

Effect of AMF Inoculation on the Growth of *Combretocarpus rotundatus* (Miq) on a Peat Soil from Central Kalimantan (For Restoration Ex-Mega Rice Project Central Kalimantan)

Pengaruh Inokulasi Jamur Mikoriza Arbuskula (JMA) pada Pertumbuhan Perepat (*Combretocarpus rotundatus* (Miq) di Tanah Gambut Kalimantan Tengah (Untuk Pemulihan Lahan Ex-PLG Kalimantan Tengah)

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Abstrak

Diperlukan teknik silvikultur dengan memanfaatkan jamur mikoriza arbuskula (JMA) untuk memulihkan hutan rawa gambut yang terdegradasi. JMA berperan penting pada pertumbuhan dan ketahanan hidup pohon-pohonan. Penelitian ini bertujuan untuk mengetahui keberadaan dan bentuk dari JMA di hutan rawa gambut yang sudah terdegradasi pada blok C bekas pengembangan lahan gambut satu juta ha di Kelampangan Kalimantan Tengah. Kondisi lahan hanya ditumbuhi vegetasi jenis-jenis pioner. Contoh tanah diambil dibawah perakaran lima jenis tanaman pioner (*Melastoma* sp, *Combretocarpus* sp, *Acacia* sp, *Cratoxylon* sp, dan *Nephrolepsis* sp), kemudian contoh tanah dihitung jumlah spora dengan metode saringan basah. Jumlah spora terbanyak ada di bawah perakaran tanaman *Melastoma* sp, kemudian *Acacia* sp, dan *Combretocarpus* sp. Didapatkan tiga genus JMA: *Glomus*, *Gigaspora*, dan *Acaulospora*. Penelitian di rumah kaca bertujuan untuk mengetahui pengaruh inokulasi JMA jenis *Glomus* sp TD15, *Glomus* sp D32, dan *Gigaspora* sp pada semai perepat (*C. rotundatus*). Hasil penelitian menunjukkan JMA memberikan pengaruh pada parameter semai yang diukur. Jenis JMA *Glomus* sp TD15 meningkatkan pertumbuhan semai perepat yang ditunjukkan oleh parameter tinggi semai, diameter semai, dan jumlah daun per tanaman. Pada media gambut semai *C. rotundatus* sangat tergantung pada asosiasi JMA dalam meningkatkan pertumbuhan tanaman.

Kata kunci: Peat-swamp forest, ex-MRP

Abstract

For the restoration of degraded peat swamp forest, silvicultural techniques using Vesicular Arbuscular Mycorrhizal (VAM) are considered necessary. VAM fungi symbiotic relationships play an important role for the growth and survival of trees. The objective of this study is to observe the VAM fungal status of a typical peat swamp forest in Block C ex-MRP at Kelampangan in Central Kalimantan which has been cleared and disturbed. Currently the site is occupied by various pioneer plants. Soil samples from the rhizosphere of five pioneer vegetation (*Melastoma* sp, *Combretocarpus* sp, *Acacia* sp, *Cratoxylon* sp, and *Nephrolepsis* sp) were collected and spores were isolated from the soil using a wet sieving and decanting method. Greatest spore number was observed in the rhizosphere of *Melastoma* sp, followed by *Acacia* sp, and *Combretocarpus* sp. Three genera of VAM fungi were found, namely *Glomus*, *Gigaspora*, and *Acaulospora*. A field experiment was conducted to examine the effect of the arbuscular vesicular mycorrhizal fungus *Glomus* sp TD15, *Glomus* sp D32 and *Gigaspora* sp and seedling on the growth of *Combretocarpus rotundatus* (Miq). In general the VAM fungi used in this study showed beneficial effect on plant growth parameters. The colonization by *Glomus* sp TD15 produced the highest plant height, stem diameter and leaf number per plant. On the peat soil medium, *Combretocarpus rotundatus* (Miq) showed a high degree of dependence on mycorrhizal association, increasing with the age of the plants.

Key words: Peat-swamp forest, ex-MRP

Introduction

Tropical peatlands make up approximately 12% (30–45Mha) of the total global peatlands (Immirizi and Maltby, 1992), of which Indonesia supports the greatest area, 20 Mha (Radjagukguk, 2001). In the last decade large areas of the Indonesian peatlands have experienced serious damage as a result of human activities such as logging and drainage. Peatland site development is often associated with the construction of drainage canals in order to make the land usable for agriculture or, more often, for oil palm and pulp wood plantations. Canals and ditches are not only built to control and lower the water level but also to facilitate access to the peat swamp forest and for transportation of timber logs (Jaenicke and Siegert, 2008). After forest clearance, peat becomes more susceptible to fires and flooding, resulting in a downward cycle of retro-succession, with floral diversity becoming dominated by shrubs, ferns and sedge (Page *et al.*, 2008). When undisturbed, peatlands play an important role in the global carbon (C) cycle, storing about 450 Gt C, which the amount is about 30% of the global soil carbon pool (Gorham, 1991), and peatlands act as carbon sinks (Page *et al.*, 2002), support extremely high levels of floral and faunal biodiversity (Cheyne *et al.*, 2007), maintain stable hydrological and nutrient cycling systems (Wösten *et al.*, 2006) and provide means of livelihood for local communities (Smith, 2002).

The Indonesian Government has a national program to accelerate rehabilitation of degraded peat swamp forest. Peat soil has an extremely low pH and revegetation of the peat soil is difficult because of the high concentration of toxic elements, such as aluminum, and poor nutrient availability. However, it is not easy to rehabilitate this ecosystem immediately, because it is necessary to select and produce high quality tree seedling species for rehabilitation. In order to implement successful rehabilitation programs, transferable methods need to be developed. This paper considers the use of mycorrhizae inoculation for seedling transplants as one of these potential methods.

Mycorrhizae are fungi that form symbiotic relationships with plant roots. The two most common forms of mycorrhizae are

ectomycorrhizae and arbuscular mycorrhizae fungi (AMF). In exchange for sugars produced by the plant, the mycorrhizae provide additional phosphates, nitrates, and other micro and macronutrients, can increase water supply through times of drought, and reduce susceptibility to soil-borne pathogens (Dell, 2002). Mycorrhizae play a crucial role in ecosystem processes, including plant and soil community composition and ecosystem resource capture (Rillig, 2004).

Studies in the tropics, of the relations between mycorrhizae, their plant hosts and their ecosystems have been under studied in relation to the temperate zone, though in recent years this has begun to be remedied (for example, Kiers *et al.*, 2000; Lovelock and Ewel, 2005; Tawaraya *et al.*, 2003). These studies have shown mycorrhizae are just as important in tropical as in temperate biomes. Tawaraya *et al.*, (2003) established that in TPSF 17 of 23 Dipterocarp species hosted both AMF and ectomycorrhizae. Equally, once the correct mycorrhizal species was established for a given host species, the symbiosis was shown to increase plant biomass, height, leaf production and nutrient assimilation, under nursery conditions (Turjaman *et al.*, 2005; Turjaman *et al.*, 2006).

Arbuscular Mycorrhizal Fungi (AMF) are symbiotic associations, formed between plants and soil fungi that has essential role in plant growth, plant protection, and soil quality that benefit both partners. The phyto-biont correspond to approximately 80% of plant species and the fungi are classified in the phylum Glomeromycota, including nine genera; *Glomus*, *Paraglomus*, *Acaulospora*, *Entrophospora*, *Gigaspora*, *Scutellospora*, and *Archaeospora* (Dalpe, 2004).

The symbiotic relationship between AMF and the roots of higher plants contributes significantly to plant nutrition and growth (Auge, 2001; Amaya-Carpio *et al.*, 2009). These positive responses in productivity to AMF colonization have mainly been attributed to the enhanced uptake by AMF of relatively immobile soil ions such as phosphorus, potassium, calcium, magnesium, sulfur, iron, zinc, copper, and manganese (Marschner *et al.*, 1994; Liu *et al.*, 2007), but also involve the enhanced uptake and transport of far more mobile nitrogen ions,

particularly under drought conditions (Liu *et al.*, 2007). Enhanced plant growth might also result from soil-borne pathogen (e.g. nematodes, pathogenic fungi, bacteria) protection (Cardoso and Kuyper, 2006; St-Arnaud and Vujanovic, 2007), improved soil structural development and aggregate stabilization (Miller and Jastrow, 2000; Picone, 2003; Rillig, 2004; Wright, 2005).

AMF symbiosis, a natural association between the roots of higher plants and arbuscular mycorrhizal, are important in trees, because AMF are believed to improve host plants growth, water relations and acquisition of nutrients especially P from soil (Bucher, 2007). Mycorrhizas affect the maintenance of vegetation in various ecosystems, and may play an important role in tropical peat swamp forest. AMF may be formed even in trees, which grow in the peat swamp forest.

Combretocarpus rotundatus Miq is classified into fast-growing tree species and has tolerance to strong light, drought and high soil temperature condition in their regenerate and survivorship (Saito *et al.*, 2002). This species is also an important timber tree in tropical peat swamp forest in Indonesia. *C. rotundatus* is

distributed over scattered locations of Kalimantan and Sumatra islands, and attains height of up to 30 m and diameter of up to 60 cm. This species is common and often gregarious in tropical peat swamp forest.

Study Site

Peatland of Central Kalimantan is distributed in the lowland area, about 5 – 35 m above sea level, and stretches approximately 200 km inland (Fig. 1). It holds nearly 27% of the total peatland on Kalimantan Island (Shimada, 2001). Some large rivers, such as the Kapuas, Barito, and Katingan, flow from the mountains of northern side of the peatlands through the peatlands to the Java Sea. Peat thickness varies among peatland types, from approximately 0.5 to 9.7 m (Shimada *et al.*, 2001). Tropical peat swamp forest occupies most of the Central Kalimantan area. Part of the forest has been either cleared for cultivation and logging or destroyed by peatland fires. The study site is located in the Kelampangan forest. A large part of this forest has been degraded both by cultivation and human settlement and by the enormous forest and peat fires in 1997.

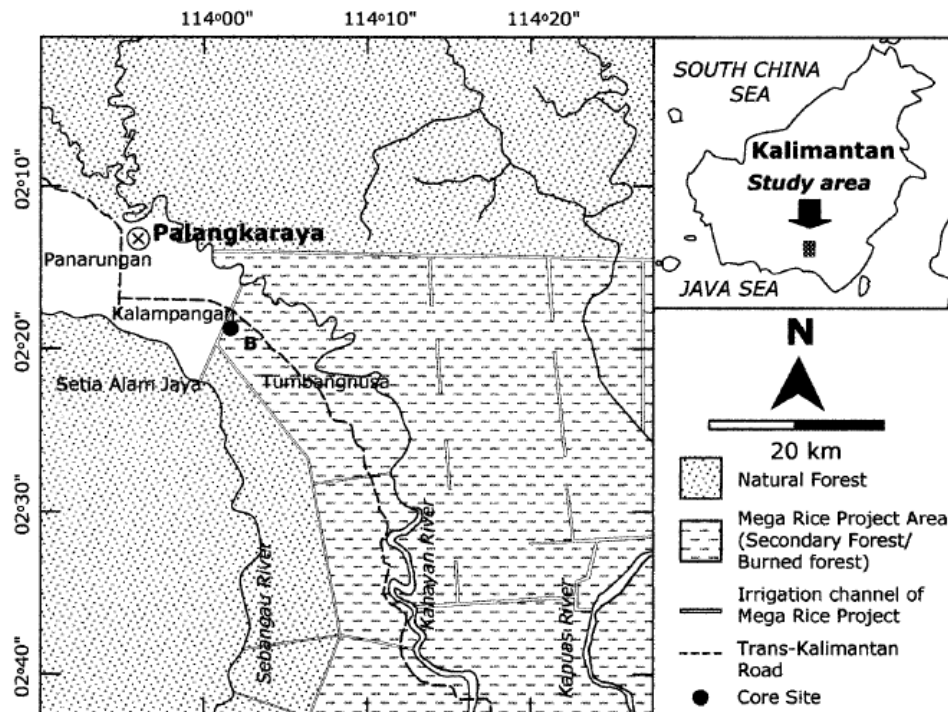


Figure 1. Map of the study site (after Yulianto *et al.*, 2004).

Materials and Methods

Isolates of VAM fungi were collected from rhizosphere of pioneer plants in Ex Block C Mega Rice Project (MRP) in Central Kalimantan (Fig. 1). Five different pioneer plants, namely *Combretocarpus* sp, *Acacia* sp, *Melastoma* sp, *Cratogeomys* sp, and *Nephrolepis* sp were compared. Isolation and identification of AM fungal spore were conducted in the Laboratory of Microbiology, Faculty of Agriculture, Gadjah Mada University in Yogyakarta. The identification followed Manual for Identification of VAM fungi and examination was conducted under a dissecting microscope (Brundrett *et al.*, 1996).

Seeds of *C. rotundatus* were the surface sterilized in 70% alcohol for 5 min, subsequently rinsed with sterilized water and germinated on sterilized peat soils. Four week old uniform seedlings were transplanted into plastic pots (30 x 30 cm) containing 2.0 kg autoclaved (0.11 MPa, 121°C, 2 h) peat soil with a pH of 3.3, and 5.91 mg kg⁻¹ available phosphorus. The experimental pots were placed in a plastic greenhouse under natural light conditions from September, 2007 to January, 2008, which had no controlling temperature equipment. The average day/night temperature was 29/21°C and the relative humidity was 70-90%.

The AMF used in this study were *Glomus* sp TD15, *Glomus* sp D32, and *Gigaspora* sp obtained from Block C ex-PLG Central Kalimantan. The inoculation with AMF was added as soil from pure pot cultures containing spores and mycorrhizal root fragments, 30 g/pot of inoculum was placed directly below the seedlings. Non-AMF treatments (control seedlings) received the same weight of autoclaved growth media.

The experimental treatments were made up of three AMF inoculations (*Glomus* sp TD15, *Glomus* sp D32, *Gigaspora* sp, and non-AMF) under greenhouse nursery condition and were arranged in a completely randomized design. Each treatment had six replicates and, hence, there were a total of 24 pots with one seedling per pot.

Five months after planting, plant height, stem diameter and leaf number per plant were recorded and the AMF and non-AMF seedlings were harvested. All of the seedlings were separated into roots and shoots, dried in hot-air oven at 75°C for 2 d, and dry weights of shoots and roots were recorded. Part of fresh roots was carefully washed, cut into 1 cm long root segments and fixed by FAA at least 24 h. The root samples were cleared with 10% KOH solution, stained with 0.05% trypan blue in lactophenol (Phillips and Hayman, 1970), and examined microscopically for root colonization. The AMF infected percentage was calculated by the following formula: AMF colonization (%) = 100 x root length/root length observed (Smith and Read, 2008).

The experimental data were subjected to analysis of variance (ANOVA) with Statistical Analysis System (SAS) 8.1 software, and Fisher's Protected Least Significant Difference (LSD) ($\rho=0.05$) was used to compare treatment means.

Results and Discussion

The results showed that AM fungi was abundant in rhizosphere of pioneer plants and identification to the spore revealed that there were three genera of AM fungi, namely *Glomus*, *Gigaspora*, and *Acaulospora* (Table 1 and Fig. 2, 3).

The greatest spore number was obtained in the rhizosphere of *Melastoma* sp (263 spores), followed by rhizosphere of *Acacia* (108 spores), and rhizosphere of *Combretocarpus* sp (68 spores). AM fungi are ubiquitously associated with the large majority of plant families in different ecosystems across the world ranging from the tropics to subtropics (Gai *et al.*, 2006). Opik *et al.*, (2006) suggested that the location effect on AM fungal communities might be significant through a meta-analysis of 26 publications that reported AM community compositions in different locations of the world. They also pointed out, however, that there were too few studies on fungal communities of the same plant species at different site.

Table 1. Type and spore number of VAM fungi in the rhizosphere of pioneer plants.

No.	Plant	Spore number per 100 g soil	AMF species
1.	<i>Combretocarpus</i> sp	68	<i>Glomus</i> sp; <i>Acaulospora</i> sp; <i>Gigaspora</i> sp
2.	<i>Acacia</i> sp	108	<i>Glomus</i> sp; <i>Gigaspora</i> sp
3.	<i>Melastoma</i> sp	263	<i>Glomus</i> sp; <i>Acaulospora</i> sp; <i>Gigaspora</i> sp
4.	<i>Cratoxylon</i> sp	8	<i>Glomus</i> sp; <i>Acaulospora</i> sp
5.	<i>Nephrolepsis</i> sp	7	<i>Glomus</i> sp

Table 2. Plant growth and AMF colonization of *C. rotundatus* inoculated with three VAM types 5 months after transplanting under nursery conditions.

Treatment	Plant growth				AM Colonization (%)
	Shoot Height (cm)	Stem Diameter (mm)	Leaf Number per (plant)	Dry Weight (g/plant)	
Control	10.26 c*	2.16 c	10 c	4.45 c	9 c
<i>Glomus</i> sp TD15	41.96 a	5.16 a	36 a	39.51 a	74 a
<i>Glomus</i> sp D32	26.30 b	4.33 a b	28 a b	35.23 b	68 b
<i>Gigaspora</i> sp	16.43 b c	4.16 a b	27 a b	11.34 c	63 b

• Values followed the same letter are not significantly different ($p < 0.05$).

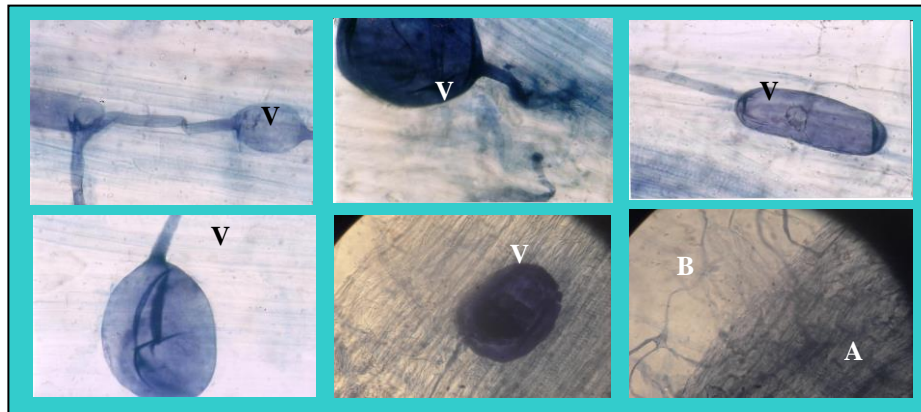


Figure 2. Vesicular (V), intraradical hyphae (A), and extraradical hyphae in root (B).

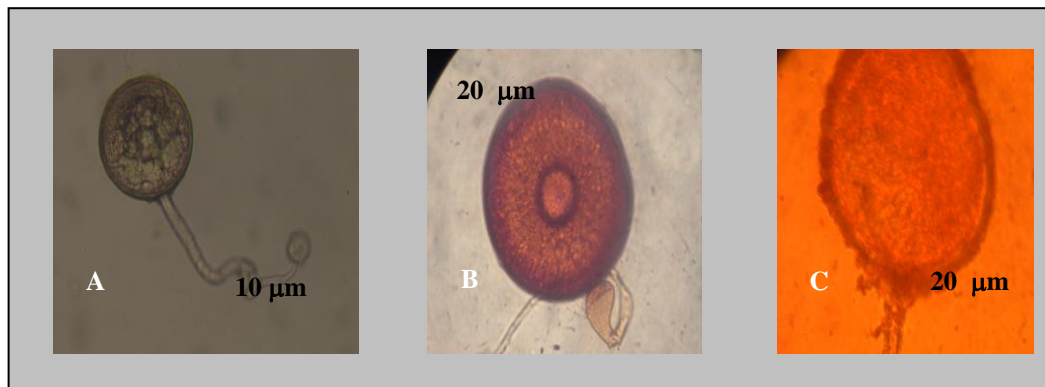


Figure 3. Spores of *Glomus* sp (A), Color: a continuum from white to yellow-brown, Shape: globose, Spore wall: three layers. *Gigaspora* sp (B), Color: yellow-brown, Shape: Globose to subglobose, Spore wall: consisting of three layers. and *Acaulospora* sp (C), Color: orange-brown, Shape: globose, Spore wall: Three layers.

In the nursery conditions, *Glomus* sp TD15, *Glomus* sp D32, and *Gigaspora* sp, formed AMF in *Combretocarpus rotundatus* Miq seedlings (Table 2). AMF colonization was higher than 60% in all inoculated seedlings. There was a significant difference between three AMF species and control seedlings. AMF colonization was higher in seedlings colonized by *Glomus* sp TD15 than with the other AM fungi species. AM fungi colonization of *C. rotundatus* using spores of *Glomus* sp TD15, *Glomus* sp D32, and *Gigaspora* sp increased plant height, stem diameter (Fig. 4, and 5).

Even though the AM fungi of trees in peat swamp forest has been reported (Tawarayama *et al.*, 2003), the role of AM fungi in species originating from peat swamp forest needs to be clarified. This result demonstrate for the first time that AM fungi have positive effects on *Combretocarpus rotundatus* Miq after five months under nursery conditions. Colonization by three AMF species was identified as the most appropriate fungi for improving early growth of *Combretocarpus rotundatus* Miq after five months under greenhouse nursery conditions. Therefore, *Glomus* sp TD 15 is to be AMF candidate for production of *C. rotundatus* under nursery conditions. Similar result has also been reported by Morte *et al.*, (2008) inoculation with *Glomus mosseae* significantly increased the growth of *Phoenix canariensis*. The positive effect of *Glomus* sp colonization on Canary palm has also been observed under different growth condition (Morte and Honrubia, 2002) and in other palms like *Bactris gasipaes*. Smith and Read, (2008) concluded that, the arbuscular mycorrhizal fungi supply nutrients to their host plant, especially phosphorus. The fungi absorb phosphate from the soil through their extraradical hyphae and incorporate it into the cytosolic pool, while the excess of phosphorus (P) is transferred to the vacuoles and translocated to the intraradical hyphae (Saito, 2000). The forms of P involved in long distance transport along the hyphae are probably inorganic orthophosphate (Pi), poly-phosphate and organic phosphate such as Phosphate-ester (Ezawa *et al.*, 2002). Ecological categories of AMF may be more useful for summarizing fungal performance in the nursery conditions (Brundrett *et al.*, 2005).

The AM fungi expand their filaments in soil and plant roots. This filamentous network promotes bi-directional nutrient movement where soil nutrients and water move to the plant and plant photosynthates flow to the fungal network. The structures produced by fungi are intraradical and extraradical spores, intraradical hyphae, and extraradical hyphae. Intraradical AM fungal mycelium form a network around and inside cortical cells of plant roots, extraradical AMF mycelium can spread throughout the soil surrounding the root system and increase the ability to explore soil areas, accessing water and nutrients for plant roots. Benefits to plants are improved water and nutrient uptake, enhanced P transport, and drought and disease resistance (Dalpe, 2004).

Survival rates of seedlings are important for the first establishment of plant growth of *Combretocarpus rotundatus*. The ability of AM fungi to increase crop growth and yield, attributed to better nutrition in phosphorus-poor and phosphorus-fixing soils, have been reported for hundreds of plants species (Auge and Moore, 2005). Mycorrhizal symbiosis can also improve host responses to other environmental limitations, such as drought (Brundrett *et al.*, 1996; Alguacil *et al.*, 2006; Aliasgharzag *et al.*, 2006; Aroca *et al.*, 2007), salinity (Auge, 2004; Gupta and Rautaray, 2005; Cho *et al.*, 2006).

In conclusion, colonization by *Glomus* sp TD15 consistently increased the growth of five months old *Combretocarpus rotundatus* seedlings. *Glomus* sp TD15 could, therefore, be used to inoculate *Combretocarpus rotundatus* Miq in nurseries because this fungi was well adapted to the environmental conditions and had a superior effect on plant growth as compared with the other AMF types. Indigenous AM fungi are suggested to be potentially good inoculants for native peat swamp tree species under greenhouse nursery conditions and would be beneficial for the restoration of degraded peat swamp forest.

This study may also contribute to the future application of AM fungi to restoration of peatlands. Our finding that “generalists” can colonize host plants over a low of pH values is novel, and it is expected that these fungi may have a great potential as “universal fungi” for environmental restoration if they can be isolated successfully.

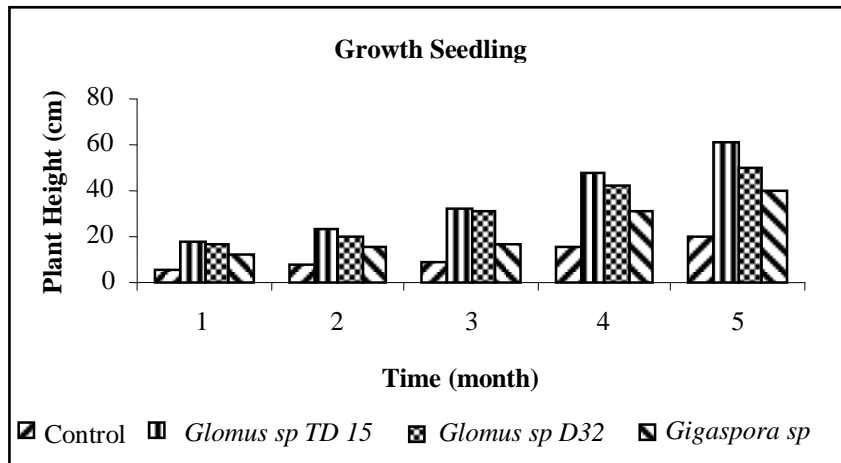


Figure 4. Height increase with time of *Combretocarpus rotundatus* plants inoculated with three AMF types.

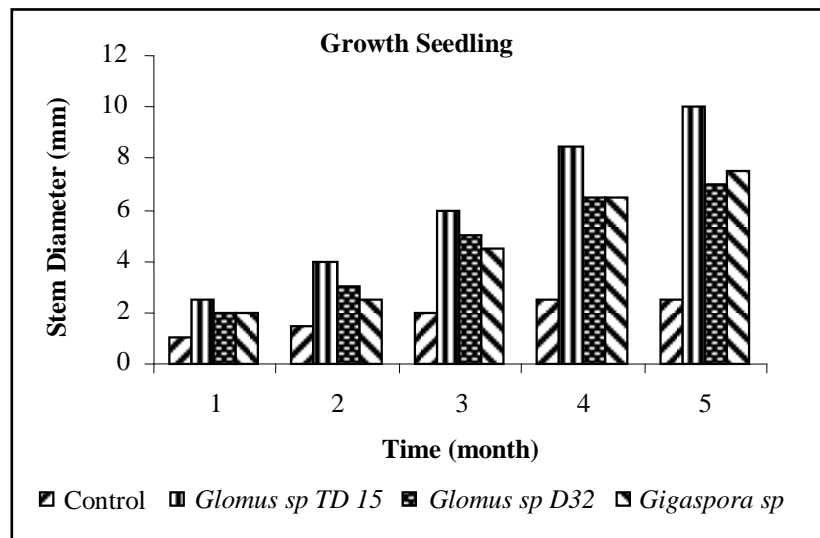


Figure 5. Stem diameter increase with time of *Combretocarpus rotundatus* plants inoculated with three AMF types.

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