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Genera of arbuscular mycorrhiza occurring within the rhizospheres of *Octomeles sumatrana* and *Anthocephalus chinensis* in Niah, Sarawak, Malaysia

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ABSTRACT: *Octomeles sumatrana* and *Anthocephalus chinensis* are two non-commercial tree species with future potential as plantation species in Malaysia. In order to understand the habitat in which such species grow, a study on the species as well as organisms related to them is crucial. The objectives of this study were to investigate the soil properties in which the two species grow and the associated mycorrhiza occurring within their rhizospheres. Results revealed that the properties of rhizosphere soils and the composition of arbuscular mycorrhiza varied with location. Based on the spore count method, the mean number of spores ranged from 45–142 per 50 g dry soil. The rhizosphere of *O. sumatrana* at the Niah Forestry Research Station recorded the highest number of spores. Meanwhile, the most probable number method showed values ranging from 6.5–16.0 per gram of dry soil, with the highest value recorded for *O. sumatrana* at the Niah National Park. *A. chinensis* showed the lowest values for both methods. *Glomus* was found to be dominant in the rhizospheres of both species followed by *Acaulospora* and *Gigaspora*. *O. sumatrana* was found to be a better host plant than *A. chinensis* in terms of supporting the sporulation of mycorrhiza. This is believed to be closely related to the ability of the root system to make the rhizosphere more suitable for reproduction and development of mycorrhiza spores, besides being affected by soil properties.

KEYWORDS: host plants, most probable number, mycorrhiza composition, soil properties, spore count

INTRODUCTION

Mycorrhiza is said to be the most dominant organism among the many microbial community components of the rhizosphere. It has been known to form a symbiotic relationship with the fine roots of plants[1] while enhancing plant capabilities to absorb nutrients[2]. The importance of mycorrhiza has been acknowledged in the fields of agriculture[3], forestry, and other land use[4].

The relationship between arbuscular mycorrhiza (AM) and host plants has been documented extensively for a number of species covering various habitats. Bohrer et al[5] noted that effects of mycorrhiza on host plants were often generalized but lately, more studies have highlighted differences in the effects of using different mycorrhiza. For example, Klironomos et al[6], reported that AM showed specialization in terms of soil type, pH, and mineral content. Kiers et al[7], on the other hand, acknowledged that an individual AM species can have a broad spectrum effect on a plant species or different host.

Thus, the selection of the most suitable AM for a specific host plant[8,9] and appropriate planting conditions are deemed necessary. A study on the natural conditions in which the host plant grows is therefore important before any planting activity can be conducted. This research was conducted with the objectives of investigating the soil properties in which two non-commercial tree species, namely *Octomeles sumatrana* and *Anthocephalus chinensis*, grow in the natural forest and the associated mycorrhiza as well as the AM propagules occurring within their rhizospheres.

MATERIALS AND METHODS

Site location

Two sites within the vicinity of the Niah River watershed in Miri, Sarawak, Malaysia were selected for sampling. The first site was a secondary forest at the Niah Forestry Research Station (FRS, latitude 3°40′ N, longitude 113°43′ E, altitude 23 m). The second site was a primary forest at the Niah National Park (NP, latitude 3°49′ N, longitude 113°45′ E, altitude 400 m). Both sites have a temperature of 22 °C before sunrise that increases up to 32 °C in the afternoon while the mean annual rainfall is approximately 2000 mm[10]. These sites have a tropical moist climate with the rainy season occurring from October to February. The relative humidity throughout the year in Niah is above 85%[11].

Soil sampling

We selected 5 *O. sumatrana* trees at the NP and 5 *O. sumatrana* and 5 *A. chinensis* trees at the FRS. Differences in terms of species composition were observed between the two sites. Soil was collected from randomly selected trees with diameter at breast height of more than 30 cm. Soil sampling was done up to a depth of 25 cm from the soil surface within a 60 cm radius from the trunk where no other plants were found to be growing. Each hole provided approximately 1 kg of soil and 3 samples were taken at each rhizosphere site. Each tree contributed about 3 kg of soil which was later mixed to form one bulk sample. Thus a total of 15 bulk samples were collected.

Soil physical and chemical analyses

Soil texture was determined using the hydrometer method while the water-soil paste technique[12] was adopted to determine soil pH. The Kjeldahl digestion method[13] was used to extract soil N and 0.1 M HCl was used in the titration process. The molybdenum blue method[14] and a UV-Vis spectrophotometer (Scinco) at a wavelength of 882 nm were used to determine soil P. Soil K was determined using a flame photometer at a wavelength of 766.5 nm whereas soil Ca and Mg were obtained using an atomic absorption spectrophotometer (AAS, Perkin Elmer) at wavelengths of 422.7 and 285.2 nm, respectively. The Walkley-Black[15] method was used to determine the total organic carbon (TOC).

Vesicular arbuscular mycorrhiza spore extraction, identification, and spore count

The wet sieving and decanting method was adopted[16]. First, 50 g of soil was filtered through two sieves with aperture sizes of 425 and 63 µm. Centrifugation was done twice using a 1.17 M sucrose solution at a speed of 2000 rpm ($626g$) for 5 min and then filtered and washed through a filter paper (Whatman No. 1). Extracted spores for each sample were kept in a petri dish and separated according to size and colour under a dissecting microscope. Identification of the genus was made according to descriptions by Brundrett et al[16]. The total number and percentage of spores for each sample was calculated.

Most probable number

A ten times dilution factor with six dilution levels was adopted with *Setaria anceps* as a host plant[17]. *Setaria anceps* was selected as a trap plant due to its rapid growth and tolerance towards different growing conditions. Each dilution level was represented by five replicates. Plant were grown for 12 weeks before being harvested for root infectivity inspection. Roots were treated according to the method described by Brundrett et al[16] using Chlorazol Black E as a staining dye. Root samples were arranged on a glass slide, covered with a glass coverslip, and inspected under a compound microscope to determine whether they were positive or negative for vesicular arbuscular mycorrhiza (VAM) infection.

Data analysis

Data for the TOC in the form of percentages were transformed to the arcsine values before being analysed. Statistical analysis was carried out using SAS Version 9.1.3 (SAS Institute). One-way analysis of variance was used to determine significant differences between means for the two sites in terms of soil pH, nutrient content, spore number, and most probable number (MPN). Differences between means were compared with the Duncan Multiple Range Test ($p < 0.05$).

RESULTS

Soil physical and chemical analyses

Soil samples from the rhizosphere of the two plant species consisted of two different soil textures. Soil collected from the rhizosphere of *O. sumatrana* trees at the NP was clay while those collected from the rhizosphere of *O. sumatrana* and *A. chinensis* at the FRS were sandy clay loam. Higher pH was recorded for soil collected from the rhizosphere of *O. sumatrana* at the FRS than for soil from *A. chinensis* and *O. sumatrana* at the NP (Table 1). Soil nutrients were found to be significantly different between sites in terms of N, P, K, Ca, and Mg content. The

TOC and organic matter content were also found to be significantly different at both sites and between host plants. Generally, values obtained from the rhizosphere of *O. sumatrana* at the NP were higher than those obtained for *O. sumatrana* and *A. chinensis* at the FRS.

Spore count and MPN

Significant differences in spore counts were observed for all rhizospheres (Table 2). Soils collected from the rhizosphere of *O. sumatrana* recorded a higher number of spores than for *A. chinensis*.

The MPN method gave similar patterns to the spore count method with all rhizospheres showing significant differences from each other. However, the number estimated using the MPN method was higher than that obtained using the spore count method (Table 2). Soil from the rhizosphere of *O. sumatrana* at the NP had a higher MPN than from the FRS. *O. sumatrana* rhizospheres were also found to have higher MPN estimates than *A. chinensis*.

VAM genus identification

Three mycorrhiza genera were found within the rhizosphere of *O. sumatrana* at both study sites while only two were observed for *A. chinensis* (Table 3, Fig. 1). The NP recorded a higher mycorrhiza composition with three *Glomus*, one *Acaulospora*, and two *Gigaspora* detected. Meanwhile, *O. sumatrana* at the FRS recorded only a single species for each genus. On the other hand, soil from the rhizosphere of *A. chinensis* consisted of a single species of *Glomus* and *Acaulospora*, respectively.

In this study, *Glomus* was found to be dominating all tree rhizospheres. Soil from the rhizosphere of *A. chinensis* at the FRS recorded a higher percentage of *Glomus* followed by *O. sumatrana* at the same site. Meanwhile, the rhizosphere of *O. sumatrana* at the Niah National Park showed a lower percentage of *Glomus*. A similar trend was observed for *Acaulospora*. A higher percentage of *Gigaspora* was recorded within the rhizosphere of *O. sumatrana* at the NP than at the FRS.

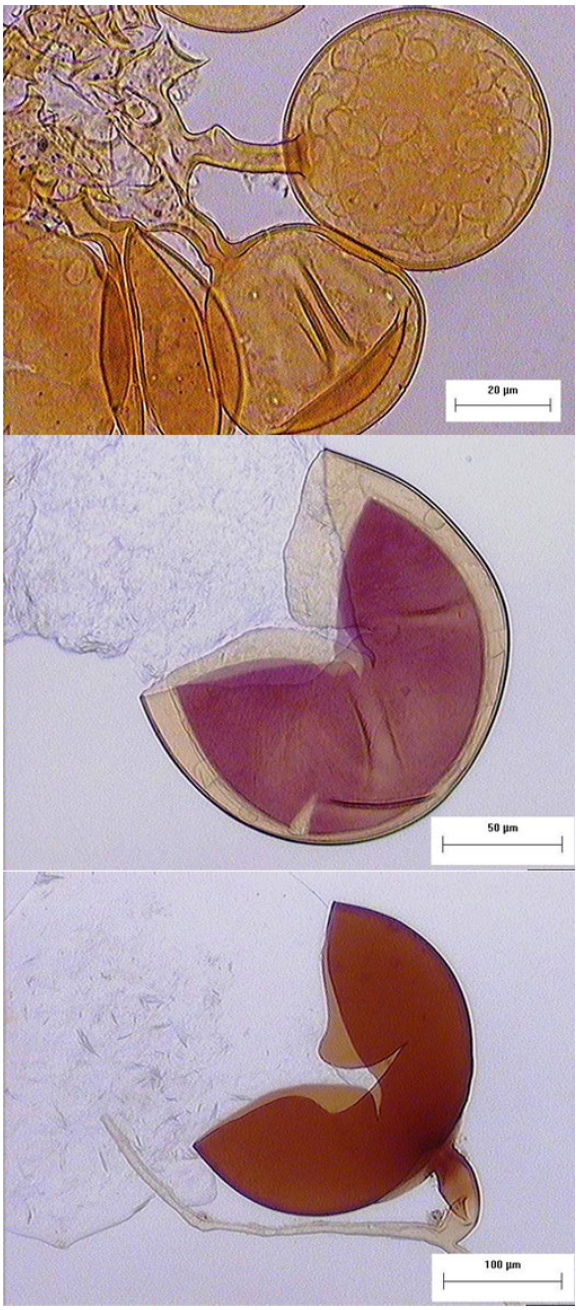

Fig. 1 *Glomus sp*. (top, bar = 20 µm), *Acaulospora sp*. (middle, bar = 50 µm) and *Gigaspora sp*. (bottom, bar = 100 µm) found in both study sites.

DISCUSSION

The number of spores recorded in this study was found to be low but in concordance with those reported for other tropical moist forests[18,19]. Values obtained were found to be within those recorded by Muthukumar et al[20] who recorded values of 1.36–19.32 per 10 g soil. Zhao et al[21] reported the number to be 5.50–19.08 per 10 g soil in a primary forest in Xishuangbanna, China. Such low spore density was believed to be influenced by death and parasitism factors which frequently affect mycorrhizal spores available in the field[22]. The fact that only healthy and perfect spores were included in the spore count assessment in this study could also provide some explanation for the low spore count.

Table 1 Soil pH, nutrient contents, total organic carbon, and organic matter of rhizosphere soils at the two forest sites.

Location	Plant species	pH	N (%)	P (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	TOC (%)	OM (%)
NP	*O. sumatrana*	5.6[b]	0.47[a]	0.46[a]	195[a]	2304[a]	260[a]	1.9[a]	3.8[a]
			(0.02)	(0.03)	(16.2)	(230.8)	(31.3)		
FRS	*O. sumatrana*	6.0[a]	0.27[b]	0.25[b]	126[b]	1222[b]	205[a]	1.5[b]	3.1[b]
			(0.02)	(0.10)	(22.2)	(256.4)	(69.0)		
FRS	*A. chinensis*	5.4[b]	0.27[b]	0.16[b]	105[b]	1137[b]	216[a]	1.4[c]	2.9[c]
			(0.02)	(0.03)	(3.08)	(37.4)	(30.1)		

Means within a column with different letters indicate significant differences ($P<0.05$) between treatments according to the Duncan Multiple Range Test. Values in parentheses indicate standard error.

Table 2 Mean spore count (MSC) and MPN estimates for rhizosphere soils at the two forest sites.

Location	Plant	MSC per 50 g dry soil (range)	MPN per g of dry soil (95% conf lims)
NP	OS	98 (90–107) [b]	16.0 (6.2–43.0)
FRS	OS	142 (104–179) [a]	16.0 (5.9–41.0)
FRS	AC	45 (35–60) [c]	6.5 (2.0–21.0)

OS = *O. sumatrana*; AC = *A. chinensis*
Means within a column with different letters indicate significant differences ($P<0.05$) between treatments according to the Duncan Multiple Range Test.

Table 3 Number (N) of mycorrhiza species and percentage of spores in rhizosphere soils at the two forest sites.

Location	Plant	Mycorrhiza	N	% of spores
NP	OS	*Glomus*	3	63.0
		Acaulospora	1	9.6
		Gigaspora	2	27.4
FRS	OS	*Glomus*	1	67.4
		Acaulospora	1	17.7
		Gigaspora	1	15.0
FRS	AC	*Glomus*	1	75.1
		Acaulospora	1	24.9

Brundrett[23] explained that sporulation of VAM fungi was influenced by the environment, host, and fungi factors. Stutz and Morton[24] supported this explanation and stressed that the relationship between sporulation and colonization of VAM fungi was different depending on the mycorrhizal species, host plant and soil nutrient content. In this study, the number of spores recorded for *A. chinensis* was very much lower than that obtained for *O. sumatrana*. Soil from the rhizosphere of *O. sumatrana* at the FRS was also found to be giving higher spore number than the NP. This could be due to the higher nutrient content detected in the rhizosphere of *O. sumatrana* at the NP (Table 1). High P content in particular has been known to suppress the growth and infection of mycorrhiza[25].

Moreover, the different soil textures found in the two sites showed that sandy clay loam soil may have promoted better spread for the mycorrhiza spores to colonize *O. sumatrana* roots at the FRS than at the NP. Mathimaran et al[26] reported that the small size of pores in clay soil may have hampered the growth of the mycorrhiza hyphae in the soil[27] either mechanically through the formation of a penetration barrier[28] or by affecting the oxygen concentration in the soil[29].

Spore numbers obtained through the spore count method were found to be lower than the MPN estimates with values 6–10 times higher. Higher inoculum potential estimates with the MPN method have been discussed by Wilson and Trinick[30] whereas Adelman and Morton[31] have reported otherwise. Abbott and Robson[32] reported that the spore count method did not provide an actual soil infectivity index, while Porter[33] believed that the MPN provided a more realistic estimate as it considers only the active mycorrhiza propagules. MPN takes into account the life cycle of the AM fungi in the soil including the non-sporulating AM fungal species that survive and propagate by means of hyphae and host root fragments[34].

Glomus was found to be dominant in all rhizosphere soils collected, followed by *Acaulospora* and *Gigaspora*. Muthukumar et al[21] reported *Glomus* (93%) to be more dominant than *Acaulospora* (53%), *Gigaspora* (23%) and *Scutellospora* (18%) in their study. Muthukumar and Udaiyan[35] and Zhao et al[21] also noted that *Glomus* and *Acaulospora* can better dominate soils in the tropics than other mycorrhiza genus. Shi et al[36] recorded similar results while studying the family Meliaceae in the Hainan Island, China. According to Ananthakrishnan et al[37], the ability of *Glomus* to dominate soil rhizosphere indicated that *Glomus* has a broad host range and is able to cover

vast environmental conditions as compared to other mycorrhiza genus.

The host factor also plays an important role in determining the mycorrhiza species available within the rhizosphere. Sieverding[38] proposed that the diversity of VAM fungi was influenced by variation in the host species within the natural ecosystem. In this study, mycorrhiza composition, spore number, and MPN values were found to be higher within the rhizosphere of *O. sumatrana* than *A. chinensis*. Eom et al[39] reported that the variation in spore density and VAM fungi colonization in relation to host plants can be linked to factors such as plant phenology, dependency on mycorrhiza, changes in the soil microenvironment, or unknown host characteristics. Hetrick and Bloom[40], in their investigation on the effect of host plant on spore colonization and production of VAM fungi spores, found that the development of *Glomus fasciculatum* was affected by the host plant whereas *G. mosseae* and *G. macrocarpum* were not. The findings thus showed that certain host plants can influence the ability of mycorrhiza to form symbiotic relationships.

Results of this study indicated that the rhizosphere of *O. sumatrana* contained higher mycorrhiza composition than *A. chinensis* with *Glomus* dominating both tree species. *Gigaspora* was however found to be absent from the rhizosphere of *A. chinensis*. The study also showed that *O. sumatrana* has a greater ability to enhance the development and sporulation of mycorrhiza than *A. chinensis*. The root system of *O. sumatrana* may have affected the rhizosphere environment of the plant, making it more suitable for the reproduction and development of mycorrhiza spores besides being influenced by the soil texture and nutrient contents. The fact that *O. sumatrana* at the FRS recorded higher spore count and composition, despite its status as a secondary forest, indicated that soil texture has a minimal effect on mycorrhiza sporulation at the site.

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