



Application of Arbuscular Mycorrhiza from Senaru Forest Rhizosphere for *Gyriopsis versteegii* Germination and Growth

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Abstract

The aims of this research are to apply Senaru forest rhizosphere on Kabupaten Lombok Barat West Nusa Tenggara as Mycorrhiza inoculants for *Gyriopsis versteegii* germination and growth. Rhizosphere sample was taken from ten sampling spot on Senaru forest between Latitude: 08°18.808' S – 08°19.174' S and Longitude: 116°24.138' E – 116°24.181' E. This study employed Factorial Experiment Design with 2 Factor including: Medium Composition (M) and Mycorrhiza Inoculant (I). There were 5 media composition: M1 (sand), M2 (soil:sand = 1:2), M3 (soil:sand = 1:1), M4 (soil:sand = 2:1), M5 (soil). There were 2 types of Inoculation: I1 (without inoculant) and I2 (inoculant from senaru Rhizosphere). Growth parameters observed in this study were: germination percentage, stem length, stem diameter and root colonization. Germination percentage of *G. versteegii* seeds in all growth media are below 60 % which could be classified as low germination rate. Also germination from media without rhizosphere is higher than germination from media with rhizosphere. On the other hand, *G. versteegii* growth on rhizosphere media is slightly higher than growth of *G. versteegii* on media without rhizosphere based on stem diameter and length measurement. It tends that medium composition with higher sand proportion tended to gives better germination and growth rate of *G. versteegii*. Mycorrhiza colonization on *G. versteegii* root was higher in media with rhizosphere addition. It could be concluded that Application of Senaru rhizosphere containing Mycorrhiza increases *G. versteegii* growth but not its germination percentage. This research enrich knowledge in biological science about association of mycorrhiza with *G. versteegii* especially on its growth and germination

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INTRODUCTION

Gyriopsis versteegii is one of endemic agarwood species to Lombok Island (Mulyaningsih & Yamada, 2007; Subehan *et al.*, 2005). This species has a high economic value that was expected could be a good solution for poverty problem in Lombok (Siddik, 2010). On the other hand, wild type of this species was in vulnerable status due to overexploitation and also made this species listed as CITES Appendix II (Schmidt, 2011). Thus, it is needed to develop this species by cultivation method to conserve its wild type. Cultivation also could improve agarwood production from *Gyriopsis versteegii* species as a main non-timber commodity of Lombok Island.

The most important aspect of cultivation is providing artificial environment to *G. versteegii* population as similar as their natural environment in forest. Among others environment factor, growth media has the most important role as the main nutrition supplier (Setiti *et al.*, 2016). For that reason, growth media selection needed to be done for sustainable *G. versteegii* cultivation. Growth media for *G. versteegii* should be designed as similar as its natural environment to ensure that growth media contains good nutrition. After natural growth media already been set up, improvement by adding substance that could give beneficial effect for *G. versteegii* growth. One of that beneficial substances is Mycorrhiza that also could be a good soil fertility indicator (Syib'li *et al.*, 2013).

Mycorrhiza arbuscular is Fungus from Glomeromycetes that improves plant growth by increasing absorption of water and mineral from growth media (Turk *et al.*, 2006). Agarwood from Aquilaria Group reported to grow better in medium with mycorrhiza. This group has better stem height, stem diameter and biomass compare to the same group grown in medium without Mycorrhiza. (Turjaman *et al.*, 2011). Same as Aquilaria group, agarwood from *Gyriopsis* group could be improved its growth by application of Mycorrhiza. Mbaubedari (2011) concluded that *G. versteegii* needs a high compatibility with Mycorrhiza as symbiont. So it is important to applied indigenous Mycorrhiza to improve growth of *G. versteegii*.

Senaru forest is one of potential place in Lombok Island as a source of indigenous Mycorrhiza associated with *G. versteegii*. Typical rainforest rhizosphere usually has complex ecological environment which rich of microorganism biodiversity, especially Mycorrhiza (Nottingham *et al.*, 2013). Moreover, this forest is well known

as *G. versteegii* natural habitat in Lombok Island. It is expected that arbuscular mycorrhiza isolated from this place has a high compatibility with *G. versteegii*. Thus, the aimed of this research are to apply Senaru forest rhizosphere as Mycorrhiza inoculants for *Gyriopsis versteegii* germination and growth.

METHODS

Senaru rhizosphere sampling

Rhizosphere sampling was conducted in 10 different spot. Coordinate of each spot were recorded by GPS receiver. Rhizospheres were taken from 10 cm - 20 cm depth. Root sample of *G. versteegii* growth in spot area also been taken for further analysis (Worabai, 2009).

Spore extraction from rhizosphere

Ten gram of rhizosphere sample were mix with 100 mL aquadest and shake vigorously. Filtration of mixture were conducted with 100 µm and 38 µm filter. Filtrate were centrifuge 2000 rpm for 20 minute. Pellet were collected and added by 50% sucrose solution, being centrifuged 2000 rpm for 5 minute. At this time, supernatant were collected by filtration using filter paper Advantec No. 2. Collected spore were identified using morphological character from INVAM website (<http://invam.wvu.edu/>)

Root preparation and screening

Root preparation were conducted based on Wangiyana (2009). *Gyriopsis versteegii* root were cut into 2 cm length, washed with aquadest and then soaked in 10% KOH for 48 hours. After 48 hours, root samples were washed with aquadest and soaked in 2% HCl for 30 minutes. After washed with aquadest, root samples were stained with 0.05 % Lactoglycerol Tripam Blue. After 12 hours staining, sample washed with aquadest and soaked in 50 % glycerin solution.

Growth Medium preparation

Growth media used in this research are combination of sand, soil dan rhizosphere with different composition. Sand and soil were taken from University of Nusa Tenggara Barat experiment field and sterilize by autoclave temperature 121°C, 2 atm for 15 minutes. Composition media were designed based on Randomize Complete Design with two factors.

First factor: composition of sand and soil

M1 : sand

- M2 : soil : Sand = 1:2
 M3 : soil : Sand = 1:1
 M4 : soil : sand = 2:1
 M5 : soil
 Second factor: Rhizosphere inoculation
 I1 : without rhizosphere inoculation
 I2 : with rhizosphere inoculation 1 : 1

Each media were used both in germination experiment and growth experiment. Senaru Rhizosphere were mix with all media in the same proportion (for second factor I2)

Preparation of *G. versteegii* seed

G. versteegii fruit was taken from *G. versteegii* cultivation field at Balai Penelitian Teknologi Hasil Hutan Bukan Kayu West Nusa Tenggara Province. Selection was conducted based on fruit size and colorization. After fruit wall has been peeled, seed were soaked with Atonic and Delsene Fungicide for 15 minutes (Utami, 2006). Seeds then spread on growth media based on experiment design.

Germination and growth experiment

Germination of *G. versteegii* seed were conducted for 21 days. Amount of germination were recorded every day. The percentage of germination was calculated by divided the number of germinate seed by the number of total seed on particular growth media (Aji et al., 2015). On the other hand growth experiment was conducted for 8 weeks. Growth parameter including: stem height and stem diameter measured every week. After 8 weeks, *G. versteegii* seedling were cultivated for colonization observation

$$\text{Germination Percentage} = \frac{\text{Number of germinated seed}}{\text{total seed in particular medium}} \times 100\%$$

Mycorrhiza colonization observation

Mycorrhiza colonization from *G. versteegii* were observed by staining with lactoglycerol tripam-blue based on the same method as root preparation and screening. Colonization of arbuscular Mycorrhiza observation were conducted by gridline intersect method (Wangiyana, 2009). Colonization percentage were measured by divided the number of root with colonization to the number of total root sample.

Data Analysis

Germination percentage and root colonization percentage were analyzed using Analysis of Variance (ANOVA) and then continued by

Honest Significant Different (HSD) if the result was significant. Error Standard analysis also conducted to analyzed interaction between factors. ANOVA, HSD and Error analysis using 5% significant error ($\alpha=0.05$). All analysis conducted by Co-stat for windows program. Growth of *G. versteegii* seed was analyzed with line graphic for growth pattern analyzes.

RESULTS AND DISCUSSION

Sampling Location

Sampling location at Senaru Forest, Kabupaten Lombok Barat, West Nusa Tenggara Province. Basaed on the survey, we could define the boundary location of sampling is at coordinate: Latitude: 08°18.808' S – 08°19.174' S and Longitude: 116°24.138' E – 116°24.181'E. This location is chosen because of high population of *G. versteegii* on that area. This could be good data information for further mapping study especially using Geographic Information System.

Beside coordinate location, depth of sampling also play important role for Mycorrhiza biodiversity study. Thus, samples were taken from depth less than 20 cm of rhizosphere. This sampling depth is an ideal location for mycorrhizal sampling and also ideal place for growth medium sampling due to high of mineral nutrition (Liu et al., 2016)

Spore and root from Senaru Rhzosphere

Based on the microscopic observation, Senaru forest rhizosphere contain Mycorrhiza arbuscular spore with medium abundance. Stem sample from rhizosphere also confirmed that Mycorrhizal colonization occurred in mostly all stem sample. It could give specific location for further study about endemic mycorrhiza study from *G. versteegii* natural habitat that needed for sustainable *G. versteegii* cultivation (Mbaubedari, 2011). It was confirmed based on the morphological character that spore belong to *Glomus* genus

Germination Percentage

Addition of Senaru rhizosphere gave no significant result on germination process. However, it does not always mean that growth media composition has no important role in germination process. Based on figure 2 composition media M2 give the highest percentage germination of *G. versteegii* seed. M2 media contains more sand then soil (sand : soil = 2 : 1) that means this medium has great porosity but also contains enough nutrition.

Table 1. Sampling Location Spot on Senaru Forest

Location code	Sampling depth (cm)	Latitude	Longitude
1	0 – 10	S: 08° 19.174'	E: 116° 24.138'
2	0 – 10	S: 08° 19.116'	E: 116° 24.148'
3	0 – 10	S: 08° 19.077'	E: 116° 24.167'
4	0 – 10	S: 08° 18.835'	E: 116° 24.166'
5	0 – 10	S: 08° 18.850'	E: 116° 24.164'
6	0 – 10	S: 08° 18.831'	E: 116° 24.169'
7	10– 20	S: 08° 18.827'	E: 116° 24.169'
8	10– 20	S: 08° 18.814'	E: 116° 24.181'
9	10– 20	S: 08° 18.808'	E: 116° 24.166'
10	10– 20	S: 08° 18.821'	E: 116° 24.170'

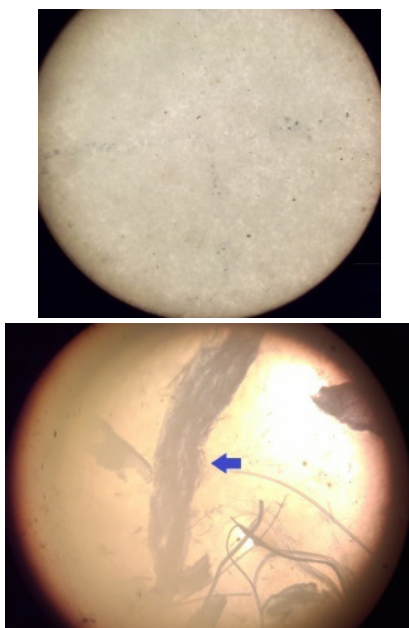


Figure 1. Mycorrhiza spore (left) and Mycorrhiza colonization (right) from Senaru Forest

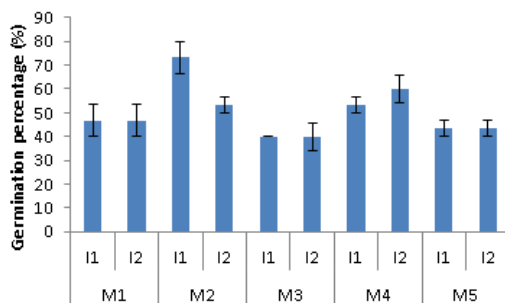


Figure 2. Germination percentage of *G. versteegii* on different growth media Seed Growth (M1: sand, M2: soil:sand = 1:2, M3: soil:sand = 1:1, M4: soil:sand = 2:1, M5: soil, I1: without inoculant and I2: inoculant from senaru Rhizosphere 1:1)

Application of Senaru rhizosphere contains mycorrhiza should be able to improve *G. versteegii* germination percentage due to a lot of benefit given by mycorrhiza for its host. However, based on the result, the germination percentage of *G. versteegii* on rhizosphere media is not significantly different from its germination on media without rhizosphere. There are two possibilities that could describe this phenomenon. First, association with mycorrhiza was not significantly needed by seed in the initial stage of germination. The most important factor for seed germination is water concentration because it could activate enzymatic process in the seed. Second, it could possibly that *G. versteegii* seeds using in this research has a very low viability. Based on the result, germination percentage average of *G. versteegii* is still below 70% which mean that its germination percentage is still low. That is why Agar wood seed including *G. versteegii* is classified into recalcitrant seed that has short viability period and hard to germinate (Tabin & Shrivastava, 2014)

Internal factor play important role germination because in the beginning stage of growth plant cannot do photosynthesis. Plant also cannot absorb nutrition from growth media just like what mature plant do. Hormonal factor play important role in the beginning of seed germination more than what environment factor can do (Miransari & Smith, 2014). This could be one of the reasons why germination percentage of *G. versteegii* seed at germination media with and without Senaru rhizosphere is not significantly different. Addition of Senaru rhizosphere containing mycorrhiza may lead to better nutrition absorption but it will not give significant impact if the seed could not adsorb nutrition optimally.

G. versteegii Growth

Based on stem height *Gyrinops versteegii*

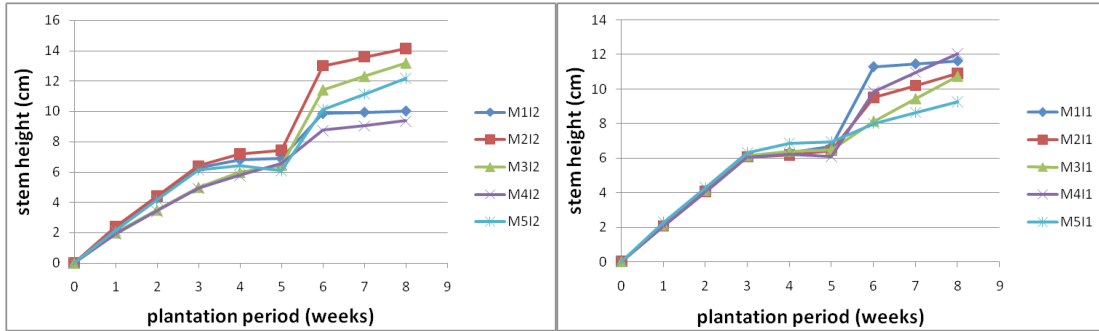


Figure 3. Comparison of *G. versteegii* growth in medium without rhizosphere (left) and with rhizosphere (right) based on stem height parameter. (M1: sand, M2: soil:sand = 1:2, M3: soil:sand = 1:1, M4: soil:sand = 2:1, M5: soil, I1: without inoculant and I2: inoculant from senaru Rhizosphere 1:1)

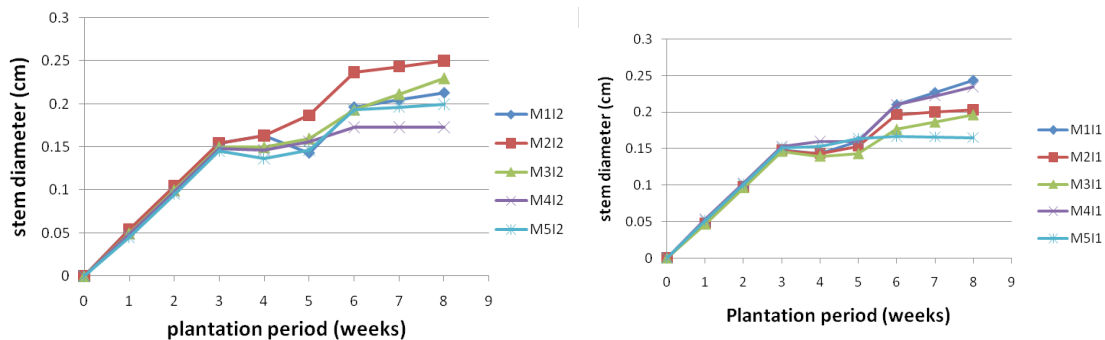


Figure 4. *G. versteegii* growth based on stem height (top) and stem diameter (bottom). (M1: sand, M2: soil:sand = 1:2, M3: soil:sand = 1:1, M4: soil:sand = 2:1, M5: soil, I1: without inoculant and I2: inoculant from senaru Rhizosphere 1:1)

growth in medium with Senaru forest rhizosphere slightly better than its growth in medium without rhizosphere (Figure 2). Maximum height of *G. versteegii* on growth medium with rhizosphere is 2 – 5 cm higher than *G. versteegii* maximum height on medium without rhizosphere after 8 week sowing. This margin could be increase after longer plantation period.

Medium composition also play important role on *G. versteegii* growth. *G. versteegii* has a better stem height on medium that contain more sand than soil (M2 and M1). Higher concentration of sand than concentration of soil in medium give a better aeration on the medium. Seed on the early stage of growth (1 – 8 weeks after sowing) need a good aeration to grow optimally. Growth medium aeration has a negative correlation with solidity level of medium. Medium with less solid structure have better aeration than medium with high solid structure (Chen et al., 2011). Moreover medium with better aeration also support the growth of young root that need less solid medium.

On the other hand, based on stem diameter, there is no significant different between *G. versteegii* growth on media with rhizosphere and without rhizosphere (Figure 3). Maximum

stem diameter of *G. versteegii* both on medium with and without Senaru rhizosphere are 0.25 cm. As a woody tree plant, *G. versteegii* seedling has a very slow diameter growth in the beginning of growing state (Turjaman et al., 2006). Several months after germination is the beginning growing state, that means *G. verasetegii* stem diameter grow in a very slow rate at this time. On a slow growth rate period, it is logic that *G. versteegii* stem diameter has no significant different.

Mycorrhizal Colonization on *G. versteegii* root

Inoculation Factor give significant effect on *G. versteegii* root colonization. Mycorrhiza colonization from *G. versteegii* roots on media with Senaru rhizosphere is better than those on media without Senaru rhizosphere (I factor). On the other hand, there is no significant result of root colonization on different media composition (M factor) especially on M2, M3 and M4 (Figure 5).

It is indicate that mycorrhiza from Senaru rhizosphere compatible with *G. versteegii* as its host. This compatibility was shown by the ability of mycorrhiza from Senaru rhizosphere to colonize *G. versteegii* root in all different growth media composition (M2, M3 and M4). The lowest

colonization percentage is on the growth media without a mixing composition of sand and soil (only contains sand or only contains soil). It is suggested that mixing sand and soil in growth media is important to optimize mycorrhiza root colonization of *G. versteegii*. Addition another component of media beside sand and soil is also possible (for example: compost, manure or cocopeat). However, soil and sand are still natural and basic medium growth for most plant.

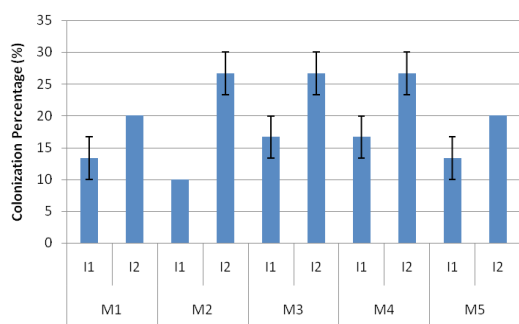


Figure 5. Colonization Percentage of Mycorrhiza on *G. versteegii* seed. (M1: sand, M2: soil:sand = 1:2, M3: soil:sand = 1:1, M4: soil:sand = 2:1, M5: soil, I1: without inoculant and I2: innoculant from senaru Rhizosphere 1:1)

Mycorrhiza from Senaru rhizosphere were taken from natural habitat of *G. versteegii*. Mycorrhiza from its natural habitat has a good compatibility with host (Mbaubedari, 2011). However, even mycorrhiza of Senaru rhizosphere has good compatibility with *G. versteegii*, its colonization percentage is still approximately low (less than 50%). This phenomenon could be explained by the fact that colonization of mycorrhiza is increase in a positive correlation with the growth of root (Smith & Read, 2008). For woody plant like *G. versteegii*, 8 week after sowing is an early stage of growth. That means colonization percentage of Senaru rhizosphere mycorrhiza could be increase along with the increase of *G. versteegii* growth parameter. For that reason this early stage study of root colonization still could give important information about association of *G. versteegii* with Mycorrhiza from Glomus group.

This research is preliminary study about association of mycorrhiza from Senaru rhizosphere with *G. versteegii*. All data was taken from *G. versteegii* in a very early stage of growth. It is important to continue measuring data (especially root colonization) at least until *G. versteegii* has entered generative stage. However observation until this species has reached its generative stage give a potential problem since *G. versteegii* need up to 2 – 3 years to produce flowers and fruit (Isnaini *et al.*,

2010). Once again, for that reason most representative study about association of *G. versteegii* and Mycorrhiza is at the seedling stage of *G. versteegii*.

CONCLUSION

It could be concluded that application of Senaru rhizosphere containing Mycorrhiza increase *G. versteegii* growth based on stem height. Mycorrhiza from Senaru rhizosphere also has good compatibility with *G. versteegii* that shown by its colonization percentage on *G. versteegii* root. However this mycorrhiza gives no significant effect on *G. versteegii* germination

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