

PAPER • OPEN ACCESS

The effects of arbuscular mycorrhizal inoculation to growth and survivability of micropropagated *Eucalyptus pellita* and *Acacia crassicarpa* in nursery

To cite this article: L Agustini *et al* 2020 *IOP Conf. Ser.: Earth Environ. Sci.* **533** 012028

View the [article online](#) for updates and enhancements.

A promotional banner for the PRiME 2020 meeting. The background features a blue and white globe. On the left, there are three circular logos: the top one is 'ECS' in a circle, the middle one is 'The Electrochemical Society' with a stylized 'ECS' logo, and the bottom one is 'THE KOREAN ELECTROCHEMICAL SOCIETY'. The main text in the center reads 'Joint International Meeting PRiME 2020 October 4-9, 2020' in white and blue. Below this, a blue bar contains the text 'Attendees register at NO COST!' in white. On the right side, there is a logo for 'PRiME™ PACIFIC RIM MEETING ON ELECTROCHEMICAL AND SOLID STATE SCIENCE 2020'. At the bottom right, a blue bar contains the text 'REGISTER NOW' with a white right-pointing arrow.

ECS

Joint International Meeting

PRiME 2020

October 4-9, 2020

Attendees register at NO COST!

PRiME™
PACIFIC RIM MEETING
ON ELECTROCHEMICAL
AND SOLID STATE SCIENCE
2020

REGISTER NOW ▶

The effects of arbuscular mycorrhizal inoculation to growth and survivability of micropropagated *Eucalyptus pellita* and *Acacia crassicarpa* in nursery

L Agustini¹, R S B Irianto¹, H Indrayadi², R D Tanna², Fahrizawati², S A Faulina¹, A Hidayat¹, B Tjahjono², D Priatna^{3,4} and M Turjaman¹

¹ Forest Research and Development Center, Jl. Gunung Batu No.5, Bogor – Indonesia

² R & D Department of PT.Arara Abadi, Sinar Mas Forestry, Jl. Raya Minas KM 26, Perawang, Riau – Indonesia

³ Department of Forest Landscape Conservation of PT. Asia Pulp and Paper Group, Sinarmas Land Plaza, Jl. MH Thamrin No.51, Jakarta – Indonesia

⁴ Study Program of Environment Management, Post Graduate Program of Pakuan University, Jl. Pakuan Kotak Pos 452, Bogor – Indonesia.

E-mail: luciagustini2014@gmail.com

Abstract. Inoculation of arbuscular mycorrhizal (AM) fungi into micropropagated *Eucalyptus pellita* and *Acacia crassicarpa*, that may have poor root structures, was conducted. The study aimed to investigate the effects of *Acaulospora* sp., *Acaulospora tuberculata*, *Entrophospora* sp., *Gigaspora* sp., and two different isolates of *Glomus maculosum* inoculations on the growth parameters of 21-days old plantlets of *Eucalyptus pellita* and *Acacia crassicarpa*. After 120 days of acclimatization in the nursery, *E. pellita* seems to be more responsive to mycorrhizal inoculation than *A. crassicarpa*. The survival rate of *E. pellita* was almost three times of the control. Although there was no significant difference between treatments, inoculation of *Glomus maculosum* RD.1.5.1 and *Acaulospora* sp. GB.10.A2 showed the highest impact on plant height (54 – 56 cm), stem diameter (4.3 – 4.4 mm), and root dry-weight (2.03 – 2.05 gr) of *E. pellita*; and *Entrophospora* sp. RB.10.3.1 on plant height, stem diameter, and root dry-weight of *A. crassicarpa* (41.46 cm, 2.96 mm, and 1.34 gr, respectively). This study also revealed that the benefits of AM fungi association were not always related to the level of root colonization.

1. Introduction

Micropropagation through plant tissue culture techniques has been widely applied in forestry plantation industries, either of exotic- and indigenous- plant species, including the planting materials derived from genetic improvement programs [1]. The success rate of micropropagation of the plants is determined by the stage of plantlets transfer from in vitro conditions to the field through an acclimatization process [2]. Acclimatization allows the plants to adapt to the ambient conditions that will improve plant survivability when they are planted in the field [3]. However, on the commercial scale, cost-effective acclimatization with high survival rates of the plants is still a big challenge for plant stock production [4]. Plantlets that grow heterotrophically in nutrient-rich media and micro-environments with low light intensity and very high humidity can produce individual plants with



morphological and physiological abnormalities. For example, non-functional stomata, weak root structures, low photosynthetic efficiency, anatomical abnormalities of leaf palisade tissue and others [2 - 4].

Root organogenesis in plant micropropagation technique is often problematic. The fragile root system that is easily broken when the process of separating individual plantlets from the cluster in the culture bottle and the presence of non-functional roots due to the disconnection of the vascular tissue in the roots with the one in the stem are a couple of the in vitro root structure constraints which affect the plant's survival rate [4]. Thus, in order to improve root structures and morphology as well as its function, several efforts at the acclimatization stage are required. Applying the plant growth regulator of auxin groups, especially IBA (Indole Butyric Acid) and inoculation of arbuscular mycorrhizal (AM) fungi, may induce and strengthen roots structure and increase plant survival rates [5, 6].

Arbuscular mycorrhizal (AM) fungi are obligate biotrophs that form mutualistic symbioses with the majority of terrestrial plants by improving phosphorus (P) nutrition of the host plants via increased P-uptake through in-depth hyphal networks within the soil [7]. AM fungi application has numerous beneficial impacts for both horticulture crops and forestry plantation industries, such as improving plant nutrient cycles and soil aggregates [8, 9]. The association of AM fungi with plant roots can enhance plant growth and improve their ability on nutrient uptake from the soil, especially the element of P, Ca, N, Cu, Mn, K and Mg [9]. The symbiosis of AM fungi and plants can induce noticeable changes in the host traits, such as root architecture, growth, flowering, and stress tolerance; All of which are regulated by phytohormones [10]. Mycorrhizae may alter concentration and concerted action of cytokinins, auxins, and strigolactones, which regulated the formation of root architecture [11]. The symbiosis can also alter root hydraulic properties that are regulated by abscisic acid (ABA) and influence the increase of plant water uptake under unfavorable conditions [12]. Moreover, the association between AM fungi and host plants induces many mechanisms that would affect plant tolerance to avoid stress damages such as drought stress, contaminated soil, and pathogens infection, which may offer the possibility of minimizing the application of chemicals for fertilizer and pesticides [8, 13].

The benefits of mycorrhizal applications in plantlets have been demonstrated in several herbaceous and woody species [14], such as *Spilanthes acmella* (ornamental medicinal herbs), *Persea Americana* (avocado), *Punica granatum* (pomegranate) and *Prunus* spp. [14 - 17]. However, scientific reports on the influence of AM fungi on growth and survival of micropropagated forest tree species are limited, whereas the supply of rootstock through micropropagation has been widely applied by several forestry plantation companies. One of the biggest industrial plantation companies in Indonesia who planted *Eucalyptus pellita* and *Acacia crassicarpa*, rely on in vitro micropropagation technique to ensure the sustainable supply of the rootstocks of *E. pellita* and *A. crassicarpa* which were developed from the company's tree improvement programs. However, as commonly happened in other plants that were developed through in-vitro micropropagation, *E. pellita* and *A. crassicarpa* plantlets have fragile root structures and may exhibit abnormality in morphology and physiology in other organs as well [4]. Therefore, related to the role and benefits of AM fungi-plant root associations and as an effort to improve the rootstock quality of *E. pellita* and *A. crassicarpa*, this study investigates the effects of six indigenous AM fungal isolates on survivability and growth parameters of the plantlets in the nursery.

2. Materials and Methods

2.1. Plant materials and preparation of AM fungi inoculant

Plantlets of *E. pellita* and *A. crassicarpa*, aged 21-days old, were obtained from the Tissue Culture Laboratory of PT. Arara Abadi in Perawang, Riau province. Five isolates of AM fungi used in this study were collected from the rhizosphere of *E. pellita*, *Cratogeomys arborescens* and *Shorea balangeran* stands growing in the concession area of three different plantation companies belonging to Sinarmas Forestry group [18] (Table 1). AM fungal propagules from the collected rhizosphere soil samples were trap-cultured in 200 mL pots using sterilized zeolite media and *Pueraria javanica* as the host plant for approximately three months, then the AM fungal spores were collected from the

rhizosphere of *P. javanica* using wet sieving and decanting technique [19]. The spores were classified and separated based on their morphological features under a stereomicroscope. The isolated AM fungal spores with similar morphology were then inoculated into several pots of new germinating *P. javanica* for mass multiplication of the AM fungal cultures. The pure cultures of each AM fungal isolates were grown under greenhouse conditions for another three months as preparation of starter inoculums. At the end of the AM fungi propagation stage, the produced spores were harvested and quantified for the subsequent inoculation process.

Table 1. Source of arbuscular mycorrhizal (AM) fungi in this study.

Isolate code	Host plant	Locality	Morphological Identification
GB.10.A2	<i>Cratoxylon arborescens</i>	PT.Arara Abadi's concession area	<i>Acaulospora</i> sp.
SB.1.A2	<i>Shorea balangeran</i>	PT.Arara Abadi's concession area	<i>Glomus maculosum</i>
EP.8.A.1	<i>Eucalyptus pellita</i>	PT.Arara Abadi's concession area	(a) <i>Gigaspora</i> sp. (b) <i>Acaulospora tuberculata</i>
RD.1.5.1	<i>Eucalyptus pellita</i>	PT. Wirakarya Sakti's concession area	<i>Glomus maculosum</i>
RB.10.3.1	<i>Eucalyptus pellita</i>	PT. Tripupa Jaya's concession area	<i>Entrophospora</i> sp.

2.2. Experimental design

The experiment involved two factors, *i.e.*, AM fungi isolates (six AM fungal isolates – as listed in Table 1 and un-inoculated control) and host plant species (plantlets of *E. pellita* and *A. crassicarpa*). It has resulted in 14 different treatments with 12 replications, giving in total 168 pots.

2.3. Inoculation procedure and growing conditions

Before the AM fungi inoculation, sterilized media containing cocopeat and husk charcoal (2:1) were prepared and put into 350mL pots, then watered with sterile water to obtain appropriate humidity and density of the media.

Plantlets were carefully taken out from the culture flasks and washed using tap water to remove the agar media. AM fungal spores were carefully placed/inoculated onto the surface of the plantlet's root (Figure 1). The inoculated plants were then transplanted in the pots containing a sterilized mix of cocopeat and husk charcoal (2:1). The pots were placed in the nursery for 120 days with three sequential steps. Firstly, the inoculated plantlets were acclimatized for 21 days under tissue culture conditions, grown in an air-conditioned room, and exposed the plants to the out-door condition (along the laboratory corridor) every morning, for approximately three hours. Translucent plastic bags covered the pot in order to maintain high humidity and prevent damage to the newly transferred plantlets. After five days, the plastic bags were pored (1-2 pore (s) per day for the next 16 days) to gradually decrease the humidity and acclimatize the plants to the external atmosphere. At the end of this stage, the plastic covers were removed, allowing the plants to be fully exposed. Secondly, at 22 days after inoculation (DAI), the plants were transferred to the shading house in the nursery until 45 DAI. To cope with the absence of humidity- and temperature- regulators in the shading house, the plants were sprayed with sterilized water 2-3 times a day for 15 minutes each, depending on the ambient temperature and humidity. Finally, on 46 DAI, the pots were transferred to the open area, which allowed the plants to be exposed to the full outdoor conditions until 120 DAI. The plants were watered with unsterilized tap water twice a day.



Figure 1. Spores inoculation onto plantlet of *A. crassicarpa*'s root under a stereo-microscope with 15 times magnification.

2.4. Assessment of plant growth parameter

Plant growth parameters, such as plant height, stem diameter, number of leaves per plant, were recorded five times during the experiments with approximately three weeks interval between assessments. Plant dry weight and AM fungi root colonization were assessed at the end of the experiment. Plant dry weight was determined after drying the tissue in an oven at 70°C for 72 hours.

2.5. Estimation of AM fungi root colonization

Detection of fine root systems that were colonized by mycorrhizae was carried out by a staining procedure [20], and the percentage of the total root length colonized by the AM fungi was determined using the gridline intersect technique [21].

2.6. Statistical analyses

Statistical analyses were performed using one-way ANOVA on the online statistical calculators <https://astatsa.com> to quantify possible differences of plant growth parameters among treatments. The posthoc Tukey's HSD Tests were subsequently applied to determine which groups were different.

3. Results and Discussion

AM fungi form mutual symbioses with the majority of terrestrial plants, including the genera of *Eucalyptus* and *Acacia* [22, 23]. The AM fungi enhanced supply and absorption of macro- and micro-nutrients of the plants as well as increased disease resistance and the water relation [24]. The application of mycorrhizal technology to these two micropropagated plant species (*E. pellita* and *A. crassicarpa*) was expected to reduce transplantation shock during acclimatization. Thus, it may improve plant growth and survivability.

Survivability observations demonstrated that all six AM fungal inoculation treatments had a higher survival rate of micropropagated *E. pellita* plantlets than the un-inoculated plantlets with the various magnitude of response. At the end of observation, only 25% of the uninoculated control of *E. pellita* plants survived. In contrast, survival rates of the inoculated ones were about 2 – 3 times better than the control plants, ranging between 41.67 – 83.33% (Table 2). The highest survival rate was observed in *E. pellita* plants, which were inoculated by *Acaulospora* sp. GB.10.A2 and *G. maculosum* RD.1.5.1 spores.

AM fungi inoculations were reported to reduce mortality in various micropropagated plantlets, e.g. *Gerbera jamesonii*, *Nephrolepis exaltata*, *Syngonium podophyllum*, and *Spilanthes acmella* [25, 14]. High mortality in micropropagated plantlets might be due to underdeveloped root system [25] or the difference in microclimate where the plantlets were designed to grow in. The AM fungal hyphae may

act as an extension to the likely underdeveloped root system and enhance the nutrient uptake from the media and thus the plantlets were able to improve their tolerance towards the environment and increase their survivability [7].



Figure 2. Rhizobia nodulation of *A. crassicaarpa*'s root. Arrows pointed the nodules.

In contrast to *E. pellita*, survival rates of *A. crassicaarpa* plantlets were quite high throughout all treatments, ranging between 83.33 – 100%, *i.e.*, AMF inoculations did not appear to affect *A. crassicaarpa* survivability (Table 2). When the roots of *A. crassicaarpa* was harvested, the rhizobial nodulation was observed in the roots (Figure 2). Rhizobia contamination is predicted to occur when the plants were grown in the open area of the nursery (since 46 DAI), which used unsterile tap water for watering and being exposed to rains. This condition allows nitrogen-fixing bacteria to accidentally spread into the planting media and induced nodulation of the legume plants – *A. crassicaarpa*. This incidence may play a significant influence on the high survival rates of the plants in this trial.

Rhizobia is a generic name for diverse groups of bacteria, such as *Allorhizobium*, *Aminobacter*, *Azorhizobium*, *Bradyrhizobium*, *Devosia*, *Sinorhizobium*, *Mesorhizobium*, *Methylobacterium*, *Microvirga*, *Ochrobactrum*, *Phyllobacterium*, *Rhizobium*, *Burkholderia*, *Cupriavidus*, *Herbaspirillum*, and *Pseudomonas*, that fix nitrogen in association with roots of legumes [26]. Due to the considerable biodiversity of nitrogen-fixing bacteria, the chance to have unintentional rhizobial association into the *A. crassicaarpa* root is quite high. Even just one species may induce root nodulation of the compatible plant hosts.

A. crassicaarpa, as a leguminous plant, is known to associate with nitrogen fixing bacteria [27] naturally. Rhizobia have various strategies and interactions in the rhizosphere [28]. At the initial process of legume–rhizobia symbioses, reciprocal biochemical signaling, and recognition between the hosts and symbionts are required. Plants roots release exudates such as sugars, amino acids, flavonoids and phenolic compounds that induce chemostatic reaction from rhizobia and act as nodulation gene inducer (Nod- factor) of the micro-symbionts [26]. These compounds modulate rhizobial response to release complex lipo-chitoooligosaccharides that cause morphological changes in legume root hairs, nodule organogenesis and symbiotic N₂ fixation [29]. In addition, most rhizobial strains also produce phytohormone (such as indole acetic acid), siderophore, and organic acids, which can enhance mineral nutrition for the plants [30]. Siderophore takes a role as a Fe-chelating agent and organic acids as P- and Mn- solubilizing agents [29]. These mechanisms support optimum plant growth and health.

Rhizobia and AM fungi appear to have a synergic interaction. A study reported that ectomycorrhizal and endomycorrhizal symbioses with *A. crassicaarpa* significantly improved the rhizobial nodulation of *Bradyrhizobium* sp. strain Aus13C, which may further benefit the growth of *A.*

crassicarpa [27]. Moreover, the rhizobial Nod- factor seems to play major roles in AM establishment since the bacterial metabolite was found to promote mycorrhizal colonization of both nodulated- and un-nodulated plants [31]. Further investigation on multiple inoculations to *A. crassicarpa* plantlets with ectomycorrhizae AM fungi, and rhizobia, particularly the provenance to be transplanted in peatland, is necessary.

Despite the influence of AM fungi inoculation in *E. pellita* survival rates, the percentage of AM fungi colonization of all treatments were very low or even undetected (0 – 4.05%) (Table 2). AM colonization inside the roots might be very sparse, which resulted in a very low detection of colonization, if any, based on the conducted method. However, this does not necessarily translate as no- or low- existence of AM fungi in inoculated pots. Spores and mycelium in the media/soil might still be functioning. There are factors determining root colonization, such as P level in the soil, inoculum density and compatibility [32, 33]. However, propagule in the inoculum does not necessarily mean spores as AM fungal colonization was repeatedly shown to not reflect the spore density in the soil [34, 35].

Our results showed that no obvious correlation was observed between *E. pellita*'s survival rates and AM fungi colonization. Similar results were also observed in *A. crassicarpa* plantlets, where AM fungal colonization varied between 0 to 3.33% (Table 2). Although in a reverse manner, in which inoculated plants attained higher levels of colonization, AM fungal colonization levels were similarly reported to be uncorrelated to survival, growth, or biomass accumulation in *Dyera polyphylla* [36].

Table 2. Influence of AMF on the survival rate of micropropagated *Eucalyptus pellita* and *Acacia crassicarpa* after 120 days of inoculation.

Treatments	<i>Eucalyptus pellita</i>		<i>Acacia crassicarpa</i>	
	AMF colonization (%)	Survival rate (%)	AMF colonization (%)	Survival rate (%)
Un-inoculated control	0	25.00	0.33	100
<i>Acaulospora</i> sp. GB.10.A2	0	83.33	3.00	100
<i>G. maculosum</i> SB.1.A2	4.05	41.67	0	83.33
<i>Gigaspora</i> sp. EP.8.A1.a	0.7	66.67	0	100
<i>A. tuberculata</i> EP.8.A1.b	0	75.00	0	100
<i>G. maculosum</i> RD.1.5.1	0	83.33	1.35	100
<i>Entrophospora</i> sp. RB.10.3.1	0	50.00	3.33	91.67

The AM fungal symbioses are generally not exclusive to particular plant species. However, it may show host preferences. The results showed that even though no significant difference in the growth parameters between the inoculated plantlets and the control plants was observed, *Glomus maculosum* RD.1.5.1 and *Acaulospora* sp. GB 10.A2 isolates visually have a better effect on plant height, stem diameter and root dry weight of the *E. pellita* plantlets (Table 3), while *Entrophospora* sp. RB.10.3.1 induced better growth of plant height, stem diameter, shoot- and root- the dry weight of *A. crassicarpa* plantlets (Table 4). The host preference phenomenon was obviously displayed by the AM isolate *Glomus maculosum* RD.1.5.1. In comparison to the uninoculated control plants, the association of *Glomus maculosum* RD.1.5.1 with *E. pellita* resulted in positive growth responses (the inoculated plants showed the best growth responses – Table 3), while the association with *A. crassicarpa* exhibited the opposite result (the inoculated plants showed inferior growth responses – Table 4). It is worth to note that the number of plants for growth parameters, particularly for *E. pellita*, varied due to the variation of survival rates.

Table 3. Influence of AMF on several growth parameters of micropropagated *Eucalyptus pellita* after 120 days of inoculation (average \pm standard error).

Treatments	Plant height (cm)	Stem Diameter (mm)	Leaves (no./plant)	Dry weight (gr/plant)	
				Shoot	Root
Un-inoculated control	47.70 \pm 1.32 ^{abc}	3.88 \pm 0.53 ^{ab}	47.67 \pm 9.68 ^a	9.17 \pm 1.09 ^a	1.80 \pm 0.10 ^a
<i>Acaulospora</i> sp. GB.10.A2	54.79 \pm 1.13 ^a	4.41 \pm 0.17 ^a	39.20 \pm 4.43 ^{ab}	8.22 \pm 0.33 ^{ab}	2.05 \pm 0.20 ^a
<i>G. maculosum</i> SB.1.A2	45.04 \pm 2.53 ^{bc}	4.04 \pm 0.28 ^{ab}	39.00 \pm 4.21 ^{ab}	6.70 \pm 0.38 ^{abc}	1.94 \pm 0.23 ^a
<i>Gigaspora</i> sp. EP.8.A1.a	48.09 \pm 1.79 ^{ab}	3.17 \pm 0.12 ^{bc}	36.88 \pm 4.39 ^{abc}	6.94 \pm 0.48 ^{abc}	1.50 \pm 0.08 ^{ab}
<i>A. tuberculata</i> EP.8.A1.b	36.34 \pm 4.10 ^{cd}	2.73 \pm 0.39 ^{cd}	30.56 \pm 5.04 ^{bcd}	5.72 \pm 0.98 ^c	1.51 \pm 0.26 ^{ab}
<i>G. maculosum</i> RD.1.5.1	55.81 \pm 1.50 ^a	4.31 \pm 0.13 ^a	43.40 \pm 3.98 ^{ab}	8.39 \pm 0.16 ^{ab}	2.03 \pm 0.16 ^a
<i>Entrophospora</i> sp. RB.10.3.1	40.13 \pm 2.18 ^{cd}	3.33 \pm 0.24 ^{bc}	34.67 \pm 7.77 ^{abc}	5.90 \pm 0.67 ^{cb}	1.62 \pm 0.18 ^{ab}

The values in each column with the same letter are not significantly different at $p < 0.05$ as determined by Tukey's HSD test

Table 4. Influence of AMF on several growth parameters of micropropagated *Acacia crassicarpa* after 120 days of inoculation (average \pm standard error).

Treatments	Plant height (cm)	Stem Diameter (mm)	Leaves (no./plant)	Dry weight (gr/plant)	
				Shoot	Root
Un-inoculated control	31.43 \pm 3.02 ^{ab}	2.70 \pm 0.21 ^{ab}	6.67 \pm 0.69 ^{ab}	4.43 \pm 0.43 ^{bc}	1.24 \pm 0.16 ^a
<i>Acaulospora</i> sp. GB.10.A2	27.78 \pm 2.36 ^{bc}	2.08 \pm 0.14 ^{bc}	5.75 \pm 0.45 ^{abc}	4.62 \pm 0.67 ^{bc}	1.05 \pm 0.16 ^{ab}
<i>G. maculosum</i> SB.1.A2	31.93 \pm 1.80 ^{ab}	2.25 \pm 0.15 ^{abc}	6.30 \pm 0.50 ^{ab}	5.40 \pm 0.38 ^{ab}	1.06 \pm 0.16 ^{ab}
<i>Gigaspora</i> sp. EP.8.A1.a	33.28 \pm 2.65 ^{ab}	2.62 \pm 0.18 ^{abc}	7.50 \pm 0.64 ^a	4.96 \pm 0.27 ^{abc}	1.04 \pm 0.10 ^{ab}
<i>A. tuberculata</i> EP.8.A1.b	33.33 \pm 2.51 ^{ab}	2.22 \pm 0.16 ^{abc}	5.50 \pm 0.40 ^{bcd}	5.22 \pm 0.45 ^{ab}	0.96 \pm 0.11 ^{ab}
<i>G. maculosum</i> RD.1.5.1	20.44 \pm 1.93 ^c	1.91 \pm 0.21 ^{cd}	5.08 \pm 0.53 ^{bcd}	4.70 \pm 0.49 ^{bc}	1.14 \pm 0.19 ^a
<i>Entrophospora</i> sp. RB.10.3.1	41.46 \pm 1.99 ^a	2.96 \pm 0.18 ^a	6.45 \pm 0.59 ^{ab}	6.81 \pm 0.18 ^a	1.34 \pm 0.20 ^a

The values in each column with the same letter are not significantly different at $p < 0.05$ as determined by Tukey's HSD test

Interaction between the host plant and AM fungi relied on the species of both parties [37]. Plant–mycorrhizal association commonly considered as the C–P trade between the symbionts, *i.e.*, organic carbon (C) delivery to the fungi by the plants and phosphorus (P) delivery in the opposite direction [38]. P is an essential nutrient for all organisms, including plants and fungi, but it is difficult to obtain from soil [39]. External mycelium of the AM fungi plays a role in finding and absorbing P effectively, which frequently leads to higher P uptake and improve the growth of plants. These plants are categorized as having a positive mycorrhizal response. However, some plant species can be unresponsive to mycorrhizal inoculation, *i.e.*, indicating low or no positive growth responses under the same conditions, such as some varieties of barley, wheat and some prairie grasses [38].

Growth depression of the plants was thought to be the result of the excess drain of C to the AM fungus with no nutritional benefits (P-uptake) given by the fungus as a return [7]. However, based on investigations using radioactive P, this assumption was inaccurate. The studies showed that the AM pathway displayed a major contribution to plant P-uptake, whether the symbioses result in massive growth improvement of the host plants or not [39, 40].

4. Conclusion

Inoculation of six individual AM fungal isolates increased the survivability of *E. pellita* almost three times more than that of the control plants, but not the *A. crassicarpa*, in the nursery. Even though both plant species exhibit insignificant responses in all observed growth parameters, a host-preferences phenomenon of AM fungi was observed. *Acaulospora* sp. GB.10.A2 and *G. maculosum* RD.1.5.1 were the most preferred inoculants to support *E. pellita*'s growth, while *Entrophospora* sp. RB.10.3.1 was the most preferred inoculums to support *A. crassicarpa*. However, the benefits of mycorrhizal association were not always related to the degree of root colonization. As unintended rhizobial nodulation was observed on the root of *A. crassicarpa* plants, further investigation on the co-inoculation of AM fungi with rhizobium should be considered.

Acknowledgment

We thank our co-workers (Aryanto, Najmulah, Resti Ariantari, Sira Stephanandra, Herni Yuniar, Sugeng Santosa, and Ahmad Yani) for their assistance in laboratory and nursery in Bogor. We also thank anonymous reviewers for many helpful comments on previous versions of this paper. This project was conducted in collaboration between Forest Research and Development Center and Sinar Mas Forestry.

References

- [1] Yasodha R, Sumathi R and Gurumurthi K 2004 Micropropagation for quality propagule production in plantation forestry *Indian J. Biotechnol.* **3** 159 – 170
- [2] Chandra S, Bandopadhyay R, Kumar V and Chandra R 2010 Acclimatization of tissue cultured plantlets: from laboratory to land *Biotechnol. Lett.* **32** 1199 – 1205
- [3] Hazarika B N 2003 Acclimatization of tissue-cultured plants *Curr. Sci.* **85** 1704 – 1712
- [4] Pinto G, Silva S, Loureiro J, Costa A, Dias M C, Araújo C, Neves L and Santos C 2011 Acclimatization of secondary somatic embryos derived plants of *Eucalyptus globulus* Labill.: An ultrastructural approach *Trees-Struc. Funct.* **25** 383 – 392
- [5] Aggarwal D, Kumar A, Sharma J and Reddy M S 2012 Factors affecting micropropagation and acclimatization of an elite clone of *Eucalyptus tereticornis* Sm. *In Vitro Cell. Dev. Biol. - Plant* **48** 521 – 529
- [6] Sulichantini E D, Sutisna M, Sukartiningsih S and Rusdiansyah R 2014 Clonal propagation of two clones *Eucalyptus pellita* F. Muell by mini-cutting *International Journal of Science and Engineering (IJSE)* **6** 117 – 121
- [7] Smith S E and Read D J 2008 *Mycorrhizal symbiosis* 3rd ed (San Diego: Academic Press)
- [8] Corkidi L, Allen E B, Merhaut D, Allen M F, Downer J, Bohn J and Evans M 2004 Assessing the infectivity of commercial mycorrhizal inoculants in plant nursery conditions *J. Environ. Hort.* **22** 149
- [9] Syafruddin S, Syakur S and Arabia T 2016 Propagation techniques of mycorrhizal bio-fertilizer with different types of mycorrhiza inoculant and host plant in Entisol Aceh *Int. J. Agric. Res.* **11** 69 – 76
- [10] Pozo M J, López-Ráez, J A, Azcón-Aguilar C and García-Garrido J M 2015 Phytohormones as integrators of environmental signals in the regulation of mycorrhizal symbioses *New Phytol.* **205** 1431 – 1436
- [11] Fusconi A 2014 Regulation of root morphogenesis in arbuscular mycorrhizae: What role do fungal exudates, phosphate, sugars and hormones play in lateral root formation? *Ann. Bot.* **113** 19 – 33
- [12] Ruiz-Lozano J M, Porcel R, Azcon C and Aroca R 2012 Regulation by arbuscular mycorrhizae of the integrated physiological response to salinity in plants: new challenges in physiological and molecular studies *J. Exp. Bot.* **63** 4033 – 4044
- [13] M'barki N, Chehab H, Aissaoui F, Dabbaghi O, Attia F, Mahjoub Z, Laamari S, Chihaoui B, del Giudice T, Jemai A, Boujnah D and Mechri B 2018 Effects of mycorrhizal fungi

- inoculation and soil amendment with hydrogel on leaf anatomy, growth and physiology performance of olive plantlets under two contrasting water regimes *Acta Physiol. Plant.* **40** 1 – 10
- [14] Yadav K, Singh N and Aggarwal A 2012 Arbuscular mycorrhizal technology for the growth enhancement of micropropagated *Spilanthes acmella* Murr *Plant Protect. Sci.* **48** 31 –3 6
- [15] Vidal M T, Azcón-Aguilar C, Barea J M and Pliego-Alfaro F 1992 Mycorrhizal inoculation enhances growth and development of micropropagated plants of avocado *Hort. Sci.* **27** 785–787
- [16] Singh N V, Singh S K, Singh A K, Meshram D T, Suroshe S S and Mishra D C 2012 Arbuscular mycorrhizal fungi (AMF) induced hardening of micropropagated pomegranate (*Punica granatum* L.) plantlets *Sci. Hortic.* **1** 122 – 127
- [17] Monticelli S, Puppi G and Damiano C 2000 Effects of in vivo mycorrhization on micropropagated fruit tree rootstocks *Appl. Soil Ecol.* **15** 105 – 111
- [18] Turjaman M, Herdyantara B, Faulina S A, Agustini L, Irianto R S B, Hidayat A, Wahno I, B Tjahyono and Indrayadi H 2019 Mycorrhizal colonization of indigenous tropical tree species grown in peat swamp forests of Sumatera, Indonesia *IOP Conference Series: Earth and Environmental Science* **308** 012049
- [19] Pacioni G 1992 Wet-sieving and decanting techniques for the extraction of spores of vesicular-arbuscular fungi *Methods in Microbiology* **24** 317 – 322
- [20] Brundrett M C, Bougher N, Dell B, Grove T and Malajczuk N 1994 Working with mycorrhizas in forestry and agriculture (Canberra: ACIAR, 1994)
- [21] Giovannetti M and Mosse B 1980 An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots *New Phytol.* **84** 489 – 500
- [22] de Araujo Pereira A P, Santana M C, Bonfim J A, de Lourdes Mescolotti D and Cardoso E J B N 2018 Digging deeper to study the distribution of mycorrhizal arbuscular fungi along the soil profile in pure and mixed *Eucalyptus grandis* and *Acacia mangium* plantations *Appl. Soil Ecol.* **128** 1 – 11
- [23] Zhang Q, Sun Q, Koide R T, Peng Z, Zhou J, Gu X, Gao W and Yu M 2014 Arbuscular mycorrhizal fungal mediation of plant-plant interactions in a Marshland Plant Community *The Scientific World Journal* **2014** Article 923610
- [24] Karti P D M H, Prihantoro I and Setiana M A 2018 Evaluation of arbuscular mycorrhizal fungi inoculum on production and nutrient content of *Pennisetum purpureum* *Trop. Anim. Sci. J.* **41** 114–120
- [25] Wang H, Parent S, Gosselin A and Desjardins Y 2019 Vesicular-arbuscular mycorrhizal peat-based substrates enhance symbiosis establishment and growth of three micropropagated species *J. Am. Soc. Hortic. Sci.* **118** 896 – 901
- [26] Lira M A (Jr.), Nascimento L R S and Fracetto G G M 2015 Legume-rhizobia signal exchange : promiscuity and environmental effects *Front. Microbiol.* **6** Article 945
- [27] Lesueur D and Duponnois R 2005 Relations between rhizobial nodulation and root colonization of *Acacia crassicarpa* provenances by an arbuscular mycorrhizal fungus *Glomus intraradices* Schenk & Smith or an ectomycorrhizal fungus, *Pisolithus tinctorius* Coker & Couch *Ann. For. Sci.* **62** 467 – 474
- [28] Barrett L G, Bever J D, Bissett A and Thrall P H 2015 Partner diversity and identity impacts on plant productivity in Acacia – rhizobial interactions *J. Ecol.* **103** 130 – 142
- [29] Dakora F D 2003 Defining new roles for plant and rhizobial molecules in sole and mixed plant cultures involving symbiotic legumes *New Phytol.* **158** 39 – 49
- [30] Dakora F D and Phillips D A 2002 Root exudates as mediator of mineral acquisition in low-nutrient environments *Plant Soil* **245** 35 – 47
- [31] Xie Z P, Staehelin C, Vierheilig H, Wiemken A, Jabboun S, Broughton W J, Vogeli-Lange R and Boller T 1995 Rhizobial nodulation factors stimulate mycorrhizal colonization of nodulating and non-nodulating soybeans *Plant Physiol.* **108** 1519 – 1525

- [32] Cheng Y, Ishimoto K, Kuriyama Y, Osaki M and Ezawa T 2013 Ninety-year-, but not single, application of phosphorus fertilizer has a major impact on arbuscular mycorrhizal fungal communities *Plant Soil* **365** 397 – 407
- [33] Verbruggen E, van der Heijden M A, Rillig M C and Kiers E T 2013 Mycorrhizal fungal establishment in agricultural soils: Factors determining inoculation success *New Phytol.* **197** 1104 – 1109
- [34] Clapp J P, Young J P W, Merryweather J W and Fitter A H 1995 Diversity of fungal symbionts in arbuscular mycorrhizas from a natural community *New Phytol.* **130** 259 – 265
- [35] Hempel S, Renker C and Buscot F 2007 Differences in the species composition of arbuscular mycorrhizal fungi in spore, root and soil communities in a grassland ecosystem *Environ. Microbiol.* **9** 1930 – 1938
- [36] Graham L L B, Turjaman M and Page S E 2013 *Shorea balangeran* and *Dyera polyphylla* (syn.*Dyera lowii*) as tropical peat swamp forest restoration transplant species: Effect of mycorrhizae and level of disturbance *Wetl. Ecol. Manag.* **21** 307 – 321
- [37] Weremijewicz J, Lobo S, Sternberg O R and Janos D P 2016 Common mycorrhizal networks amplify competition by preferential mineral nutrient allocation to large host plants *New Phytol.* **212** 461 – 471
- [38] Smith S E and Smith F A 2012 Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth *Mycologia* **104** 1 – 13
- [39] Smith S E and Smith F A 2011 Roles of arbuscular mycorrhizas in plant nutrition and growth: New paradigms from cellular to ecosystem scales *Annu. Rev. Plant Biol.* **62** 227–250
- [40] Smith F A, Grace E J and Smith S E 2009 More than a carbon economy: Nutrient trade and ecological sustainability in facultative arbuscular mycorrhizal symbioses *New Phytol.* **182** 347 – 358