



Communication Arbuscular Mycorrhizal Communities in the Roots of Sago Palm in Mineral and Shallow Peat Soils

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Abstract: Communities of arbuscular mycorrhizal fungi (AMF) in plant roots improve host plant growth. In this study, AMF communities in the roots of the sago palm (*Metroxylon sagu* Rottb.) were investigated in mineral soil (MS) and shallow peat soil (SPS) in Sarawak, Malaysia. MS exhibited lower moisture content (MS, 38.1; SPS, 79.8%), higher pH (H₂O) (MS, 4.6; SPS, 4.1), higher soil bulk density (MS, 1.03; SPS, 0.20 g cm⁻³), and higher nitrogen content (MS, 16.9; SPS, 2.7 kg m⁻³) than SPS at the same soil depth, while the phosphorus (P) content (Bray II) (MS, 1.6; SPS, 1.9 g P₂O₅ m⁻³) was similar. The AMF colonization rate was significantly lower in SPS (39.2 ± 12.5%) than in MS (73.2 ± 4.6%). The higher number of AMF operational taxonomic units (OTUs) was detected by amplicon sequencing of the partial small-subunit rRNA gene (MS, 78; SPS, 50). A neighborjoining tree of obtained OTUs revealed that they belonged to Acaulosporaceae, Ambisporaceae, Claroideoglomeraceae, Gigasporaceae, and Glomeraceae. The lower abundance and diversity of AMF in SPS are possibly caused by abiotic factors, including soil physicochemical properties. *Glomus* and *Acaulospora* species detected in SPS might have strong tolerance against acidity and high soil moisture content.

Keywords: amplicon sequencing; arbuscular mycorrhizal fungi; peat soil; sago palm; soil physicochemical properties

1. Introduction

Sago palm (*Metroxylon sagu*, Arecaceae) produces more than 300 kg of dry starch per plant in its trunk. It is distributed across parts of Southeast Asia and northwestern Melanesia, including Papua New Guinea and the Solomon Islands [1]. Sarawak, Malaysia, is one of the most prominent areas for sago flour production. In this region, sago cultivation has been conducted mainly in peatland [2]. Tropical peat contains a vast amount of soil organic matter, and is characterized by low pH and low soil fertility. While almost no other major crops can grow without drainage or soil improvement, sago palm species can grow in tropical peat soil [3], although the period of growth required before the first harvest of sago palms is delayed in peat soil (12.7 years) as compared to mineral soil (9.8 years) [4].



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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). According to Kakuda et al., (2000) [5], the lower nutrient in unit volume in peat soils is one of the reasons for this delay in the growth in peat soils.

The majority of terrestrial plants establish symbiotic associations with arbuscular mycorrhizal fungal communities. In an agricultural ecosystem, AMF play an essential role in plant growth performance and soil health for sustainable management [6]. They form mycelium networks in the soil, enhancing the stability of soil aggregates [7]. They also improve the uptake of plant nutrients, including the biologically essential nutrients phosphorus (P) and nitrogen (N), and improve resistance to drought, salinity, heavy metals, pollution [8], and diseases [9]. AMF are classified in the phylum Glomeromycota, which consists of three classes [10], 12 families, and 43 genera [11–14]. This wide range of species builds a diverse AMF community structure in agricultural ecosystems, and its abundance and diversity affect plant growth performance [15]. The diversity of AMF enhances P uptake in the host [16], while co-inoculation by AMF belonging to different families improves growth of host plants exposed to abiotic stress [17].

Recent studies have revealed that the inoculation with AMF improves the growth of the Arecaceae family [18,19]. In the case of the sago palm, Chan et al., (2002) [20] detected AMF belonging to Glomales of the Zygomycetes in tropical peat soil. Furthermore, Asano et al., (2019) [21] revealed that sago palm could have a symbiotic relationship with AMF when commercial mycorrhizal inocula were applied. These findings led to the formulation of the following hypotheses: (i) the abundance and community structure of AMF in naturally grown sago palms present a specific pattern; (ii) the abundance of AMF colonization and the community structure in the roots of sago palms vary due to cultivation in different soil type; (iii) AMF is a strategy of the sago palm to enhance plant growth performance. However, the abundance and community structure of AMF colonization and the community unclear. In this study, the abundance of AMF colonization and the community structure in the root of sago palms cultivated in mineral soil (MS) and shallow peat soil (SPS) were investigated comparatively using a next-generation sequencing technique.

2. Materials and Methods

2.1. Sample Collection

Soil and roots were collected from sago palms grown in mineral soil (MS) in the research field of Universiti Teknologi MARA Sarawak Branch, Samarahan Campus (1°26.26' N, 110°27.11' E) and shallow peat soil (SPS) in Dalat (2°51.14' N, 111°49.38' E). In September 2019, five individual palms were randomly selected from each site, and fine roots 1 m from the mother palm were collected using a shovel at a soil depth of 0–15 cm. The root samples were then washed in sterile water to remove soil and stored at -20 °C for subsequent staining and DNA extraction. For each of the five trees, MS samples were taken at a depth of 15 cm from the soil surface using a shovel, while SPS samples were collected at a depth of 50 cm from the surface using a peat sampler (Eijkelkamp Soil & Water, Giesbeek, The Netherlands).

2.2. Soil Physicochemical Properties

The soil bulk density was determined by collecting soil in a cylindrical can and weighing the contents after drying at 105 °C for one day. The soil moisture content (%) was calculated by dividing the weight loss after drying at 80 °C for three days by the wet weight of field-moist soil. The soil pH (a soil–water ratio of 1:7.5 w/v) was determined with a pH meter. The amount of available soil phosphorus was extracted according to the Bray II method [22] and measured by the molybdenum blue method at 710 nm using a Perkin Elmer Lambda 35 UV–VIS spectrophotometer (PerkinElmer Life and Analytical Sciences, Waltham, MA, USA). Carbon (C) and N content were analyzed with a Vario Microcube analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany).

2.3. Visual Assessment of AMF Colonization

The roots were bleached in 10% potassium hydroxide and stained with fuchsin acid following the procedure of Asano et al., (2019) [21]. Stained roots were observed under an optical microscope to determine the AMF colonization rate, according to the method used by Giovannetti and Mosse (1980) [23]. Statistical differences were determined by Student's *t*-test.

2.4. DNA Extraction and Sequencing

Total genomic DNA from the roots was extracted using cetyltrimethylammonium bromide (CTAB) and polyvinylpyrrolidone (PVP) [24]. The five DNA solutions were then pooled in equal proportions by concentration. For the PCR procedure, the pooled DNA was used as a template for the AMF-specific primer pair AMV4.5NF/AMDGR (coding SSU rRNA gene) [25] that was attached via the Illumina MiSeq adapter sequences. PCR was performed using GoTaq Green Master Mix (Promega Co., Madison, WI, USA) with the following settings: an initial 10 min step at 95 °C; 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min; and a final elongation step at 74 °C for 9 min. The PCR products (300 bp) were electrophoresed on 2% agarose gel and visualized on a FASIV ultraviolet transilluminator (Nippon Genetics Co. Ltd., Tokyo, Japan); they were then used for next-generation sequencing (Bioengineering Lab Co. Ltd., Kanagawa, Japan). Illumina libraries were constructed and pared-end sequenced (2 × 300 bp) on the Illumina MiSeq platform following the manufacturer's instructions (Illumina Co., San Diego, CA, USA).

2.5. Bioinformatic Processing

All sample data obtained by MiSeq amplicon sequencing were filtered using the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/index.html (accessed on: 11 November 2020). By using the FASTX-Toolkit's fastq_barcode_splitter, only read sequences precisely matching the primers used were extracted. The processing of sequence reads using QIIME2 (version 2020.8; http://qiime2.org/ (accessed on: 11 November 2020) was performed. The forward and reverse primers' sequences, 70 bp at the 3' end, chimera, and noise sequences were removed by using the dada2 plugin.

BLASTN (version 2.9.0) was utilized to determine and group the operational taxonomic units (OTUs) at 97% similarity. Non-AMF sequences were removed. Additional taxonomic estimation was conducted with known AMF taxa from NCBI-GenBank by building a neighbor-joining phylogenetic tree (1000 bootstrap replicates) using MEGA X [26]. All of the sequence data used in this study were deposited in public DNA databases under Accession Number PRJDB11168.

3. Results and Discussion

3.1. Soil Physicochemical Properties

The soil physicochemical properties of all samples are shown in Table 1. The soil pH (H₂O) in both soil samples was low, indicating acidic soil, while MS was relatively less acidic than all SPS layers, which were 4.6 and 4.0 to 4.3, respectively. The soil moisture content was lower in MS (38.1%) than in SPS (ranging from 50.6 to 79.8%), and it decreased with the increasing soil depth in SPS. The soil bulk density in SPS in the 0–15 cm and 15–30 cm layers was lower (0.20, 0.23 g/cm³), as compared with that in the 30–50 cm layer (0.96 g/cm³) and MS. The soil C content per square unit of soil volume was similar in all soil samples (ranging from 52.7 to 69.7 kg m⁻³). However, the N content was higher in the 30–50 cm soil layer in SPS (18.7 kg m⁻³) than in MS (16.9 kg m⁻³). The P₂O₅ content (Bray II) in SPS was higher than that in MS, ranging from 1.9 to 4.3 and 1.6 g m⁻³, respectively. It was found that the peat layer in SPS was in the 0–30 cm soil layer, and the 30–50 cm soil layer displayed physicochemical characteristics similar to those of MS.

Soil Type	Depth (cm)	pH (H ₂ O)	Moisture Content (%)	Bulk Density (g cm ⁻³)	C Content (kg 1	N Content n ⁻³)	P ₂ O ₅ (Bray II) (g m ⁻³)
MS	0–15	4.6	38.1	1.03	66.2	16.9	1.6
SPS	0–15	4.1	79.8	0.20	69.7	2.7	1.9
	15-30	4.0	77.7	0.23	64.9	1.4	3.2
	30–50	4.3	50.6	0.96	52.7	18.7	4.3

Table 1. Soil physicochemical properties.

3.2. The Abundance of AMF Colonization

AMF-specific structures, hyphae, arbuscules, vesicles, and spores were observed (Figure 1). The abundance of AMF in the root of the sago palm, indicating the AMF colonization rate, was significantly lower in SPS ($39.2 \pm 12.5\%$) than in MS ($73.2 \pm 4.6\%$) at the 0.0004 probability level (Figure 2). It has been suggested that soil temperature, moisture content, pH, organic, and inorganic nutrient contents affect AMF spore germination [27-30]. In this experiment, the soil moisture content was different between MS and SPS. Miller (2000) [31] showed that flooding partially inhibits AMF colonization. Wang et al., (2009) [32] found a negative correlation between AMF colonization and moisture content, since AMF need oxygen to thrive. Considering these previous findings, SPS's higher moisture content might be one of the factors limiting the AMF colonization rate.



Figure 1. Photomicrograph of root samples stained with acid fuchsin. The top pictures and the bottom pictures were roots in MS and SPS, respectively. Bar = 100μ m; arrow 1: hypha; arrow 2: vesicle; arrow 3: arbuscule; arrow 4: spore.



Figure 2. AMF colonization rates (%) in the roots of sago palm grown in MS and SPS. Bars indicate standard deviation of the means (n = 5). *** indicate levels at the 0.001 probability level according to the Student *t*-test.

3.3. AMF Community Structure

A total of 122 AMF operational taxonomic units (OTUs) containing 78 OTUs from the MS sample and 50 OTUs from the SPS sample (six shared OTUs) were obtained. Those OTUs were incorporated into the neighbor-joining tree with AMF reference sequences from the NCBI-GenBank, and 17 clades were formed (Figure 3). The 122 AMF sequences were classified into Acaulosporaceae, Ambisporaceae, Claroideoglomeraceae, Gigasporaceae, and Glomeraceae; *Glomus* was dominant in MS (76%), and *Acaulospora* and *Glomus* were dominant in SPS (37 and 63%, respectively) (Table 2). Clades Aca 2, Aca 3, Cla, Glo 2, Glo 3, Glo 5, Glo 6, and Glo 8 only have OTUs from the MS sample, while clades Aca 4 and Gig only have OTUs from the SPS sample. Furthermore, the number of belonging clades was higher in MS (15) than in SPS (7).



Figure 3. A neighbor-joining tree of partial SSU rDNA sequences obtained from the roots of the sago palm. Bootstrap values (only values > 60 are shown) were estimated from 1000 replicates. Reference sequences were incorporated from the NCBI Genbank.

	MS	5	SPS	5
Clades	No. of OTUs	RA (%)	No. of OTUs	RA (%)
Aca 1	10	3.7	11	25.3
Aca 2	7	7.1	0	0.0
Aca 3	11	2.0	0	0.0
Aca 4	0	0.0	15	11.8
Amb	2	0.2	3	0.1
Cla	1	0.1	0	0.0
Gig	0	0.0	1	0.2
Glo 1	7	1.7	16	53.4
Glo 2	3	1.4	0	0.0
Glo 3	2	2.8	0	0.0
Glo 4	5	1.2	2	9.2
Glo 5	7	6.3	0	0.0
Glo 6	6	36.5	0	0.0
Glo 7	6	32.0	2	0.0
Glo 8	5	1.3	0	0.0
Rhi	3	0.7	0	0.0
Unknown	3	2.9	0	0.0
Total	78	100	50	100

Table 2. The number of operational taxonomic units (OTUs) and relative abundance (RA) in each clade.

In this study, a high abundance of *Glomus* spp. was found in the roots of sago palm grown in both MS and SPS. This finding showed a similar tendency to the previous reports that Glomeraceae including *Glomus* spp. is generally dominant in agricultural field conditions [33–35] and high abundance of *Glomus* spp. within the rhizosphere of tree species in Sarawak [36]. Among the Arecaceae family, it is also reported that *Glomus* spp. and *Acaulospora* spp. are dominant in the soil of oil palm plantations in Thailand [37], and *Glomus* spp. were dominant in date palm [38]. From this information, our results indicate that the AMF community of sago palm in MS and SPS show a similar pattern with other palm species, but the soil type changes the composition and abundance of AMF community.

It is well known that AMF communities are affected by soil physicochemical properties. Hazard et al., (2013) [39] reported that soil pH has a marked effect on AMF communities in agroecosystems and crops, and Deepika and Kothamasi (2015) [40] reported that higher moisture content significantly reduced the diversity of AMF. In the case of oil palm, a positive correlation between AMF biodiversity and pH is reported [37]. Considering this information, the lower soil pH and higher moisture content in SPS might reduce the diversity of AMF species, resulting in the lower number of clades distribution in SPS compared to MS in this experiment (Figure 3). So far, it has been reported that some species belonging to *Acaulospora* and *Glomus* showed tolerance to flooding [31,32] and acidity [41–44]. Sahmat and Chan (2011) [45] suggested the specificity of the *Glomus* species of the sago palm, which thrives at pH 4.1. In this study, OTUs belonging to clades Aca 1, Aca 4, and Glo 1 in SPS showed a high relative abundance (over 10%), so these AMF species might be tolerant to flooding and acidity.

Although we found out the AMF colonization rates and community structure in MS and SPS, we cannot conclude that the AMF is a strategy of the sago palm to enhance plant growth performance. However, the results from this study support the idea that AMF improves the growth performance of sago palms. Indeed, growth-improvement of date palm and oil palm by AMF were reported [18,19] and salt and drought tolerance in date palm were confirmed [46,47]. To ensure the relationships between sago palm and AMF, inoculating tests of AMF and the correlation analysis of plant growth performance and AMF community structure in the field is needed in the future.

4. Conclusions

This investigation of the abundance of AMF and its community structure in the root of sago palms in MS and SPS revealed that the AMF colonization rate was lower in SPS than in MS. It was concluded that the diversity and abundance of the AMF community were higher in MS than in SPS, possibly caused by soil physicochemical properties, including the soil moisture content and soil pH. AMF species belonging to *Glomus* and *Acaulospora* obtained from SPS might be the key to enhancing the growth of sago palms in peatlands. Evaluating the growth-promoting impact of AMF on the sago palm is a subject for further investigation.

Author Contributions: K.A. and H.E. conceived and designed the experiments; K.A. and W.V.A.K. performed the experiments; K.A., K.S. and T.I. analyzed the data; S.M.B.M., M.S.A.T., S.S.S., M.K.Y.C. and M.K.-N. contributed materials and tools; K.A. wrote the paper; M.K.Y.C. and T.I. and H.E. contributed in giving input for this paper. The interactions of the listed authors represented a true collaborative effort in this publication. All authors have read and agreed to the published version of the manuscript.

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