubated for four weeks. The treatment was inoculation (20 spores) and uninoculated (as control). Each treatment was repeated twelve times. Maintenance of each treatment is carried out by watering the grass and also applying NPK (16:16:16) fertilizer at a dose of 10 ppm every three days for four weeks.

Transfer of AMF colonized signal grass to pot

Signal grass seedlings that had been colonized with AMF on spore culture were transferred to the pots. The growth media was 500 g sterilized zeolite. Uninoculated plant seedlings were used as controls. Maintenance of each treatment is carried out by only watering the grass without adding the nutrient. Each treatment had twelve replications. Plants were grown for eight weeks in a greenhouse condition. Measured

parameters to determine the effectiveness of *C. etunicatum* inoculation were plant height, stem diameter, number of leaves and tillers, shoots and root fresh weight, and shoot dry weight that formed in signal grass plants. Moreover, Dodd *et al.* (2001) said that the percentage of root colonization of AMF becomes the basis in determining the status of AMF infection in plant roots (Dodd *et al.*, 2001). The effectiveness of *C. etunicatum* root colonization was observed by staining the roots (Tawaraya *et al.*, 1998). The roots were cut approximately 1-1.5 cm as much as ten and placed in line on the glass object, then each piece of root was observed under a microscope. The AMF root colonization was shown by the formation of external and internal hyphae structures, spores, vesicles, and arbuscules on the roots. The percentage of AMF root colonization was measured using the formula:

$$\text{Percentage of AMF} = \frac{\text{Number of infected root}}{\text{Total root observed}} \times 100 \%$$

Experimental design

The normality distribution of the data (plant height, stem diameter, number of leaves and tillers, shoots and root fresh weight, and shoot dry weight) was analyzed using the Shapiro-Wilk's normality test. The data that had been obtained were analyzed using an Independent Samples T-test to find out the effect of treatments. Meanwhile, the correlation between the two variables (the number of spores with the vegetative growth) was analyzed by using the Pearson test. Each treatment had twelve replications. Data analysis was performed using SPSS Statistics 22.0 software at a significance level of 0.05.

Results and Discussion

Inoculation of AMF in spore culture

Purification of AMF *C. etunicatum* successfully carried out by grouping the spores. Spore of *C. etunicatum* had a globose shape, 64-65 µm in diameter, dark yellow, and two layers of walls. Following Talbi *et al.* (2015), *C. etunicatum* spore morphology was globose, oval up to ellipsoid, 40-111 µm, yellow to dark yellow up to brown, spore surface smooth to granular. Srimathi *et al.* (2014), also reported that *C. etunicatum* spore color light brown, globose, and had two layers of walls. Becker & Gerdemann (1977), stated *C. etunicatum* spore pale yellow to yellow, globose to subglobose, 75-135 µm in diameter, occasionally ovoid and spore wall composed of two layers.

Inoculation of *C. etunicatum* using spore culture techniques was aimed to obtain effectively a method of grass root inoculation. In spore culture, the type of planting medium and temperature were the essential

success factors of this stage. The planting medium that used in spore culture was zeolite. Zeolite is already known used in producing the AMF spores. Baghaie *et al.* (2019) reported that the response of grain biomass and grain P and N uptake to mycorrhization were all positive and stimulated by zeolite addition. Based on his research, the result of X-ray diffraction (XRD) analysis indicated that the zeolite is mainly from clinoptilolite micronized, which is a hydrated aluminosilicate of alkaline and alkaline earth metals (Na, K, Ca, and Mg). The zeolites use to neutralize the soil acidity, release and bind the water reversibly, and able to control the release of nitrogen and potassium ions from fertilizers. Zeolites also use to improve nutrient uptake, and nutrient use efficiency on *Zea mays* plant cultivating at acid soils (Ainaa *et al.*, 2014; Jakkula and Wani, 2018). Application of zeolite treatment increased plant height of 42%, above-ground dry biomass of 61%, and total dry matter biomass of 66%, which were higher than control treatment (Azimi *et al.*, 2019). Rosalina *et al.* (2019) stated that using ameliorant material in the form of zeolite, compost, or both could improve the chemical properties of the planting medium in the form of quartz sand, and affect the growth of *Brassica Juncea*. Zheng *et al.* (2019) reported combined application between zeolite and P under AWD (alternate wetting and drying) system reduced water use, improved P uptake and grain yield in rice, and also decreased environment risk.

In addition to the type of growing media, storage temperature also has an essential role in AMF symbiosis. Spore culture was stored at room temperature (27°C) and not allowed to interact directly with the sun. The storing aimed to reduce the increasing heat of the growing medium in large quantities and to stimulate the spore germination. According to Brundrett (1991), light intensity and temperature significantly affect the growth and development of AMF and determine the success of symbiosis between plant roots and AMF. The high intensity of sunlight will increase the soil temperature, further increasing soil temperature will affect the capacity and development of AMF when infecting the roots of a host plant. Gavito *et al.* (2005) stated that AMF growth decreased in the temperature range between 6-18°C. Schenck & Smith (1982) also stated that *Glomus claroideum*, *Glomus clarum*, *Gigaspora pellucida*, and *Gigaspora gregaria* produced the most significant number of spores per gram when roots are colonized at temperature 24°C, while maximum root colonization of *Glomus mosseae* and *Acaulospora laevis* at 30°C. *Gigaspora gregaria* has a maximum percentage of root colonization at 36°C while *Glomus mosseae* reached maximum colonization at 24°C.

Transfer of AMF colonized signal grass to pot

Inoculation of AMF to signal grass by spore culture method effectively increased root colonization and spore number. The percentage of AMF root colonization was $55 \pm 0.06\%$ while the number of spores collected from the rhizosphere of the signal grass growing media increased from 20 spores per 500 g zeolites to 252 ± 9.82 spores per 10 g zeolites after 12 weeks of inoculation. It indicates that the signal grass used in this study was responsive to the AMF inoculation. These spore numbers were higher compared to those reported by Covacevich & Berbara (2011) that stated that AMF spores produced in *Braquiaria* grass as host plant during 90 days after inoculation were only 237 spores per 100 g soil. Moreover, Gomes *et al.* (2015) reported that AMF *Acaulospora scrobiculata* inoculation on soil without adding the Arsenic (control) using a signal grass as a host plant, produced 66 spores per 100 g soil with percentage root colonization was 58% after 30 days incubation.

The data of plant height, stem diameter, number of leaves and tillers, shoots and root fresh weight, and shoot dry weight were normally distributed according to the Shapiro-Wilk's normality test. There was a significant difference between treatment without inoculation and inoculation AMF to the growth response of signal grass. Table 1 showed that AMF *C. etunicatum* inoculation significantly affected the plant height, stem diameter, number of leaves, number of tillers, shoot fresh weight, root fresh weight, and shoot dry weight compared to those without AMF inoculation ($p < 0.05$) according to Independent T-test.

The results showed that *C. etunicatum* was potential to enrich signal grass. It has an opportunity to be applied in post-mining land. Arbuscular Mycorrhizal Fungi (AMF) has been reported to increase the plant growth by surrounding their hyphae in facilitating the nutrient uptake. The AMF mycelium that appeared from the plant root system can be used to obtain nutrients from soil volumes that are inaccessible to roots by colonizing the root cortex. Arbuscular Mycorrhizal Fungi interactions provide other benefits to plants, such as improved drought and salinity tolerance. Arbuscular Mycorrhizal Fungi are known to alleviate heavy metal toxicity in the host plants, and to tolerate high metal concentrations in the soil, improve the soil structure and aggregation. This result supported by Rahayu (2014) that reported *C. etunicatum* had an optimal effect on plant growth, the absorption of phosphate, and the percentage of mycorrhizal infection. The ability of *C. etunicatum* in increasing the dry weight of *Alpitonia neocaledonica* and *Cloezia artensis* at 12 months (Amir *et al.* 2014). Among AMF, *C. etunicatum* had known more effective in reducing drought stress symptoms compared to *G. mosseae* (Behrooz *et al.*, 2019). Mycorrhizal maize plants had higher relative water content and water use efficiency under drought stress compared to those non-mycorrhizal plants (Zhu *et al.* 2012).

Application of AMF increased plant growth performance also reported by Halim *et al.* (2019); Rini & Efriyani (2016) AMF application increased the weight of shoots and roots of *Pueraria javanica*, and *Elaeis guineensis* Jacq. Research of Basuki (2013) showed that AMF inoculation on sugarcane plant could enhance seedling height 48.41%, and leaf wide 22.2% compared to control. Sugarcane seedlings that had been colonized with AMF

Table 1. Vegetative growth of signal grass in each treatment
Tabel 1. Pertumbuhan vegetatif rumput bede pada setiap perlakuan

Treatment Perlakuan	Plant height (cm)	Stem diameter (cm)	Number of leaves	Number of tillers	Fresh weight (g)		Shoot dry weight (g)
	Tinggi tanaman (cm)	Diameter batang (cm)	Jumlah daun	Jumlah anakan	Shoots Tajuk	Roots Akar	Bobot kering tajuk (g)
Without inoculation (control) Tanpa inokulasi (kontrol)	$37.08 \pm 9.97^{b*)}$	0.31 ± 0.12^b	5.25 ± 2.96^b	0.33 ± 0.32^b	1.44 ± 1.09^b	0.62 ± 0.30^b	0.45 ± 0.44^b
Inoculation Inokulasi	48.92 ± 4.06^a	0.43 ± 0.06^a	11.67 ± 5.00^a	1.92 ± 1.44^a	5.81 ± 0.66^a	5.15 ± 1.67^a	1.98 ± 0.13^a

*) Means in the same column followed by the same letter are not significantly different according to Independent samples t-Test at $\alpha = 0.05$
*) Rata-rata angka dalam kolom yang sama diikuti oleh huruf yang sama berarti tidak berbeda nyata menurut uji t sampel bebas pada $\alpha = 0,05$

produced fresh root weight two times higher than that of uninoculated seedlings. The colonized seedling hopefully could survive in dry conditions (Basuki 2013). According to Samanhudi *et al.*, (2014), AMF treatment at various doses (5, 10, and 15 g/plant) could increase the number of tillers of ginger rhizome. Saidi *et al.* (2014) stated that symbiosis mutualism between AMF and plants could increase the capacity of plants to absorb nutrients and water from the soil. AMF hyphae containing glomalin as a glycoprotein that serves to glue the between dispersed soil particles. The content of glomalin in soil was positively correlated with soil aggregate stability. Production of glomalin, it also related to the AMF response in handling the environmental stress. Salinity and osmotic stress enhance glomalin production in the mycelium of the AMF *Glomus intraradices* (Hammer & Rillig, 2011).

The results of the correlation analysis showed that all growth parameters correlated with an increase in the number of spores, although with different levels. However, a significant correlation was shown by shoots fresh weight with an increase in the number of spores. From the results of the study, it is suspected that photosynthates are produced with high shoots biomass, so there is a large amount of energy

for spore formation. Spores are AMF organs originating from the tip of the external hyphae, and their formation requires high energy and nutrients. Bago *et al.* (2002; 2003) state that AMF requires a high amount of energy and carbon for its growth, which is mostly transported in the form of lipids and glycogen for the development of AMF hyphae. Mycorrhizae are obligate biotrophs that depend on the carbon supply of host plants (Smith & Read, 2008). Previous research on ¹³C labeled tracer based NMR showed that hexose sugar was the primary vehicle used to transfer carbon from plant hosts (Shachar-Hill *et al.* 1995). High glucose transport activity is used for the development of AMF arbuscular and root colonization (Helber *et al.*, 2011).

Meanwhile, the absence of a significant positive correlation between the increase in the number of spores and root biomass may be due to the formation of spores not related to AMF infection (at the root) but instead to the number of photosynthates needed for spore formation and environmental conditions such as temperature, light intensity, and nutrient content of media.

Table 2. Coefficient correlation between vegetative growth parameter of signal grass and number of spores

Tabel 2. Koefisien korelasi antara pertumbuhan vegetatif rumput bede dengan jumlah spora

Vegetative growth parameter vs spore number <i>Parameter pertumbuhan vegetatif vs jumlah spora</i>	Coefficient correlation (<i>koefisien korelasi</i>)	
	r	Sig. (2-tailed)
Plant height <i>Tinggi tanaman</i>	0.143	0.918
Stem diameter <i>Diameter batang</i>	0.378	0.226
Number of leaves <i>Jumlah daun</i>	0.366	0.243
Number of tillers <i>Jumlah anakan</i>	0.124	0.701
Shoot fresh weight <i>Bobot basah tajuk</i>	0.652	0.021*
Root fresh weight <i>Bobot basah akar</i>	0.268	0.399
Shoot dry weight <i>Bobot kering tajuk</i>	0.299	0.345

*) Correlation is significant at the 0.05 level (2-tailed)

*) Korelasi signifikan pada tingkat 0,05 (2-tailed)

Conclusion

The results showed that *C. etunicatum* is the potential to enrich signal grass's roots. The AMF inoculation significantly increased the plant height, stem diameter, number of leaves, number of tillers, shoots fresh weight, root fresh weight and shoot dry weight ($p < 0.05$). It is expected that the availability of signal grass seedling colonized by AMF *C. etunicatum* supports the growth for Silvopasture preparation in post-mining land.

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