BIODIVERSITAS
Volume 20, Number 8, August 2019
Pages: 2410-2416

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d200840

Indigenous endomycorrhizal fungi at salak (*Salacca zalacca*) plantations in Bali, Indonesia and their colonization of the roots

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Manuscript received: 31 May 2019. Revision accepted: 31 July 2019.

Abstract. *Rai IN, Suada IK, Proborini M, Wiraatmaja IW, Semenov M, Krasnov G. 2019. Indigenous endomycorrhizal fungi at salak (*Salacca zalacca*) plantations in Bali, Indonesia and their colonization of the roots. Biodiversitas 20: 2410-2416.* Cultivation of snake fruit, commonly known as salak usually done organically on dry land with limited fertilizer in Bali. This research aimed to observe and to identify the indigenous endomycorrhizal fungi on salak roots. The exploration was carried out by collecting soil and root samples in salak producing areas in Bali, i.e. Bebandem and Selat of Karangasem Regency, Payangan of Gianyar, and Pupuan of Tabanan Regency. At each location, 9 random samples were taken, resulting in a total of 36 samples. Spore extraction was carried out using a wet filtration technique followed by centrifugation according to the method by Brunndrett et al. (2009). Morphological identification was carried out at the genus and species level using the Manual for Identification of Mycorrhiza Fungi for identifying Vesicular-Arbuscular-Mycorrhiza (VAM) fungi (Schenk and Perez, 1990), while molecular identification was carried out according to Tedersoo et al. (2014). The percentage of root infections was carried out using the coloring method with trypan blue. The results showed there were only two genera of endomycorrhizae (*Glomus* and *Entrophospora*) identified at the locations of study sites. The results also showed that samples from Bebandem and Selat regions had 3 *Glomus* species, Payangan had 3 *Glomus* species and 1 *Entrophospora* species, while in Pupuan had only 2 *Glomus* species. Identification results based on morphological characters showed that all species in the genus *Glomus* consisted of 3 species, namely *Glomus sp-1*, *Glomus sp-2*, and *Glomus sp-3*, while one species in the genus *Entrophospora* was *Entrophospora sp*. Genetic identification results based on the nucleotide arrangement showed that *Glomus sp-1* concluded as *Glomus cubence*, *Glomus sp-2* concluded as *Glomus custos*, and *Glomus sp-3* concluded as *Glomus indicum*, while *Entrophospora* species concluded as *Entrophospora_sp_SH197095.06FU*. The average of root colonization/ infection was very high, reaching 93.33% in Bebandem and Selat, 95.00% in Pupuan, and 100% in Payangan. The very high root infection rates indicated that the indigenous endomycorrhiza found in these areas was very adaptive in salak plantation, so there is an opportunity to be developed as biofertilizers.

Keywords: Genetics, indigenous endomycorrhiza, infection rates, morphology, spores

INTRODUCTION

Snake fruit (*Salacca zalacca*) known as salak in Indonesia is a species of the palm tree (family Arecaceae) which is organically cultivated on dry land with limited production inputs in Bali. It is usually fertilized with leaf litters or other organic fertilizers with a minimum application and erratic administration time (Sukewijaya et al. 2009). This pattern was carried out by snake fruit farmers is due to the difficulty in obtaining adequate fertilizer. Therefore, soil fertility of salak plantations is usually low, and according to Rai et al. (2010), it is indicated by the low levels of C-organic and NPK in soil and leaf tissue. Endomycorrhizal fungal biofertilizers are being developed using isolates from the salak root area to overcome low fertility in salak plantations. The diversity of endomycorrhizal fungi, also known as Vesicular-Arbuscular Mycorrhizal (MVA) fungi, is very large (Hempel et al. 2007; Wang and Yong Shi 2008; Avio et al. 2009; Baslam et al. 2011; Proborini 2013; Suamba et al. 2014; Sarah and Ibrar 2016; Choosa-nga et al. 2019), and the application in salak plantation has a positive effect on salak production (Juliadewi et al. 2014; Rai et al. 2015).

Research conducted by Rai et al. (2015) showed an increase in the production of salak Gula Pasir that fertilized with mycorrhizal biofertilizer, as well as producing off-season fruits which are caused by increased soil fertility, photosynthesis, total sugar content, N, P, K, and Mg in leaf tissue. Rai et al. (2015) also stated that biofertilizer had a positive correlation with the length and breadth of the root in terms of coverage, so it absorbed more water and nutrients. Juliadewi et al. (2014) reported that the use of microfluidic biofertilizers up to 75 g per tree increased salak production as well as off-season production due to the increment in fruit set.

Mycorrhizae is a fungus that lives in a symbiotic relationship with spermatophytes. It colonizes the host's roots to create a mutually beneficial relationship without causing any harm. According to Menge (1985), Finlay (2008), Hernadi et al. (2012), and Zasvari et al. (2012), the

hosts obtain nutrients and water from mycorrhiza, while the fungi, in turn, obtain carbohydrates or nutrition from the hosts. Smith and Read (2008), Lee et al. (2012), and Yinan et al. (2017) reported that mycorrhizae enhance plant growth because it increases its ability to obtain water and nutrients such as phosphorus, nitrogen, and potassium. In addition, mycorrhiza is capable of increasing host resistance to root pathogens (Pozo et al. 2010), increasing host tolerance to environmental stress due to salinity and drought (Latef et al. 2016; Quiroga et al. 2017), and heavy metal contamination (Upadhyaya et al. 2010). Moreover, mycorrhizal abundance and activities in the soil rhizosphere are largely determined by climate, host type, and soil water content or drought level (Schubler et al. 2001; Moreira et al. 2007; Hernadi 2012; Sadhana 2014; Quiroga et al. 2017; Mathimaran et al. 2017; Choosa-nga et al. 2019), type and level of soil fertility, as well as altitude (Allen and Boosalis, 1983; Tahat and Sijam 2012; Kavitha and Nelson 2013; Mo et al . 2016).

Based on the infection method, mycorrhizae are divided into two groups-ectomycorrhiza and endomycorrhiza. The ectomycorrhiza infects the outer surface of the roots and the tips, which then forms a white braid of hyphae/mycelia on the root hairs (Hartig's nets). This infection causes changes in the morphology of the root-shortening, swelling, dichotomous branching, and pigment formation. According to Smith and Read (2008), Finlay (2008), and Brundrett (2009), the hosts of ectomycorrhizae are generally forestry plants. However, endomycorrhiza infects the inside and between the cells of root tips. The hyphae from outside the roots enter the cell and fill the intercellular spaces, thereby forming arbuscles-hyphae tissues that penetrate the cells through plasmalemma and vesicles-small bubbles like granules in the cytoplasm that contain lipids and useful for vegetative reproduction. Infections of endomycorrhiza form external hyphae, help to expand the space covered by the roots for absorbing nutrients. Endomycorrhizal infection does not change the morphology of the roots but changes the appearance of its cells and tissues. According to Schenck and Perez, (1990); Aguilar and Barea (1997); Hempel et al. (2007); Smith and Read (2008) & Brundrett (2009), majority of these hosts are important agricultural commodities and seasonal crops like beans, rice, corn; horticulture plants like fruits, vegetables, and ornamental plants; and industrial plants like cocoa, rubber, coffee, cashew, etc. Based on their characteristics, the development of the mycorrhizal bio-fertilizer for agriculture predominantly uses endomycorrhizal fungi (Wang and Yong Shi 2008; Sadhana 2014).

Currently, there is ready-to-use mycorrhizal biological fertilizer in the form of powder, granules, and pellets. But according to Brundrett (2009) and Jha and Kumar (2011), the indigenous (local) mycorrhizae is more adaptive and efficient because its hyphae/mycelium and indigenous fungal spores have faster and better optimal adaptability in colonizing the root system of the host plant. This research is conducted to explore, and to identify the indigenous fungi from salak roots from several regions in Bali and to determine the potential of indigenous endomycorrhizae as biological fertilizer and their potential in colonizing roots.

MATERIALS AND METHODS

Sampling locations

The soil and root samples were taken from salak plantations in 4 locations in Bali (Bebandem and Selat of Karangasem Regency, Payangan of Gianyar Regency, and Pupuan of Tabanan Regency). Bebandem and Selat are the two major producers of salak in Bali were located in the eastern of Bali Island, while Payangan and Pupuan were located in the central and western of Bali.

Roots and soil sampling

Soil and root samples were taken from nine points at each location, and there were 36 samples in total. Soil samples were taken from the rhizosphere around the base of the bark. Samples were taken 30-40 cm around the base of the stem and at a depth of 10-15 cm from the ground surface to collect about 2 kg of soil (Chaurasia and Khare 2005; Nurhandayani et al. 2013). Root samples (10 cm) were taken from the same points were put into polyethylene bags and stored at 4^{o}C in the laboratory. The level of the endomycorrhizal infection was observed as soon as the sample arrived in the laboratory.

Extraction and isolation of spores

The spore extraction was carried out using a wet filter technique followed by centrifugation according to the method by Brunndret et al. (2009). Exactly 100 g of soil samples was dissolved and evenly stirred in 1000-1200 mL of water, then sieved with a multi level sieves sized of 1 mm, 500 µm, 212 µm, 106 µm, and 53 µm. Dissolution and sieving were repeated 2-3 times, and the remaining soil was poured into the top filter. The top sieve was sprayed with water to facilitate the sieving and filtering process carried out from one sieve size to another. The filtered soil in the 500 µm, 212 µm, and 53 µm sieves were transferred to the centrifuge tube, then 25-40 mL of distilled water was added and centrifuged at a speed of 2000 rpm for 5 minutes. The supernatant was removed and the pellets resuspended with 60% glucose and centrifuged again at a speed of 2000 rpm for 1 minute. The supernatant containing sugar in each sieve was rinsed with water using a 53 µm diameter filter. The results of the rinsing were placed in Petri dishes, and the spores were isolated and examined using a stereomicroscope (Zeiss Stemi 508).

Morphology and genetic identification of indigenous endomycorrhizae

The isolated spores were placed on microscope slide added with a drop of polyvinyl alcohol-lactic-glycerol acid (PVLG), to identify indigenous endomycorrhizae based on their morphology with the use of a compound microscope (Zeiss Axioscope 40) at 10 and 100x magnification. The morphological identification was carried out at the genus and species level using the Manual for Identification of Mycorrhiza Fungi for identifying Vesicular-Arbuscular-Mycorrhiza (VAM) fungi (Schenk and Perez, 1990), based on characteristics of size, color, spore arrangement, shape, hyphae attachment, and the number of spore walls. The results were compared with various libraries and an online

database description of mycorrhizal species at West Virginia University, USA (http: //invam.caf.wvu.edu).

The molecular identification was carried out according to the method by Tedersoo et al. (2014). Total DNA was extracted using MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche) based on the manufacturer's instructions. The analysis of the cells and tissues was performed using MagNA Pure Bacteria Lysis Buffer (Bacteria, Fungi) (Roche). Extracted DNA samples were stored at-20 °C for further analysis. The amplicon libraries of the ITS5F-ITS4 regions were prepared following the procedure of Illumina Metagenomic. The fragments of the ITS region were amplified using the forward primer ITS-5 F (5' GGAAGTAAAAGTCGTAACAAGG 3') and the reverse primer ITS-4 (5' TCCTCCGCTTATTGATATGC 3'). The PCR was performed using Tersus PCR kit (Evrogen, Russia) based on the manufacturer's instructions.

Observation of root colonization/infection

The level of root infection by indigenous endomycorrhiza was observed using slide method according to Giovannetti and Mosse (1980) with the formula as follows:

Percentage of infected roots = number of infected roots: total number of roots observed x 100%.

Root infection rates are classified into 5 classes; which are very low (0-5%), low (6-25%), moderate (26-50%), high (51-75%) and very high (76-100%). Observation of root infection was conducted by trypan blue staining assay. The staining process began by washing the roots and then cutting the clean roots into 2-5 cm, after which 20-30 pieces were inserted into the test tube and soaked in 10% KOH and heated in a microwave at 250°C for 10 minutes. Heated roots were left for±12 hours at room temperature. After that, 10% KOH solution was removed, and the roots were washed 3 times with water. Roots were then soaked in 3% H_2O_2 and stored for±12 hours at room temperature. After finish storing, H_2O_2 was removed and the roots were washed 3 times with water, soaked in 1% HCL, and stored for±12 hours at room temperature. HCl was removed, and the roots were immersed in trypan blue, and then heated at 250 °C for 5 minutes in a microwave and stored for±12 hours at room temperature, then trypan blue was removed, and the roots were soaked in lactoglycerol and heated at 250 °C for 5 minutes. The roots were then stored±12 hours at room temperature. After the administration of lactoglycerol was completed, the roots were then taken with tweezers and placed lined up on a glass object for observation using a microscope to determine the existence of infection and structure of mycorrhizae (vesicles, arbuscular, hyphae).

RESULTS AND DISCUSSION

Species diversity and spore density

The results in Table 1 showed that soil samples from Bebandem and Selat only have 3 species of endomycorrhizae that belongs to the *Glomus* genus. Isolation of endomycorrhizae from Payangan soil sample showed 3 species, 2 species belonging to the *Glomus* genus and one species from the genus *Entrophospora*, while the Pupuan sample has 2 species, both in the *Glomus* genus. The results of the spores identification showed that endomycorrhizae from the study sites were included in 2 genera, namely *Glomus* and *Entrophospora*. *Glomus* genus was found in all sampling locations, while *Entrophospora* was only found in Payangan. According to INVAM (2014), *Glomus* and *Entrophospora* were both belong to the *Zygomycota phylum*, the order of *Glomeromycota* and *Glomineae* sub-order. The difference between the two is that *Glomus* belongs to the *Glomaceae* family, while *Entrophospora* belongs to the family *Entrophosporaceae*.

The density of spores of the soil samples taken from the four sub-districts ranging from 13 to 30 spores per 100 g of soil (average 20.06±5.57 spores). Different locations have different spore densities ($P < 0.05$) with the highest spores density was in the salak roots from Pupuan (27.11±2.76 spores), which was significantly different from spores density obtained from the three other sub-districts. The lowest spore density was obtained from soil samples collected in Bebandem (15.44±2.70 spores), but it did not significantly different from that of Selat (15,78±2,95 spores) (Table 1).

Morphology and genetic identification of spores

Spores identification was based on characters such as size, color, the arrangement of spores, hyphae shape and attachment, and several spore walls. The results showed that all *Glomus* species collected from four locations consist of only 3 species-*Glomus sp-1*, *Glomus sp-2* and *Glomus sp-3*, as shown in figures 1A, 1B, and 1C, while one species in the *Entrophospora* genus was identified as *Entrophospora sp* as shown in Figure 1D. *Glomus sp-1* is morphologically similar to *Glomus cubense* (Roddigues et al. 2011) and *Glomus ambisporum* (INVAM 2014), as shown in figures 2A and 2B, *Glomus sp-2* is similar to *Glomus custos* (Cano et al. 2009) and *Glomus hoi* (INVAM 2014), as shown in figures 2C and 2D. *Glomus sp-3* is similar to *Glomus indicum* (Blaszkowski et al. 2010) and *Glomus pansiholus* (INVAM 2014), as shown in figures 2E and 2F.

Table 1. Spore density, genus, and number of indigenous endomycorrhizal species in salak samples from four different locations

Sub-district	Bebandem	Selat	Payangan	Pupuan
Genus	*Glomus*	*Glomus*	*Glomus* and *Entropohospora*	*Glomus*
Species	3	3	4	2
Spore density (%)	15.44±2.70 c	15.78±2.95 c	21.89±2.68 b	27.11±2.76 a

Note: In the spore density row, the numbers followed by the same letter mean that there is no significant difference in the LSD test level of 5% (T value = 1.80)

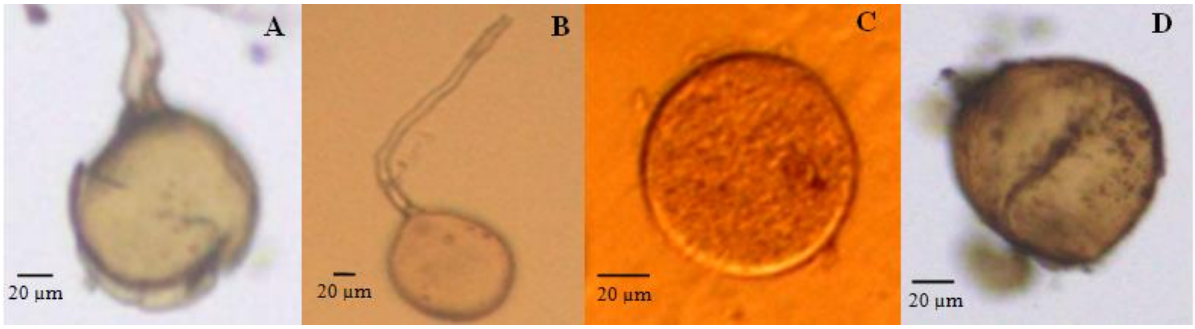

Figure 1. Three Species of *Endomychorrizae* isolated from salak roots. Two isolates belongs to the genus of the *Glomus* and one species of *Entrophospora. Glomus sp-1* (A), *Glomus sp-2* (B), and *Glomus sp-3* (C), and *Entrophospora sp*. (D)

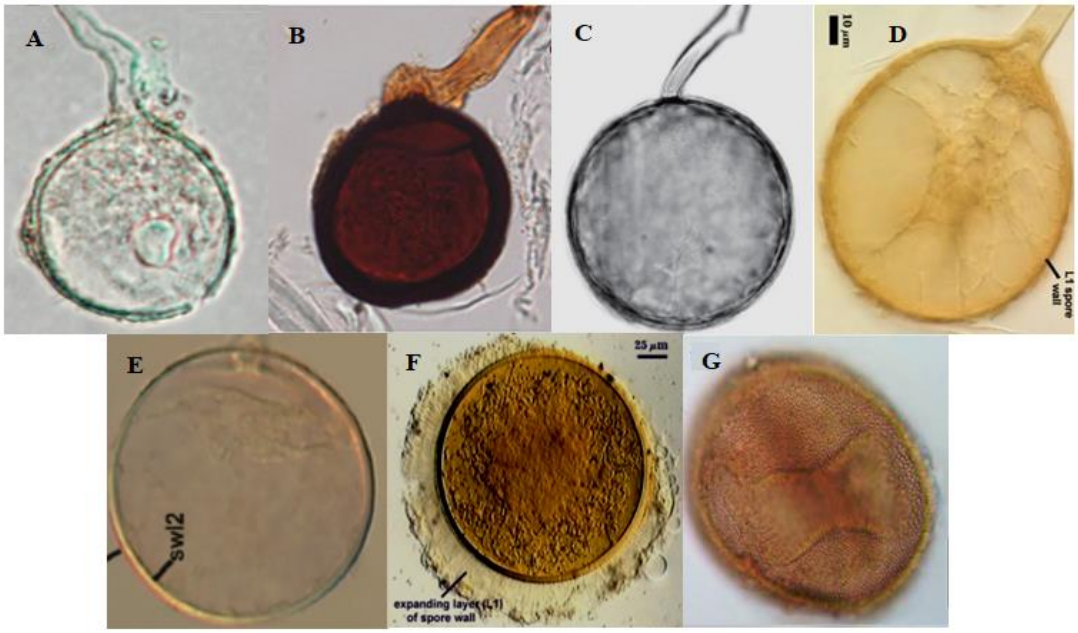

Figure 2. The indigenous *endomycorrhiza* isolate from roots of salak were similar to *Glomus* and *Entropospora* species in the library. *Glomus cubense* (Roddigues et al. 2011) (A) and *Glomus ambisporum* (INVAM 2014) (B) are similar to *Glomus sp-1* (1A), *Glomus custos* (Cano et al. 2009) (C) and *Glomus hoi* (INVAM 2014) (D) similar to *Glomus sp-2* (1B), *Glomus indicum* (Blaszkowski et al. 2010) (E) and *Glomus pansiholus* (INVAM 2014) (F) similar to *Glomus sp-3* (1C), and *Entrophospore infrequent* (Hernandes et al. 2018) (G) similar to *Entrophospore sp.* (1D)

Morphologically *Glomus sp-1* has the characteristics of a sporocarp, round spores, 50-80 µm, yellow/brownish yellow or dark brown, the surface of the spores was varies (rough and smooth), and there were hyphae attach to the spore wall. Morphological characteristics of *Glomus sp-2* are single and oval spores, 75-120 µm, light brown, smooth spore surfaces, hyphae that were still attached to spores and spore walls consisting of two separate layers. While the morphological characteristics of *Glomus sp-3* were single spheres, round, 108-180 µm, golden yellow, the surface of the spore is slightly rough, and has sub standing hyphae. According to INVAM (2014), *Glomus* spores can be single or in groups. According to Sastrahidayat (2011), *Glomus* genus has a single or double spore wall and is equipped with oily spots on mature spores of varying sizes, the surface of the smooth spore wall has no ornament, and has cylindrical straight, subtending hyphae. *Glomus* spores were formed from enlargement of hyphae to the maximal size, which eventually forms spores. The presence of hyphae which always attach to the spore wall consisted of 1-3 spore walls is a typical characteristic of *Glomus* spore. The branches of hyphae form a sporocarp (clustered spores).

The morphological features of *Entrophospora sp*. found in salak roots obtained from Payangan are elliptical, yellow or yellowish red, and the spore walls are not clear, as shown in Figure 1D. The outer appearance of the *Entrophospora sp*. is similar to *Entrophospora infrequent* (Hernandes et al. 2018) (Figure 2G). According to Oehl et al. (2011) and INVAM (2014), *Entrophospora* is a mycorrhizal genus belonging to the family *Entrophosporaceae*, having 2-3 spore walls, spores formed on the side of the soporiferous neck, round to elliptical, yellow or yellowish red, and 100-400 µm.

Genetic identification based on the nucleotide arrangement showed that *Glomus sp-1* concluded as *Glomus cubense*, *Glomus sp-2* concluded as *Glomus custos*, and *Glomus sp-3* concluded as *Glomus indicum*, while the *Entrophospora* genetically concluded as *Entrophospora_sp_SH197095.06FU*. All the *Glomus* species found in this study, i.e. *Glomus cubense*, *Glomus custos*, and *Glomus indicum* are categorized by INVAM (2014) as new species. The nucleotide arrangement of indigenous endomycoriza species found in the salak root in Bali are as follows:

1. *Glomus cubense*. >@M05275: 83: 000000000-BGVKJ: 1: 1111: 18969: 24571 [confidence = 0.98].

TCTCCGTTGGTGAACCAGCGGAGGGATCATTACAGA
GTTGCAAAACTCCCAACCATTGTGAACGAACCCGTT
ATAGTTGCTTCGGCGGGGGGTGCGCTGCAACCACCC
GCCGGCAGCATGCAAACTCTAGATTATAGTGGATCT
CTGAGTAGCTTATTTAATAAGTCAAAACTTTCAACA
ACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCA
GCGAAATGCGATANNNNNNNNNNNNNNNTAGTATT
CTGGCGGGCATGCCTGTTCGGGCGTCATTTCAACCAT
CAGCCCCTGTCTAGGTCTGTGTTGGGGGCCTGCGCCT
GCCGCAGCCCCCTAAATGCAGTGGCGGGCTCGCTGT
TACCCCGAGCGCAGTAGTGTATCTCGCTCTGGGCGT
GGCAGCGGGTGCTAGCCGTGAAACACTCACTAAGGT
TGACCTCGGATCAGGTAGGAATACCCGCTGAACTTA
A.

2. *Glomus custos*. >@M05275: 83: 000000000-BGVKJ: 1: 1106: 15949: 15504 [confidence = 1].

TCTCCGTTGGTGAACCAGCGGAGGGATCATTACCGA
GTTTTCAACTCCCAAACCCCTGTGAACATACCATTTT
GTTGCCTCGGCGGTGCCTGTTCCGACAGCCCGCCAG
AGGACCCCAAACCCAAATTTCCTTGAGTGAGTCTTCT
GAGTAACCGATTAAATAAATCAAAACTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGC
GAAATGCGATANNNNNNNNNNNNNNNGGCGGCCTT
GCCTGGTCGGGCGTCATTTCAACCCCCAAGCCCCCG
GGCTTGGTGTTGGGGATCGGCGAGCCTCTGCGCCCG
CCGTCCCCTAAATTGAGTGGCGGTCACGTTGTAACTT
CCTCTGCGTAGTAGCACACTTAGCACTGGGAAACAG
CGCGGCCACGCCGTAAAACCCCCAACTTTTGAACGT
TTGACCTCGAATCAGGTAGGACTACCCGCTGAACTT
AA.

3. *Glomus indicum*. >@M05275: 83: 000000000-BGVKJ: 1: 1105: 2880: 18372 [confidence = 0.99].

TCTCCGTTGGTGAACCAGCGGAGGGATCATTACAGAG
TTGCAAAACTCCCAACCATTGTGAACGAACCCGTTAT
AGTTGCTTCGGCGGGGGGTGCGCTGCAACCACCCGCC
GGCAGCATGCAAACTCTAGATTATAGTGGATCTCTGA
GTAGCTTATTTAATAAGTCAAAACTTTCAACAACGGA
TCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAA
TGCGATANNNNNNNNNNNNNNNTAGTATTCTGGCGG
GCATGCCTGTTCGGGCGTCATTTCAACCATCAGCCCC
TGTCTAGGTCTGTGTTGGGGGCCTGCGCCTGCCGCAG
CCCCCTAAATGCAGTGGCGGGCTCGCTGTTACCCCGA
GCGCAGTAGTGTATCTCGCTCTGGGCGTGGCAGCGGG
TGCTAGCCGTGAAACACTCACTAAGGTTGACCTCGGA
TCAGGTAGGAATACCCGCTGAACTTAA.

4. *Entrophospora_sp_SH197095.06FU*. >@M05275: 83: 000000000-BGVKJ: 1: 1106: 11344: 27377 [confidence = 1].

TCTCCGTTGGTGAACCAGCGGAGGGATCATTACAGA
GTTGTAAAACTCCCAAACCCATGTGAACATACCTGT
TGCCTCGGCGGCCTACCCGGCAGCTACCCTGTAGCT
ACCCTGTAGTCCGCCGACGGATTTCAAAACTCTTGTT
TCCAGTTGTATCTCTGAGAATAAAACAAATAAATCA
AAACTTTCAACAACGGATCTCTTGGTTCTGGNNNNN
NNNNNNNNNNGTTCGAGCGTCATTAGGTCCACTTAA
GCCCTGTTTAGTGTTGGGAGACTGCTCCGGGGGTGC
TACCCTGTTGCTCCCCTGCAGCTCCTCAAAGACAGCG
GCGGAGTTGTGGTATCCTCTGAGCTTAGTAACTTGTT
CTATCTTTCGAGTACACCACTTCCCCTGCCGTAAAAC
CCTTAATTTTTAATGGTTGACCTCGGATCAGGTAGGA
ATACCCGCTGAACTTAA.

Colonization/root infection by indigenous endomycorrhiza

Based on the results, it showed that the average root colonization by the indigenous endomycorrhiza was high; i.e., 93.33% in Bebandem and Selat, 95.00% in Pupuan, and 100% in Payangan as shown in Table 2. Although spore density was low (Table 1), the results proved that these indigenous endomycorrhizas were very adaptive in salak plants. It was indicated by a very high rate of infection and the ability to colonize roots with an average percentage of infections as high as 95.42%. Therefore, with a high root infection rates, these indigenous endomycorrhizae have great potential to be developed as biological fertilizers. It was in accordance with Pulungan (2013) that indigenous endomycorrhizae which was classified as arbuscular mycorrhizal fungi can be used as an alternative technology to help growth and improve the quality and productivity of plants, especially in infertile marginal lands. According to Brundrett et al. (2009), the level of root infection by mycorrhiza is strongly influenced by the level of soil fertility. High infections occurred when the soil around the roots was infertile. Consequently, the high mycorrhizal root infection in this study was related to the low soil fertility in the salak plantation. It was also in accordance with the results of a study conducted by Rai et al. (2010) that the level of soil fertility and nutrient content of N, P, and K in the leaves tissue of salak plants in Karangasem Regency was relatively low.

The results of the study showed that the endomycorrhizae structure isolated from the cortical root cells of salak consisted of hyphae, spores, arbuscular, vesicles, and chlamydospores (Table 2). Hyphae were found along the root cortex with an almost straight shape (Figure 3A). In the root cortex, also found arbuscular hyphae (Figure 3B), vesicular hyphae (Figure 3C), internal hyphae (Figure 3D), and external hyphae (Figure 3E). The bubble tip is turned into a spore; therefore, the spores are formed from the swelling of the hyphae. According to Navarro et al. (2012), spores originating from the development of hyphae are called chlamydospore and in each branching hyphae are formed chlamydospore called sporocarp. Kehri et al. (2018), reported that indigenous endomycorrhizal fungi are arbuscular groups of mycorrhizae which always form vesicular and arbuscular in cortical cells. Puspitasari et al. (2012) reported that the genus *Glomus* has a high adaptation to environmental conditions so that they are mostly found in a symbiotic relationship with the roots of various types of plants. The result of this study was in accordance with Puspitasari et al. (2012) that the genus *Glomus*, presence in all sampling locations, hence, it is classified as a genus with very high adaptability.

Table 2. Endomycorizal structure of *Glomus* Genus and *Entrophospora* Genus in Salak Root Cortex Cells in Four Sampling Locations

Sub-districts	Structure of endomycorrhiza					Average root infection (%)	Genus
	H	S	A	V	Ks		
Bebandem	(+)	(+)	(+)	(+)	(+)	93.33	*Glomus*
Selat	(+)	(+)	(+)	(+)	(+)	93.33	*Glomus*
Payangan	(+)	(+)	(+)	(+)	(+)	100.00	*Glomus* and *Entrophospora*
Pupuan	(+)	(+)	(+)	(+)	(+)	95.00	*Glomus*

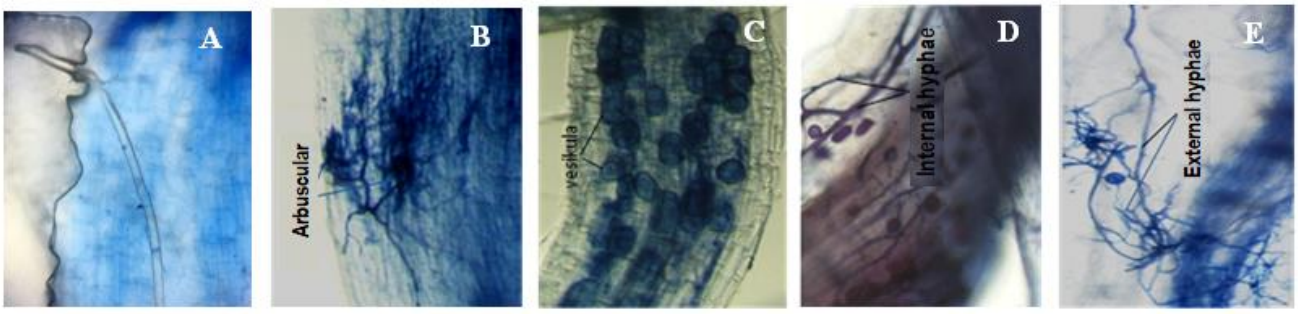

Figure 3. The structures of hyphae from endomycorrhiza in the cortical root cells of salak (A), arbuscular hyphae (B), Vascular Hyphae (C), Internal Hyphae (D), and External Hyphae (E). Note: : H = hyphae, S = spore, A = arbuscular, V = vesicular, Ks = clamidospora, and (+) = endomycorhizza infection

According to Nusantara et al. (2012), and Kehri et al. (2018) external hyphae absorb phosphorus and water from the soil and brought to the internal and arbuscular hyphae for host plant growth, while internal hyphae absorb organic material from the host plants for mycorrhizal growth. Kavitha and Nelson (2013) reported that mycorrhizae grow from outside the root and then enters the root tissue and the arbuscular hyphae cells (tissue hyphae which penetrate between cells and penetrate cells through plasmalemma). In the cells, the hyphae form vesicles, small bubbles in the cytoplasm, granular vesicles containing lipids and become vegetative mycorrhizal reproductive cells. Arbuscular is hyphae which penetrate plasmalemma and help transport nutrients to plant cells.

In addition to vesicles and arbuscules, external hyphae are formed to help expand the nutrient absorption by the roots. In certain plants, the length of the external hyphae usually reaches 80 cm per 1 cm of root length. Outside the roots, hyphae can form sporangium which produces spores as a means of reproduction. The extent of hyphae tissue in the soil helps the roots absorb nutrients and water. In this study, the formation of vesicles and arbuscules in salak root cells showed perfect symbiosis so that the plants increase the availability of nutrients absorbed from the soil.

Based on the results, it is concluded that there were 2 endomycorrhizae genera, *Glomus* and *Entrophospora*, isolated from salak roots taken from 4 different locations in Bali. *Glomus* is a genus that has a large distribution area because it was found in all sampling locations, while *Entrophospora* has a limited distribution area. There were three isolates of the genus *Glomus* genus and one isolate of the genus *Entropohospora* isolated from the roots of salak. Root infection rates are very high, reaching 100%, indicating that indigenous endomycorrhizae are very suitable to be developed as biological fertilizers, but the population needs to be increased due to low population density.

ACKNOWLEDGMENTS

Our gratitude goes to the Directorate General of Higher Education, Ministry of Research, Technology and Higher Education for the funds provided through the Higher Education Applied Research (MP3EI) in 2018. The authors also thank the Russia-ASEAN Secretary Office for funding the molecular identification of mycorrhiza through a grant of Environmentally-Friendly Technologies of Agriculture: Problems and Prospects in 2018.

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BIODIVERSITAS
Volume 20, Number 8, August 2019
Pages: 2410-2416

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d200840