

Short Communication:

Growth response and dependency of endangered nedun tree species (*Pericopsis mooniana*) affected by indigenous Arbuscular Mycorrhizal Fungi inoculation

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Abstract. Husna, Tuheteru FD, Wigati E. 2017. Short Communication: Growth response and dependency of endangered nedun tree species (*Pericopsis mooniana*) affected by indigenous Arbuscular Mycorrhizal Fungi inoculation. *Nusantara Bioscience* 9: 57-61. Arbuscular mycorrhizal fungi (AMF) were isolated from the rhizosphere of local nedun tree or *kayu kuku* (*Pericopsis mooniana* (Thw.) Thw.) and based on the results of previous studies which showed that inoculation with AMF mix enhanced growth and nutrient uptake of seedlings *P. mooniana* on ultisol and serpentine soil media. Thus study aimed to determine the effectiveness of indigenous AMF on the growth and dependence of *P. mooniana*. *P. mooniana* seedlings were grown on ultisol soil medium and inoculated with and without AMF (*Clareodeglomus etunicatum*, *Glomus* sp. (HA), *Glomus* sp. (KDI)) and mixed (*C. etunicatum*, *Glomus* sp. (HA), *Glomus* sp. (KDI) for 12 weeks under greenhouse conditions. The results showed that inoculation with *Glomus* sp. (HA) significantly increased plant height, stem diameter, number of leaves, dry weight (roots, shoots, total), nodulation, root length and leaf length. It was better than results on control of *P. mooniana* seedling but it was not significantly different from other types of AMF, except on *Glomus* sp. (HA) on plant height. The width of the leaf and shoot-root ratio was not influenced by the AMF. *P. mooniana* has a high dependency (<75%) with mycorrhizal dependency (MD) value ranging from 54-68%. *Glomus* sp. (HA) and other types of AMF have potential to be developed to support the conservation of *P. mooniana*.

Keywords: Indigenous arbuscular mycorrhizal fungi, mycorrhizae dependency, *Pericopsis mooniana*, plant growth

INTRODUCTION

An Arbuscular Mycorrhizal Fungi (AMF) is obligate symbiotic fungi of the phylum Glomeromycota and belongs to endomycorrhiza groups (Smith and Read 2008). Fungi form a symbiosis with the roots of 70-90% of land plants (Wang and Qiu 2006) and have a very broad range of ecology, from land to wetland habitat. The existence of AMF is needed by plants in condition of stressed environment. AMF can increase the tolerance of crops in biotic and abiotic stresses, such as drought (Zhang et al. 2014), salinity (Giri et al. 2003), roots pathogens (Akhtar and Siddiqui 2007), inundation (Wu et al. 2013), as well as heavy metal (Kerand Christine 2009; Orłowska et al. 2011). Inoculated crop tolerance can occur through improving nutrient status, water and biochemistry and physiology conditions of plants (Smith and Read 2008; Wu et al. 2013). Inoculated crop tolerance is influenced by the type of AMF, soil conditions, and crop types.

One type of tree that can be symbiotic with the AMF is the nedun tree or *kayu kuku*, *Pericopsis mooniana* (Thw.) Thw. (Husna et al. 2014, 2015). Fifteen types of AMF have been isolated from the rhizosphere of *P. mooniana* at six locations in Southeast Sulawesi and 4 species of them were first reported in Indonesia. In addition to ecology of AMF, inoculation of AMF, both Mycofer IPB and Indigenous

AMF, has also been reported that it can increase the growth and biomass of *P. mooniana* under greenhouse nursery (Husna et al. 2010, 2016), and in field scale (Husna et al. 2015). In addition to the growth and biomass, AMF also serves to increase the nutrient uptake, particularly N and P, and the number of total chlorophyll content and plant root nodulation of *P. mooniana* (Husna et al. 2015, 2016).

In this study, AMF applied to *P. mooniana* in the nursery was AMF of Mycofer IPB (Husna 2010) and the results of trapping AMF in the rhizosphere of *P. mooniana* in Southeast Sulawesi (Husna et al. 2016). However, information on the testing of indigenous AMF in the rhizosphere of *P. mooniana* is still lacking. Knowledge of the effectiveness of indigenous AMF and legume species dependency on AMF application is determined by the suitability of the AMF and the host plant (Baar 2008; Husna et al. 2015). Therefore, this research was conducted to determine the growth response that was affected by inoculation of indigenous AMF in Southeast Sulawesi.

MATERIALS AND METHODS

Seed planting

Pericopsis mooniana seeds were collected from the mother trees at Forestry Office of the Southeast Sulawesi

Province, Kendari, Southeast Sulawesi, Indonesia. Seeds were extracted manually after drying for 4 days. Prior to germination, seeds were sterilized with NaClO (5.25%) 2 mL/L of water. Pre-treatment of seeds was done in the form of filing on the side of the seed and soaking in hot water at a temperature of 80 °C for 24 hours. Seeds were then planted in a pot culture of dimensions 30 cm x 20 cm x 10 cm containing sterilized zeolite media (diameter 2 mm). Seeds germination was started on the fifth day.

Media preparation

Soil which was used for media was collected from the ground around the old campus of Universitas Halu Oleo, Kendari, Southeast Sulawesi, Indonesia and kept in a greenhouse. The soil was sieved and mixed with river sand, manure, and rice husk in the ratio of 1: 3: 1: 0.5. Media mixture was then sterilized for 2 hours at 100 °C.

Inoculum production

In this study, *Clareodeoglossum etunicatum*, *Glomus* sp. (KDI), *Glomus* sp. (HA), and the AMF mixture (*C. etunicatum*, *Glomus* sp. (KDI), *Glomus* sp. (HA)) were isolated from the rhizosphere of trees at natural forests in Tanggetada, Kolaka, Southeast Sulawesi, Indonesia and forest plantations at Governor office in Kendari, Southeast Sulawesi. AMF were propagated in pot culture with host plant of *Pueraria javanica*. After 90 days, AMF spores, external hypha and colonized root pieces were harvested with zeolite media.

AMF treatment and research design

A total of 10 g of each of the AMF inoculum containing pieces of colonized roots, mycelia, and ± 500 spores were placed 3-4 cm close to the seedling roots in polybags (15 cm x 20 cm) which contained 1 kg of sterilized soil mixture media. Non-inoculated plants were used as controls. Plants were watered daily at field capacity conditions. Plants were grown for 90 days under greenhouse condition. Weeds and pests were controlled manually. This study was designed using a randomized block design (RBD), which consists of 5 treatments namely: control, *C. etunicatum*, *Glomus* sp. (HA), *Glomus* sp. (KDI), and a mixture of the three types of AMF, each consisting of three replications with 5 units of the plant, and total plant population was 75 units.

Measurements and data analysis

Seedling height and stem diameter were measured from 1 cm above the surface of the media at the age of 90 days after planting. Once harvested, shoots and roots were also separated. Root length, number of nodules, as well as leaf size was measured after harvesting. Plant dry weight was measured after the shoots and roots have been dried at oven with temperature 70 °C for 48 hours. Shoot-root ratio was measured based on comparison between shoot and roots dry weight at the end of the study. Seedling Quality Index (SQI) was calculated using the formula: $SQI = \frac{[\text{shoot dry weight} + \text{root dry weight}]}{[(\text{height/diameter}) + (\text{shoot dry weight}/\text{root dry weight})]}$. Seedling was categorized as good quality if the value of SQI was ≥ 0.09 (Duryea and Dougherty 1991).

Roots colonization was calculated after root samples were cleaned with 10% KOH and were stained using *tryphan blue* solution (Brundrett et al. 1996) with the following formula : $\text{root colonization} = \frac{(\sum \text{field-of-view of inoculated root segment})}{(\sum \text{total field-of-view observed})} \times 100\%$ (Brundrett et al. 1996) while *mycorrhizal dependency* (MD) was calculated according to Habte and Manjunath (1991) by using the formula: $[(\text{dry weight of inoculated plants} - \text{dry weight of non-inoculated plant}) / \text{dry weight of inoculated plants}] \times 100\%$. Data were analyzed using analysis of variance (ANOVA) with *SAS software 9.1.3 Portable* and for comparison of treatment mean values *Duncan's Multiple Range Test* (DMRT) at confidence level of 95% was used.

RESULTS AND DISCUSSIONS

Root colonization and Mycorrhizal Dependency (MD)

In inoculated plant, root colonization by indigenous AMF was <50%, while in non-inoculated plants there was no AMF structure. MD value obtained in this study ranged from 55-68% (<75%) (Table 1). The AMF type with high MD tend to promote plant growth of nedun tree tree species, *P. mooniana* at the age of 90 days and SQI value of plants without mycorrhizal was <0.09 (Table 1).

Based on the data at Table 1, *P. mooniana* have a high mycorrhizal dependency (MD) (55-68% or MD <75%) as categorized by Habte and Manjunath (1991), as well as the Seed Quality Index (SQI) of inoculated plant is ≥ 0.09 (Duryea and Dougherty 1991). In addition to the mycorrhizae, some kind of tropical legume trees were also reported have a high dependency on mycorrhizae such as *Anadenanthera falcata*, *Albizia lebeck* (Siqueira et al. 1998), *Caesalpinia ferrea*, *Senna macranthera*, *S. spectabilis* (Siqueira et al. 1998; Siqueira and Saggin 2001), *Leucaena leucocephala* (Habte and Manjunath 1991; Manjunath and Habte, 1991; Siqueira and Saggin 2001), *L. diversifolia*, *L. trichodes* (Habte and Manjunath 1991), *Indigofera stenophylla* (Duponnois et al. 2001), *Acacia nilotica* (Sharma et al. 2001), *A. mangium* (Ghosh and Verma 2006), *Dalbergia sisso* (Bisht et al. 2009), and *Albizia saponaria* (Tuheteru et al. 2011a, 2011b).

MD value and high SQI indicated that the indigenous AMF inoculation can be utilized for the production of quality seedlings in the nursery and support the success of conservation efforts of *P. mooniana* on field scale through ex-situ conservation programs. AMF is reported to be effective in the cultivation of endangered plant species. Besides that, it can also accelerate the succession and the success of living plant species in conservation and rehabilitation programs (Turjaman et al. 2006; Fuchs and Haselwandter 2008; Zubek et al. 2009; Bothe et al. 2010; Husna et al. 2015, 2016).

Growth and biomass plants

Colonization by 4 different AMF has proven to increase the average growth of plant height, stem diameter, and the number and length of *P. mooniana* leaves compared to control treatment, or increased growth by 99-184% of plant

height, stem diameter (111-129%) (Figure 1), the number of leaves (136-182%), leaf length (46-68%), and the number of root nodules (Table 2) than control. Plant parameter such as height, was significantly different between treatments of *Glomus* sp. (HA) and *Glomus* sp. (KDI), but it was not significantly different from other mycorrhizal treatment. *P. mooniana* seedlings inoculated with *C. etunicatum* and *Glomus* sp. (HA) had higher length of roots than control. Non-mycorrhizal seedlings at 90 days after planting have not produced nodules than inoculated plants. The fourth AMF inoculation treatment did not affect the width of leaf and shoot-root ratio (Table 2). Inoculation with *C. etunicatum*, *Glomus* sp. (HA), *Glomus* sp. (KDI), and the AMF mixture also boosted the dry weight of the plants (roots, stems, and leaves) (Figure 2).

Inoculation with *C. etunicatum*, *Glomus* sp. (HA), *Glomus* sp. (KDI), and mixed AMF significantly enhanced the growth of seedlings of endangered species *P. mooniana* at 90 days after planting. Increased growth of inoculated *P. mooniana* allegedly occurred through the absorption of nutrients, particularly phosphates and nitrogen, and water by the external hyphae of AMF (Smith and Read 2008) as well as plant resistance to biotic and abiotic stresses (Wu et al. 2013; Zhang et al. 2014). Stimulation of the growth of legume crops has also been reported by previous

researchers. Inoculation with *Glomus aggregatum* stimulated the growth of 17 legume crops in Senegal (Duponnois et al. 2001), and *Glomus macrocarpum* boosted the growth of *Sesbania aegyptiaca* and *S. grandiflora* (Giri et al. 2004). Moreover, AMF also increased the growth of *Acacia nilotica* and *Leucaena leucocephala* at 12 weeks after planting (Michelsen and Rosendahl 1990). AMF and rhizobium effectively improved the growth of three legume trees (Cruz et al. 1988).

Table 1. Effect of AMF treatment on root colonization, mycorrhizal dependency (MD), and the Seed Quality Index (SQI) of *P. mooniana* at 90 days

Treatment	Root colonization (%)	Mycorrhizae Dependency (MD) (%)	Seedling Quality Index (SQI)
Control	0.0±0.00 ^b	-	0.07±0.04 ^b
<i>C. etunicatum</i>	37.8±8.68 ^a	57±8.76	0.18±0.06 ^a
<i>Glomus</i> sp. (HA)	33.3±5.11 ^a	68±3.69	0.21±0.01 ^a
<i>Glomus</i> sp. (KDI)	33.3±7.71 ^a	56±10.26	0.20±0.05 ^a
Mixed AMF	24.4±5.57 ^a	55±15.63	0.21±0.06 ^a

Note: Same letters in the same column showed that no significant difference between treatments at 5% confidence level based on Duncan Multiple Range Test

Table 2. Effect of AMF treatments on plant height, stem diameter, root growth and seedling leaf of *P. mooniana* at 90 days

Treatments	Height (cm)	Stem diameter (mm)	Root length (cm)	Total root nodules	Stem-root ratio	Total leaves	Leaf Length	Width
Control	4,1±0,35 ^c	0,77±0,37 ^b	64,7±0,37 ^b	0±0,00 ^b	3,27±0,38	3,6±0,33 ^b	5,4±0,27 ^b	3,7±0,15
<i>C. etunicatum</i>	10,5±0,93 ^{ab}	1,63±0,23 ^a	163,5±0,23 ^a	15±2,73 ^a	3,56±0,25	9,0±1,00 ^a	8,0±1,12 ^a	5,4±0,62
<i>Glomus</i> sp. (HA)	11,7±0,87 ^a	1,77±0,11 ^a	142,7±0,11 ^a	13±2,19 ^a	3,61±0,52	10,3±2,33 ^a	9,1±0,53 ^a	6,1±0,27
<i>Glomus</i> sp. (KDI)	8,2±0,78 ^b	1,66±0,26 ^a	109,2±0,26 ^{ab}	18±3,71 ^a	3,33±0,32	8,7±1,20 ^a	8,1±1,13 ^a	5,4±0,89
Mixed AMF	9,2±1,33 ^{ab}	1,03±0,36 ^a	114,7±0,36 ^{ab}	13±2,08 ^a	3,34±0,53	10,3±2,19 ^a	7,9±1,18 ^a	5,6±0,84

Note: Same letters in the same column show no significant difference between treatments at 5% confidence level base on Duncan Multiple Range Test

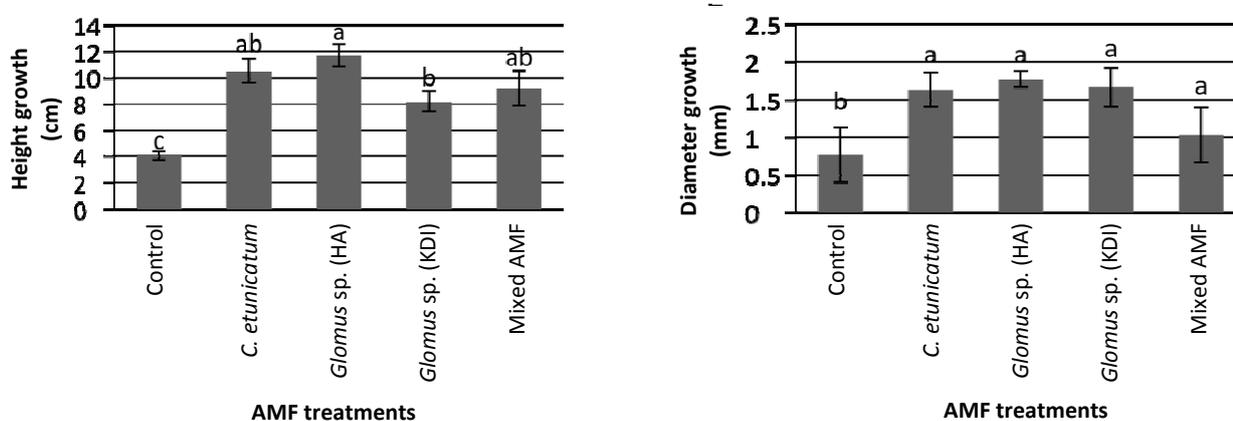


Figure 1. Effect of AMF treatment on the average height (left) and diameter (right) of *P. mooniana* seedling at 90 days after planting

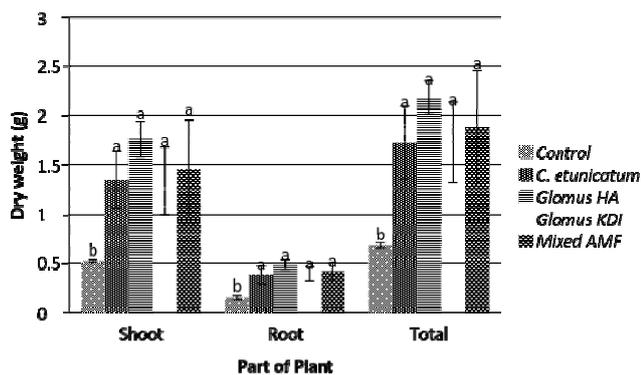


Figure 2. Effect of mycorrhiza treatment on dry weight of *P. mooniana* plant seedlings at 90 days after planting

In addition to the growth of plants, indigenous AMF are also able to stimulate the formation of nodules of legume plants compared to the controls (Table 2). The results were in accordance with several previous studies on legumes, *Sesbania aegyptiaca* and *Sesbania grandiflora* (Giri and Mukerji 2004), *Cassia siamea* (Giri et al. 2005), *Pisum sativum* cv. avola (Geneva et al. 2006), *Albizia saponaria* (Tuheteru et al. 2011a,b), *P. mooniana* (Husna et al. 2015, 2016), *Sesbania cannabina* (Ren et al. 2016), and *Cicer arietinum* L. (Garg and Singla 2016). The existence of AMF may supply P for the formation of nodules as well as increase the activity of N_2 fixation by *Rhizobium* (Geneva et al. 2006).

Based on these, it can be seen that the indigenous AMF are compatible and plays an important role in the early growth and increasing plant biomass of *P. mooniana*. Compatibility of *P. mooniana* with the indigenous AMF is determined by several factors, including: (i) The local AMF has adapted with existing field conditions, (ii) Local AMF is suitable with the root exudates produced by *P. mooniana*, mainly because the AMF is locally isolated from the rhizosphere of the *P. mooniana*, and (iii) through genotype, local AMF has the ability to absorb and deliver water and nutrients to the host plant (Johnson et al. 1997).

Based on the results, it can be concluded that *P. mooniana*, including the legumes having high dependency on local AMF and inoculation with *C. etunicatum*, *Glomus* sp. (KDI), *Glomus* sp. (HA), and mixed AMF can improve the quality of seeds (SQI > 0.09), root nodulation and growth of seedlings at 90 days after planting. Indigenous AMF has potential to be developed as a biological fertilizer based on local resources for the conservation of *P. mooniana* as well as for other purposes.

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