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Response of growth and drought tolerance of *Pterocarpus indicus* Willd. seedlings to arbuscular mycorrhizal fungi

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ABSTRACT. Drought is one of the environmental factors that can inhibit plant growth and production. Arbuscular mycorrhizal fungi (AMF) were reported to increase plant growth and tolerance to drought stress. Pterocarpus indicus seedlings were inoculated with three AMF treatments (control, *Clarodeoglomus. etunicatum,* and *Septoglomus constrictum*) at three watering intervals (watering every day, every two days, and three days) on a greenhouse scale. The results showed that the interaction of *C. etunicatum* and *S. constrictum* at various watering intervals effectively increased the height growth of Angsana seedlings. C. etunicatum and S. constrictum and daily watering each increased the diameter of the seedlings. The dry weight of seedlings and the number of root nodules of Angsana seedlings increased in various AMF treatments under daily watering conditions and tended to decrease with watering intervals. Local AMF has the potential to be developed to support the reforestation and conservation of the Angsana species in various land conditions, especially dry land.

Keywords: *Pterocarpus indicus, Claroideoglomus etunicatum,* drought stress

INTRODUCTION

Drought stress is a condition in which water availability caused by a decrease in soil water content is low so that it will affect the morphological, physiological, and biochemical phases (Hasanuzzaman et al. 2013). Drought stress can be caused by two factors, namely the lack of water supply in the root area and the rate of water loss (evapotranspiration), which is greater than water absorption even though the soil water content is sufficient. Drought can reduce germination and photosynthetic activity, decrease assimilate translocation and carbon fixation, flowering time and influence on reproductive organs, and seed production of legumes (Nadeem et al. 2019). Therefore, one alternative for improving plant growth and adaptation is using important soil microbes such as arbuscular mycorrhizal fungi.

Arbuscular mycorrhizal fungi (AMF) are fungi from the phylum Glomeromicota and are reported to be in symbiosis with about 80% of plant species (Snith and Read, 2008). AMF symbiosis can help plant adaptation and growth in drought conditions. AMF colonization increases nutrient uptake (Kapoor et al. 2013), glomalin production to increase nutrient and water absorption (Gong et al. 2013; Pagano 2014), osmotic adjustment and anti-oxidants (Rapparini and Penuelas 2014), increases stomatal conductance, transpiration, and photosynthesis during drought (Lee at al. 2012; Gholamhoseini et al. 2013). Several studies reported that AMF increased the resistance of legumes under drought conditions (Abdelmalik et al. 2020, Porto et al. 2020). One of the important tropical legume tree species that can be developed under drought conditions is the Angsana (*Pterocarpus indicus* Willd.).

Angsana is a tropical tree species and grows naturally in Southeast Sulawesi. This species has a tree height of up to 45 m with a diameter of 150 cm (Martawijaya et al., 2005), grows well at an altitude of 800 m above sea level, and grows on all types of soil except for heavy clay (Rohadi et al., 2005). Angsana has many benefits; namely, it can be used as raw material for making fine furniture, floors, cabinets, musical instruments (Joker, 2002), and medicinal plants (Fatimah et al., 2006), as well as potential species for restoration of degraded ecosystems (Husna et al. 2021a; Husna et al. 2021b). Angsana is also reported to be symbiotic with AMF (Husna et al. 2021c) and responsive to AMF (Husna et al. 2018; Husna et al. 2020; Arif et al. 2021; Husna et al. 2021b). The test on the growth of AMF inoculated with AMF in drought stress conditions has never been carried out. This research is beneficial for selecting Angsana species for ecosystem restoration and efforts to conserve Angsana species in various land conditions.

MATERIAL AND METHODS

Mycorrhizal fungi and Inoculum Propagation. The AMF inoculum used was Claroideoglomus etunicatum and Septoglomus constrictum from nail wood rhizosphere (*Pericopsis mooniana*). The AMF inoculum resulted from propagation using zeolite media and the host Pueraria javanica for three months in the plastic house of the Indonesian Mycorrhizal Association, Kendari, Southeast Sulawesi.

Experiment design. The experiment has two factors, AMF (seedlings treated with AMF *Claroideoglomus etunicatum* and *Septoglomus costrictium* and non-AMF (control) seedlings and drought treatments comprising three levels of drought cycles (every day, two and 3-day watering interval).

Plant materials, growth conditions, and drought stress treatments. Seeds of *P. indicus* were introduced by The Faculty of Forestry and Environmental Science, Halu Oleo University. The experiment was conducted in Greenhouse of Indonesia Mycorrhizal Association, Kendari, Southeast Sulawesi. Seeds were sown in plastic. The growth medium consisting of soil, sand, and arang sekam padi (2:1:1 v/v/v Before AMF inoculation, polybags (15 cm x 20 cm) were filled with soil media and ± 1.5 kg sterile husk charcoal. AMF inoculation was given according to the treatment for each polybag, which was placed near the roots of *P.indicus* wood seedlings. Seedlings that were not inoculated were used as controls. Seedlings were regularly watered as needed during the first two weeks. After that, drought treatments were applied by adding 5 ml per polybag in the interval of every day, 2 and 3 days (developed drought cycles) throughout the experiment duration (five months).

AMF colonization in the root. Mycorrhizal fungi were extracted from root samples in each treatment using the method Brundret *et al*, (1996). The roots were then soaked in 10% KOH for 24 hours, then acidified in 2% HCl for 30 minutes, and finally stained with trypan blue. Furthermore, observation activities to determine the percentage of AMF colonization in root samples were ready to be carried out under a microscope.

Data collection

Shoot growth traits. Height (cm), Height measurement is done with a ruler on the stem as high as 1 cm from the cotyledon scars to the stem apex, stem diameter (mm) at the cotyledon scar using a digital caliper (± 0.04 mm), number of leaves) and the number of leaf and nodules. **Measurement of the dry weight of shoots and roots**. The roots, stems, and leaves were separately dried at 75 °C for 48 h to obtain dry weights. **Seed quality index (SQI)**. SQI was calculated using formula by Duryea & Brown (1984): Seed Quality Index (SQI) = [Shoot dry weight + root dry weight]/[(height/diameter) + (dry weight of shoot/root dry weight)]. Seedlings have high quality if the value of SQI ≥ 0.09. **AMF colonization**. AMF colonies were calculated using the formula by Brundrett et al. (1996): [Σ number of fields of view colonized/Σ total observed field of view] x 100%.

Statistical analysis. The results of the observations in each unit of observation will be analyzed first by means of variance (F test). A different treatment test will be carried out according to the DMRT at the 95% confidence level if the test results show a real effect.

RESULT AND DISCUSSION

FMA Colonization. AMF colonization in *P. indicus* roots. The highest interaction treatment of AMF type S. constrictum was watered every three days compared to other treatments (Table 1).

Growth in height, number of leaves, and nodules of Angsana seedlings. The interaction of C. etunicaum AMF and daily and 2-day watering effectively increased plant height growth and was not significantly different from the *S. constrictum* AMF treatment in all watering treatments. The highest number of leaves and the number of nodules in the *S. constrictum* treatment were watered every day. There was no difference in the nodule number between seedlings colonized by *S. constrictum* and everyday watered and no AMF and everyday watered treatment (Table 1).

Table 1. AMF colonization and growth of *P. indicus* seedlings five months old treated with AMF and Drought.

Treatment AMF Inoculation	Sprinkling	AMF colonization (%)	Height (cm)	Number of leaves (strands)	Number of nodules (nodules)
Control	Daily watering	0.00d	34.09b	45.06b	49.00a
	Watering every 2 days	0.00d	29.05b	36.60c	14.66d
	Watering every 3 days	0.00d	17.93c	40.93bc	25.66c
C. etunicaum	Daily watering	15.30b	46.20a	48.73b	35.00b
	Watering every 2 days	8.11c	40.29a	47.60b	38.00b
	Watering every 3 days	15.18b	34.92b	38.06c	25.00c
S. constrictum	Daily watering	16.08b	44.60a	53.66a	46.00a
	Watering every 2 days	11.87c	37.91ab	42.73b	25.66c
	Watering every 3 days	24.20a	37.82ab	43.40b	32.00b
Significant level		**	*	**	**

Note: The same letter in the same row or column shows no significant difference according to Duncan's test (α=0.5).

The increase in the diameter of the Angsana seedling. The AMF treatments of *C. etunicatum* and *S. constrictum* significantly increased the diameter of Angsana seedlings compared to controls. In the watering treatment, daily watering was effective in increasing the diameter of the goose compared to other treatments (Table 2).

Table 2. Diameter of *P. indicus* seedlings. 5 months old treated with AMF and Drought.

Treatment	Diameter (mm)
AMF Inoculum (A)	
Control	3,49b
C. etunicatum	4,28a
S. constrictum	4,51a
Watering Interval	
Control	4,80a
Watering every 2 days	3,78b
Watering every 3 days	3,69b
Significant level	**

Note: The same letter in the same row or column shows no significant difference according to Duncan's test (α=0.5).

Plant Dry Weight (BKT). The interaction treatment of *C. etunicaum* AMF in daily watering significantly increased root dry weight compared to other treatments. On shoot dry weight, the control treatment on daily watering significantly increased the shoot dry weight of Angsana seedlings and was significantly different from other treatments except for *S. constrictum* AMF treatment on daily watering. The control treatment (without AMF) on daily watering effectively increased the total dry weight. However, it was not significantly different from the *C. etunicaum* AMF treatment on daily watering and *S. constrictum* AMF on daily watering.

Table 3. Dry Weight of *P. indicus* Seedlings age five Months treated with AMF and Drought

Treatment		Dry Weight (g)		
AMF Inoculum	Sprinkling	Root	shoots	Total
Control	Daily watering	3.22b	8.18a	11.40a
	Watering every 2 days	1.24c	2.30c	3.54b
	Watering every 3 days	1.17c	3.08c	4.25b
C. etunicaum	Daily watering	4.18a	6.58b	10.76a
	Watering every 2 days	1.84c	3.64c	5.48b
	Watering every 3 days	1.55c	3.24c	4.79b
S. constrictum	Daily watering	3.23b	7.20ab	10.43a
	Watering every 2 days	1.80c	3.89c	5.69b
	Watering every 3 days	1.45c	3.58c	5.04b
Significant level		**	**	*

Note: The same letter in the same row or column shows no significant difference according to Duncan's test (α=0.5).

Root Root Ratio (NPA) and Seed Quality Index (IMB). NPA in the control interaction treatment (without AMF) with daily watering and three days was higher than the other treatments. But not significantly different from the AMF treatment of *S. constrictum* on daily watering. The AMF treatment of *C. etunicaum* on daily watering increased IMB. It was significantly different from other treatments except for the interaction of control (without AMF) with watering every two days and *C. etunicaum* on watering every two days.

Table 4. Roots Ratio (NPA) and Seed Quality Index (IMB) of *P. indicus*. five months old.

Treatment AMF Inoculum	Sprinkling	NPA	IMB
Control	Daily watering	4.13a	0.39b
	Watering every 2 days	2.28b	0.55ab
	Watering every 3 days	5.06a	0.47b
C. etunicaum	Daily watering	2.84b	0.71a
	Watering every 2 days	2.56b	0.50ab
	Watering every 3 days	2.65b	0.48b
S. constrictum	Daily watering	3.66ab	0.43b
	Watering every 2 days	2.75b	0.47b
	Watering every 3 days	2.95b	0.44b
Significant level		**	*

Note: The same letter in the same row or column shows no significant difference according to Duncan's test (α=0.5).

Discussion

The study results showed that five months old *P.indicus* plants under drought stress conditions were colonized by arbuscular mycorrhizal fungi (AMF). *P.indicus* inoculated with AMF had good growth on the variables of height, diameter, and the number of leaves with low water availability conditions in plants. Mycorrhizal plants will increase nutrient and water absorption (Zhang et al. 2018) and increase P nutrient uptake (Zhang et al. 2019). The presence of external and internal fungal hyphae can function as organs for the absorption of soil and water nutrients. The use of mycorrhizae can affect the water balance in plants so that it can increase plant resistance to drought (Simel, 2013). Therefore, plants in drought conditions will experience stress and have a relatively high dependence on AMF. Research related to the administration of AMF also reported that AMF was able to increase the growth of Faidherbia albida (syn. *Acacia albida*) and *Acacia nilotica* (Osonubi et al., 1992), and Zenia insignis (Zhang et al. 2019).

AMF can assist plants in absorbing nutrients, thereby increasing plant growth and biomass. Absorption of nutrients, especially P, occurs because AMF produces phosphatase enzymes that allow an increase in P in the soil so that plants can absorb nutrients in sufficient quantities (Miransari, 2013). absorption of P nutrients can increase plant biomass and nodulation in plants (Souza, 2015; Husna, 2016). The availability of P can also stimulate the formation of root nodules. In this study, root nodules were more commonly found in the control and inoculation treatment of S. constrictum AMF under daily watering conditions. This fact shows that the formation of root nodules is highly dependent on water availability.

Angsana had a higher plant dry weight in various AMF treatments with daily watering and tended to decrease in drought treatment. The low biomass of Angsana seedlings in drought conditions is thought to be due to disturbed plant physiological processes such as photosynthesis. The higher the dry weight of the plant, the more efficient the photosynthesis process (Lizawati et al., 2014). Thus, the higher the dry weight, the plants can show an efficient photosynthetic process.

The results showed that the seeds inoculated with AMF *C. etunicaum* showed a more significant effect than other treatments. a good range of NPA values for growth and seedling ability is between 1 and 3. A balanced NPA value is needed by seedlings so that the absorption of water and nutrients by roots translocated to the shoots is balanced with a sufficient area of photosynthesis to carry out transpiration and produce carbohydrates needed for root growth. This shows that the more ready the seedlings are to be transferred to the field because there has been a balance between the ability of the roots to absorb water and nutrients and the ability of the shoots to transpiration. This is also in line with the IMB value of *P.indicus* wood, which met the IMB standard of 0.09. The suitability of the NPA and IMB values that meet the standards indicates that the presence of AMF is very much needed for *P.indicus* wood seedlings. The results of the shoot-to-root ratio study are in line with research on *P. mooniana* (Husna et al., 2015) and Aquillaria crassna (Irianto, 2015).

CONCLUSION

Based on the results of the research carried out, it can be concluded that the interaction of *C. etunicatum* AMF with two and three days of watering was effective in increasing the growth of *P. indicus* seedlings. Plant dry weight was higher in all AMF treatments under daily watering conditions. Independently inoculation of *C. etunicatum* and *S. constrictum* AMF species and daily watering treatments effectively increased the diameter of 5-month-old *P. indicus* seedlings.

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