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# ARBUSCULAR MYCORRHIZAL FUNGI INCREASED ROOT-MYCORRHIZAL ASSOCIATION AND ENHANCED SEEDLING GROWTH OF ABACA, PAPAYA, AND SUGARCANE

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## ABSTRACT

MYKOVAM® is a soil-based biological fertilizer containing spores of more than 10 species of beneficial Arbuscular Mycorrhizal (AM) fungi. Studies reported that this AM inoculant helps in the absorption of water and nutrients particularly phosphorus, prevent root infection by pathogens, and can increase plant tolerance to drought and heavy metals. This study determined if MYKOVAM® inoculation at seedling stage can establish or further enhance root mycorrhizal association in papaya, abaca, and sugarcane, and whether it results to increased root and shoot growth. Pot experiments were conducted for each crop from October 2016 to June 2017. Success of root colonization by AM was assessed one month after inoculation and biomass production were monitored periodically. Significant increase in AM fungi colonization of roots with MYKOVAM® inoculation was observed in all test crops. Plants inoculated with AM fungi also had better root and shoot growth resulting to higher plant biomass. There is advantage in integrating AM fungi inoculation at plantlet/seedling stage in nursery management of abaca and papaya.  However, for sugarcane, inoculation of large batches of seed pieces /setts technique for sugarcane seed pieces still needs to be developed. Field studies is recommended to study the persistence of the enhanced AM fungi colonization in field conditions and its effects on subsequent growth and productivity of these crops.

**Key words:** MYKOVAM®, AM fungi, root colonization

## INTRODUCTION

MYKOVAM® is a biofertilizer developed and commercialized at the National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños. It contains spores and chopped roots colonized by AM fungi belonging to the genera *Glomus, Gigaspora, Entrophospora and Acaulospora.* Bahia grass serves as the host plant for the mass production of this mycorrhizal inoculant for 5 to 6 months and was grown in soil and sand mixture. It is a soil-based biological fertilizer with beneficial active ingredient called Arbuscular Mycorrhizal (AM) fungi which is effective in improving plant growth, increasing yield and survival of plants and trees, and increasing plant tolerance against root pathogens (Aggangan et al. 2013). MYKOVAM® also promotes mutualistic symbiosis with plant roots. It was reported to not only improve plant growth but improve soil health as well. AM Fungi enhances the root system through penetration of cortical cells (Gholamhoseini et al., 2013). Symbiotic relationship between the fungi and plant roots was observed through spore germination and hyphae emergence entering the epidermis of the roots. Mycorrhizal association is known to enhance oak establishment and increase tolerance to biotic and abiotic stresses (Querejeta et al. 2003). AM fungi promotes mutualistic symbiosis with plant roots as it feed on the root exudates (Tahat and Sijam 2012); mycorrhiza infected roots will then become more efficient in absorbing water and nutrients, thus increasing plant tolerance to drought stress (Gholamhoseini et al. 2013). Moreover, it improves water uptake as the fungi help to extend the root system and modify the root architecture. Mycorrhizae also induce enzymatic activities that could mediate responses to counteract stresses and improve the extracting capability of roots to extract nutrients bound in minerals and organic matter (Gamalero et al. 2009).

Papaya (*Carica papaya* L.) is an important fruit crop in the Philippines with great economic potential. Papaya is used extensively for food and even in pharmaceutical and cosmetic industries (Boshra and Tajul 2013). Khade et al. (2010) quantified the AM colonization of papaya ranging from 26% to 77%. There were reports that mycorrhizal fungi improved the growth and development of papaya during its vegetative and reproductive stage (Trindade et al. 2006; Khade and Rodrigues 2009; Khade et al. 2010).

Sugarcane production in the Philippines comprises about 413,264 ha of the total agricultural land in the country in the year 2015-2016 (SRA, 2016). Sugarcane was reported to be responsive to mycorrhizal fungi. It was proven to have improved nitrogen use efficiency of the crop and reduced pathogenic diseases (Claassens et al. 2017). However, literature on the in-depth interaction of sugarcane and mycorrhizal fungi is scarce and needs to be explored.

Abaca, also known as “Manila Hemp” is believed to have originated in the Philippines. It is grown for its pseudostem and is considered as the most economically important fiber crop. The country trades five types of Philippine abaca products in the world such as fiber, pulp, cordage, yarns, and fabrics, and fiber craft (Lantican 2008). Abaca is widely used as a material for making tea bags, casing of meat sausages, surgical masks, and cigarette paper (Simeon 2016). Researches on the influence of mycorrhizal association on the growth and productivity of abaca are yet to be studied and published.

This study determined the enhancement of root-AM fungi association and its effect on root and shoot growth using MYKOVAM® inoculation at plantlet/seedling stage of papaya, sugarcane, and abaca.

## MATERIALS AND METHODS

**Experimental design.** Pot experiments of abaca, sugarcane, and papaya were laid out in Complete Randomize Design (CRD) with 3 replications. The treatments were Control (T1) and MYKOVAM® inoculated (T2). The study was conducted from October 2016 to June 2017 at the screen house of Fruit Crops Nursery, Institute of Crop Science, College of Agriculture and Food Science, University of the Philippines Los Baños (UPLB), College, Laguna.

**Test plants.** The varieties used in the experiment were as follows: abaca hybrid 2, Sinta papaya, and sugarcane Phil 7270 sourced from the Institute of Plant Breeding, UPLB.

**Preparation and AM fungi inoculation.** Inoculation of the seedlings/plantlets was done during potting. Following the general recommendation for applying MYKOVAM®, five grams were placed in a 5 cm deep hole made at the center of the plastic bag (10x10x16) filled with 10 kg of 1:2 coir dust and garden soil. The tested plants were approximately one month-old seedling/plantlet when planted into poly bag where MYKOVAM® was placed in band beneath the roots. The pot was watered to field capacity after potting the seedlings.

**Mycorrhizal assessment.** Root colonization analysis was done at the BIOTECH, UPLB. Freshly harvested root samples were collected 31 days after inoculation with AM fungi, cleaned under running water and blotted dry in between paper towels. Two g fresh fine roots (diameter <0.5 mm) were taken, placed in test tubes filled with 50% ethyl alcohol. The roots were then cleared with 10% (w/v) KOH solution in a water bath set at 90ºC and stained with 0.05% trypan blue (Phillips and Hayman 1970; Brundrett et al. 1996). Stained roots were placed in Petri dish and were viewed under a stereomicroscope. AM fungi colonized or uncolonized roots were counted using the grid-line intersect method (Giovannetti and Mosse 1989). The presence of attached hyphae, vesicles or arbuscules inside the roots were scored as mycorrhiza colonized roots. Spores in the rhizosphere soil sample were also isolated using the wet sieving and decanting technique (Brundrett et al. 1996). Spores were counted in Petri plates with grid lines under a stereomicroscope.

**Assessment of root growth.** Root samples were collected 31 days after MYKOVAM® inoculation and were brought to the Institute of Plant Breeding (IPB) for the root scan analysis. This was done to determine the root volume, number of root tips, root length and average root diameter. Root samples were washed thoroughly to remove the adhering soil particles and scanning automatic root measurement was done using WinRhizo™ Pro (Regent Instrument Inc.).

**Biomass production and partitioning.** Plant samples were collected 31 days after MYKOVAM® inoculation and were partitioned into roots, leaves, stem/pseudostem, and corm (abaca). The roots were washed thoroughly to remove the attached soil particles and were air-dried together with the leaves, stem/pseudostem and corm for at least 1 week prior to oven-drying at 60-70°C for 2-3 d. Root-shoot ratio was also calculated from the weights obtained from the shoot and root.

**Statistical analysis**. All data collected were analyzed using one-way analysis of variance (ANOVA) in CRD and Fischer's Least Significant Difference (LSD) for comparison of means if the confidence level is 5%. Statistical analysis was done using the Statistical Tool for Agricultural Research (STAR) program version 2.0.1.

## RESULTS AND DISCUSSION

**Root colonization.**

AM fungi inoculation increased root mycorrhizal association across all test crops. MYKOVAM® inoculated plants significantly increased AM fungi root colonization ranging from 70% to 74% (Table 1). The uninoculated plants had positive but low percent mycorrhizal root colonization ranging from 7% to 19%. The growing medium used in this study was not sterilized thus, native mycorrhizal fungi were definitely present. Only the spores were counted as infective propagules in the roots. Other finer hyphal propagules, which may additionally contribute to the extension of the root system, were not counted. This could be included in subsequent studies using the most probable number (MPN) method, which enumerates even fine endophyte propagules (Porter 1979).

This study validated and quantified root-AM fungi association for the test crops in unsterilized potting medium with and without AM inoculation. The enhanced AM fungi-root association is important particularly for papaya which has low AM infection in the field.

**Table 1.** Percent (%) mycorrhizal root colonization of four perennial tropical crops

| Treatment | Abaca | Papaya | Sugarcane |
|---|---|---|---|
| Control | 7 | 16 | 19 |
| MYKOVAM®-Treated | 74 | 72 | 70 |
| Pr (>\|t\|) | 0.0037* | 0.0147* | 0.0005* |
| Percent Increase | 951% | 350% | 272% |

*Significant at 0.05% level

**Assessment of root growth**

The results of the root scan analysis verified that generally, pre-plant inoculation of AM fungi significantly increased the root growth of the plants (Table 2), having higher root volume, longer roots, and higher root diameter compared with the untreated plants. For the number of root tips, abaca responded differently, where AM fungi treated roots had lower number of root tips. For other parameters measured (root length, root diameter, and root volume), AM fungi treated abaca had significantly higher values compared with AM fungi untreated roots, which was similar to other crops. This agreed with the results of the experiment conducted by Calvo and co-workers in 2014 that AM fungi increased the root biomass, surface area, and root hairs in corn. Having longer roots is advantageous to plants as it determines the capacity of the roots to explore soil volume (Turner and Rosales 2005).

**Table 2**. Root characteristics of MYKOVAM®-treated and untreated abaca, sugarcane, and papaya.

| Crop | Treatment | Number of Tips | Root Volume ($cm^3$) | Root Length (cm) | Root Diameter (mm) |
|---|---|---|---|---|---|
| Abaca | Control | 1509 *a* | 9.23 *b* | 993.03 *b* | 1.11 *b* |
| | MYKOVAM®-treated | 1395 b | 25.22 *a* | 1630.77 *a* | 1.55 *a* |
| Sugarcane | Control | 3018 *b* | 3.36 *b* | 1311.63 *b* | 0.53 *b* |
| | MYKOVAM®-treated | 3857 *a* | 3.81 *a* | 1713.31 *a* | 0.57 *a* |
| Papaya | Control | 540 *b* | 0.76 *b* | 456.65 *b* | 0.60 *b* |
| | MYKOVAM®-treated | 712 *a* | 1.66 *a* | 2668.71 *a* | 0.68 *a* |

Mean values with the same letter within a column are not significantly different at $p<0.5$.

**Assessment of vegetative growth and biomass partitioning**

Dry weights of abaca were higher in plants treated with MYKOVAM® than control plants (Fig. 1). AM fungi treated abaca plantlets produced higher biomass on its pseudostem compared with the control plants. This result is important, specifically for abaca growers and farmers, since abaca is grown for its fiber extracted from the pseudostem. Higher percent biomass and dry weight on this plant part could correspond to higher yields.

For papaya, MYKOVAM® treated plantlets also had higher biomass than the untreated plants. Notably, application of MYKOVAM® more than doubled the root biomass. Roots of papaya are known to be sensitive to water stress, particularly to waterlogging. Enhanced rooting due to AM fungi inoculation maybe important in stress recovery of the crop. Improved biomass also enables good establishment in the nursery or in the field (Orwa et al. 2009). AM fungi association in papaya had been reported to positively influence the root phosphatase activities (acid and alkaline) under P-deficient condition thereby enhancing the P-adsorption of the plant (Khade et al. 2010).

For sugarcane, MYKOVAM® treated sugarcane plantlets had higher biomass as compared with the untreated plantlets; and while the plants were comprised only of roots and leaves since its stem was not yet developed, root development was significantly enhanced. Jamal-Ally (2013) reported that AM fungi increases the stalk length and plant height of sugarcane. This increase is substantial for the farmers and growers since sugarcane is grown for its stalk for sugar production and/or bioethanol fuels.

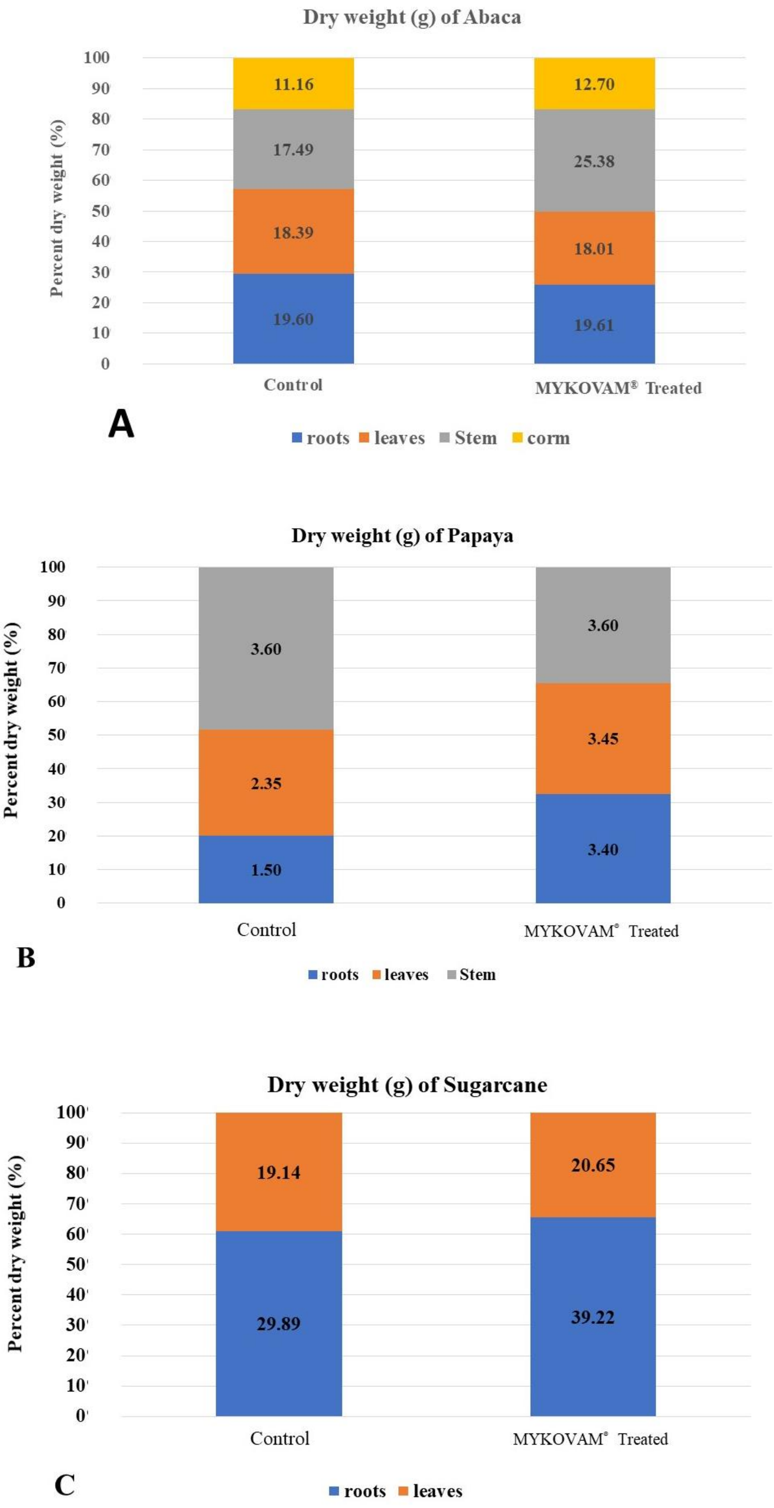


**Fig. 1**. Dry weight of abaca (A), papaya (B) and sugarcane (C) with and without MYKOVAM®

Papaya and sugarcane produced higher root-shoot ratio when inoculated with AM fungi as compared with the uninoculated plants (Table 3). For abaca, despite of the lower root-shoot ratio of AM fungi treated plants as compared with the control, it exhibited a higher shoot biomass which is important for the growers since abaca is grown for its pseudostem.

**Table 3.** Root and shoot ratio of abaca, papaya, and sugarcane

| | **Abaca** | | **Papaya** | | **Sugarcane** | |
|---|---|---|---|---|---|---|
| **Gram plant$^{-1}$** | **Control** | **With *Mykovam*®** | **Control** | **With *Mykovam*®** | **Control** | **With *Mykovam*®** |
| Root | 19.60 | 19.61 | 1.50 | 3.40 | 29.89 | 39.22 |
| Shoot | 47.07 | 56.09 | 5.95 | 7.05 | 19.14 | 20.65 |
| Total biomass | 66.67 | 75.70 | 7.45 | 10.45 | 49.03 | 59.87 |
| **R/S Ratio** | 0.42 | 0.35 | 0.25 | 0.48 | 1.56 | 1.90 |

## CONCLUSION AND RECOMMENDATION

Inoculation with MYKOVAM® at early seedling stage significantly increased root mycorrhizal association in abaca, sugarcane and papaya. This enhanced AM fungal association increased root and shoot growth of the plants. Only the spores were counted in the colonization study, thus the actual number of infective propagules may have even been underestimated.

Pre-field planting root inoculation of MYKOVAM® is recommended to be integrated in plant nursery management practices for papaya and abaca. More cost-effective inoculation technique for large batches of sugarcane setts needs to be developed (*e.g.* coating and soaking). Different varieties or genotypes may respond differently to AM inoculation and to different AM species, hence further studies could include use of different crop variety/genotype and different commercial AM fungi combinations/formulations.

For abaca, this is the first study to demonstrate that the roots of Hybrid 2 has positive but very low association to native soil AM, but it is also highly responsive to AM fungi inoculation. Further studies are being conducted to determine whether enhanced AM fungi association will persist in the field and can confer resistance to biotic and abiotic stresses.

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