

Single-Spore Culture of *Racocetra crispa* Using a Legume Tree

Faisal Danu Tuheteru, Deli, Husna, Asrianti Arif, Wiwin Rahmawati Nurdin

Forestry Department, Faculty of Forestry and Environmental Sciences, Universitas Halu Oleo, Kendari, Indonesia.

*Corresponding author: faisal.danu.tuheteru@uho.ac.id

ABSTRACT. The propagation of *Racocetra crispa* using tree-like legume host plants has not yet been widely undertaken. This study aims to investigate the sporulation of *R. crispa* in single-spore cultures using legume tree host plants, namely sengon (*Paraserianthes falcataria*), albizia (*Albizia saponaria*), johar (*Cassia siamea*), cajanus (*Cajanus cajan*), and lamtoro (*Leucaena leucocephala*). The study was conducted at the Greenhouse of the Indonesian Mycorrhiza Association (AMI) Southeast Sulawesi Branch and the Forestry Department Laboratory of Halu Oleo University from November 2025 to February 2026. The study employed a completely randomised design (CRD) with 5 treatments and 3 replicates. The variables observed included culture success, AMF colonisation of the roots, and the number of spores formed at 3 months, 5 months, and after drying 10 g of medium. The results of the study showed that the *A. saponaria* host plant treatment yielded the best results, with a culture success rate of 86.67%, the highest AMF colonisation of 35.83%, and the highest number of spores at 5 months and after drying 10g of medium. Conversely, *L. leucocephala* showed the lowest results, with a culture success rate and spore count of 0%. The best host plant for the propagation of *Racocetra crispa* is *A.saponaria*.

Keywords : *Glomeromycotina*, *Albizia saponaria*, AMF propagation

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are a group of fungi from the phylum Glomeromycota that form a symbiotic relationship with the root systems of higher plants and are found in various types of forest ecosystems (Husna, 2015). Several AMF species have been reported from post-asphalt mining areas. One AMF species reported in asphalt mining areas is *Racocetra crispa* (Tuheteru et al., 2025). This species effectively increases the growth of bitti (*Vitex cofassus*) in post-asphalt mining areas (Tuheteru, 2025). Therefore, this species needs to be propagated for development as a biofertilizer. *R. crispa* was previously reported from South America (Souza et al., 2018).

AMF propagation can be carried out using several methods, one of which is the single spore culture technique. This single culture technique uses a specific AMF spore type that is placed on the roots of a sterile host plant (Sari et al., 2024). This method is widely used because it is able to produce purer and more controlled AMF inoculum, making it effective for research purposes and field applications (Brundrett et al., 1996; Smith and Read, 2008).

The success of AMF propagation can be influenced by several factors, including the type of media, host species, and AMF species. AMF propagation generally uses crops such as *Peuraria javanica*, corn, sorghum, and others. However, in this study, we will examine the potential of forestry plants as alternative hosts: sengon (*Paraserianthes falcataria*), johar (*Cassia siamea*), Albizia (*Albizia saponaria*), cajanus (*Cajanus cajan*), and lamtoro (*Leucaena leucocephala*). These five legume species have the potential to be used as single-spore culture hosts because they have quite a lot of fibrous roots and are reported to form a symbiosis with mycorrhizal fungi and form root nodules (Delvian, 2004; Riyanto, 2011; Mansur, 2013; Selong and Lawing, 2023; Tuheteru et al., 2011; 2026). In addition, these species have mastered silvicultural techniques, are easy to grow, develop quickly, and are able to adapt well to tropical environments.

AMF propagation in Indonesia has been widely carried out, based on host species, AMF types, and fertilizer types and dosages. However, AMF propagation using legume tree habitus hosts using the single spore culture method has not been widely practiced. The purpose of this study was to determine the sporulation of *Racocetra crisca* spores in single spore culture with legume tree habitus hosts.

MATERIALS AND METHODS

Time and Place

This research was conducted at the Plastic House of the Indonesian Mycorrhizal Association (AMI) Southeast Sulawesi Branch and the Forestry Department Laboratory, Faculty of Forestry and Environmental Sciences (FHIL), Halu Oleo University (UHO), from November 2025 to February 2026.

Research design

This study was designed using a Completely Randomized Design (CRD) consisting of 5 treatments, namely A = Sengon (*Paraserianthes falcataria*), B = Johar (*Cassia siamea*), C = Albizia (*Albizia saponaria*), D = Cajanus (*Cajanus cajan*), and E = Lamtoro (*Leucaena leucocephala*). Each treatment was repeated 3 times, and each replication used 5 host plants, resulting in a total of 75 host plants.

Research variables

Table 1. Research variables

Variabel	Formula
Cultural Success (%) (Santos <i>et al.</i> , 2020)	$\% \text{ Cultural Success} = \frac{(\text{number of cultures that produce spores})}{\text{Number of cultures}} \times 100\%$
AMF colonization (%) (Brundrett <i>et al.</i> , 1996)	$\text{AMF colonization} = \frac{\sum \text{Field of View colonized}}{\sum \text{Total Field of View}} \times 100\%$
Spore count/culture	Spores were observed on both sides of the Petri dish.
Spore count/10 g (Idjo <i>et al.</i> , 2011)	$\text{Spores per gram} = \frac{\text{The number of new spores formed}}{\text{Sample weight (g)}}$

Research Procedures

AMF Spore Extraction

The technique used to extract AMF spores is the pour-filter technique from Pacioni (1992). The extracted spores are separated into a watch glass using special spore tweezers so that the collection of spores will be used in single spore cultures.

Preparation of Single Spore Culture

The preparation of the single-spore culture begins with the preparation of a Petri dish that will be used as a planting medium. Holes are made around the edges of the Petri dish to provide space for host plant growth. The dish is then filled with sterile zeolite until it is full and sufficiently compacted, allowing it to serve as a suitable growth medium that supports plant development and spore growth during the culture process.

Seed germination

Sengon, albizia, johar, lamtoro, and Cajanus cajan seeds were soaked in warm water for approximately 24 hours to enhance germination. Following the soaking process, the seeds were sown in germination trays and maintained for about 10 days until healthy seedlings emerged. The seedlings were then ready for direct planting in the prepared growing medium or field conditions.

Culture creation and maintenance

A single spore was carefully placed on the root of a seedling that had developed two leaves and was positioned on a moist tissue. The seedling was then arranged along the edge of a perforated Petri dish to facilitate growth and aeration. Subsequently, the Petri dish was covered with its lid, sealed with tape to prevent contamination, and labeled appropriately for identification. The Petri dish culture was placed inside a plastic container, and water was supplied through the container as needed to maintain adequate moisture for plant growth. The cultures were maintained under these conditions for a period of five months to allow for spore development and colonization.

Data Analysis

Observation data were analyzed using analysis of variance (F-test). If the test results showed a significant effect, a treatment difference test was conducted using the Duncan Multiple Range Test (DMRT) at a 95% confidence level.

RESULTS AND DISCUSSION

Cultural Success and AMF colonization

The results of the analysis of culture success and AMF colonization data are presented in Table 1, showing that in the culture success variable, the treatments of *P. falcataria*, *A. saponaria*, *C. siamea*, and *C. cajan* were significantly different from the treatment of *L. leucocephala*. The highest AMF colonization was in the treatments of *A. saponaria* and *P. falcataria*.

Table 2: Cultural Success and AMF colonization

Treatment	Cultural Success (%)	AMF colonization (%)
Sengon (<i>Paraserianthes falcataria</i>)	86.67 a	31.67 a
Albizia (<i>Albizia saponaria</i>)	86.67 a	35.83 a
Johar (<i>Cassia siamea</i>)	66.67 a	11.67 b
Cajanus (<i>Cajanus cajan</i>)	86.67 a	11.66 b
Lamtoro (<i>Leucaena leucocephala</i>)	0,00 b	10.83 b

Description: Numbers followed by the same letter in each row and column are not significantly different.

Number of spores

The number of spores at three months, five months, and five months after drying for 2 weeks is presented in Table 2. Table 2 shows that in the variable number of spores at three months, the *A. saponaria* treatment was not significantly different from the other treatments except with *L. leucocephala*. In the variable number of spores at five months, the *A. saponaria* treatment produced the most spores and was significantly different from *C. cajan* and *L. leucocephala*. The *A. saponaria* treatment produced the most spores after drying for 2 weeks and was significantly different from the other treatments.

Table 2. The number of spores after three months, five months, and after drying (10 g)

Perlakuan	Number of spores		
	3 month	5 month	5 months and after drying 2 weeks (10 g)
<i>Paraserianthes falcataria</i>	26.33 a	34.00 ab	10.67 b
<i>Albizia saponaria</i>	31.67 a	49.67 a	40.00 a
<i>Cassia siamea</i>	22.33 a	33.00 ab	14.33 b
<i>Cajanus cajan</i>	19.33 ab	22.67 b	6.00 c
<i>Leucaena leucocephala</i>	0,00 b	0,00 c	0,00 d

Description: Numbers followed by the same letter in each row and column are not significantly different

The results of the study showed that the highest percentage of culture success was 86.67% in the Sengon, Albizia, and Cajanus treatments, then 66.67% in the Johar treatment, and the lowest was 0% in the Leucaena treatment. The low success of culture in Leucaena is thought to be caused by physiological and biochemical factors of the plant, especially the presence of allelopathic compounds or secondary metabolites such as mimosine, saponins, alkaloids, flavonoids, and tannins known to be contained in Leucaena (Ayu and Adegun, 2013). These compounds can be toxic or inhibit the development of microorganisms, including arbuscular mycorrhizal fungi (AMF). Sanginga et al (1995) stated that the content of certain compounds in legume plants can affect symbiotic interactions with AMF, either positively or negatively depending on the type of plant and environmental conditions.

The results of the study showed that the highest percentage of root colonization was in the Sengon and Albizia treatments (31.67%- 35.83%), while in the Johar and Cajanus treatments it was 11.66%- 11.67%, and the lowest in the Lamtoro treatment (10.83%). Nurmasiyah and Khairuna (2017) stated that the increasing percentage of root colonization was influenced by nutrient levels in the soil, especially the availability of P and N nutrients. Wicaksono et al. (2014) also argued that the association between AMF and plant roots was influenced by several factors, namely the type of AMF, plant characteristics, soil pH, and P nutrient content in the soil.

The results of the spore count study showed that the average number of spores found in the Lamtoro treatment showed the lowest value, even reaching zero at all observation times (3 months, 5 months, and after drying 10 g), significantly different compared to other host plants such as Sengon, Albizia, Johar, and Cajanus, which had high spore count values. The low number of spores in Lamtoro is thought to be related to the low level of compatibility between the host plant and AMF (Arbuscular Mycorrhizal Fungi), especially *Racocetra crispera*. According to Puspitasari et al. (2012), the number of spores is not only influenced by one factor, but is influenced by the accumulation of several factors, including the mycorrhiza itself, host plant varieties, and environmental conditions, such as light and temperature. Smith and Read (1997) stated that it cannot be ascertained that plants with a high percentage of root infection will produce a high number of spores in the rhizosphere or vice versa.

Based on the research results, in general, legume plants can be used as host plants except Leucaena. Albizia plants are good because of the high success of culture and colonization of AMF in Albizia plants, allegedly because this plant has a large and fine root system that facilitates AMF infection. In addition, Albizia is a legume plant that can form root nodules and produce root exudates; Albizia roots are known to contain active secondary metabolite compounds such as saponins, flavonoids, alkaloids, tannins, and triterpenoids that can stimulate hyphal development and AMF sporulation (Himaniarwati, 2025). Research by Tuheteru et al. (2011) shows that Albizia has a high level of mycorrhizal dependence and is able to increase plant growth after AMF inoculation. The low success of AMF culture and colonization in Leucaena plants is thought to be caused by the low compatibility between the host plant and AMF, so that

hyphal development and spore formation do not take place optimally. Furthermore, the relatively slow initial growth of Lamtoro due to seed dormancy may influence the AMF infection process in young roots. Another factor suspected of contributing to low colonization is the presence of secondary metabolites in the form of mimosine, phenolic compounds, flavonoids, and alkaloids in lamtoro, which are allelopathic. Mimosine is known to inhibit cell division, enzyme activity, and the growth of several organisms, thus possibly influencing the development of AMF hyphae in the root zone (Baharuddin, 2023). Delvian and Hartanto (2021) also reported that environmental stress conditions can reduce AMF root colonization in Lamtoro.

CONCLUSION

Based on the research results, it can be concluded that the host plants Sengon (*Paraserianthes falcataria*), Albizia (*Albizia saponaria*), johar (*Cassia siamea*), and Cajanus (*Cajanus cajan*) have a high percentage of successful culture and AMF colonization of 66.67–86.67%. The highest number of spores was at the ages of 3 months, 5 months, and 10 g after drying in the host plant Albizia (*Albizia saponaria*).

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