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Research Article

Association of *Glomus Intraradices in Sorghum Bicolor*

Abstract

Arbuscular mycorrhizae (AM) are beneficial symbionts for plant growth. They are associated with higher plants by a symbiotic association, and benefit plants in uptake of phosphorus nutrients, production of growth hormones, increase of proteins, lipids and sugars levels, helps in heavy metal binding, salinity tolerance, disease resistance, and even in the uptake of radionuclides. Mycorrhizal genes are also applicable in improvement of crop plants, due to their delivery into plants, by a process called, particle bombardment. The influence of *Glomus intraradices* inoculation on growth performance of *Sorghum bicolour* in three different potting material such as Vermicompost, soil: sand and coir pith compost was studied in a screen house experiment. The results obtained indicated the dependence of *Sorghum bicolor* on mycorrhizal symbiosis. Inoculation with vesicular-arbuscular mycorrhiza in vermicompost significantly improved the growth performance of *Sorghum bicolor* by showing significant increase in shoot and root length. Vesicular arbuscular mycorrhiza inoculation has a high potential in agroforestry as a bio-fertilizer.

Introduction

Plant roots provide an ecological niche for many of the microorganisms that abound in soil. They play an important role in soil fertility, not only because of their ability to induce biochemical transformation, but also because of their importance as a source and store house for mineral nutrients. Several groups of microorganisms have their ability to enhance growth and to improve the health of the plants. Of the various microorganisms colonizing the rhizosphere, mycorrhizae, the mutualistic symbiont, play an important role in mobilizing phosphorous from the deeper layers of the soil and supplying it to the host plants. Among the symbiotic microorganisms, arbuscular mycorrhizal fungi (AMF) form mutual associations with more than 80% of the plant species, improve mineral nutrition [1] enhance resistance or tolerance to stress [2] and protection against pathogens [3]. This association is a mutually beneficial event, the plant supplies the fungus with carbon, while the fungus increase the ability of plant to uptake nutrients (mainly phosphorous) [1]. The most acceptable reason for the obligate biotrophy that, the fungi lost most of its carbon fixing capabilities or the genetic machinery that supports them during the long evolution of its symbiotic relationship with the host. The fungi became completely dependent on the host plant for fixed carbon supply. Mycorrhizal association start when soil hyphae respond to the presence of a root by growing towards it, establishing contact and growing along its surface. It initially establishes an entry point called appressorium on host root epidermis. This will act as a contact point between internal hyphae and mycelium present in the soil. The beneficial effects of vesicular arbuscular mycorrhiza (VAM) on crop growth is now well established in a variety of crop plants. The present study was undertaken to assess the frequency and level of VAM colonisation on sorghum plantlets.

Materials and Methods

Extracting AMF spores from soil

Wet-sieving and decanting [4]: Soil samples from field sites should be taken from the rhizosphere of mycorrhizal native or crop plants at a soil depth where the most root proliferation occurs, usually 0–20 cm [5]. A 100–200-g soil sample (dry weight) is transferred to a beaker. If the soil is dry at sampling, make sure it is soaked for 30–60 minutes before attempting to extract spores. Distilled or deionized water is added to obtain a 1-L suspension, is poured through a stack of sieves (750, 250, 100, 53, and 37 µm), the finest sieve being at the bottom of the stack. A stream of tap water is added to facilitate the movement of spores. The material that remains in the 100, 53 and 37-µm aperture sieves are washed away from the sieves to petri dishes of a diameter of 10 cm.

Separation into morphotypes [6]: Spores of AMF can be transferred to a petri dish for microscopic examination and separation. Fine-tipped forceps or Pasteur pipettes can be used to transfer spores into vials or micro-dishes with water for subsequent evaluation and identification. Alternatively, spores

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can be collected on a filter paper and picked up from it singly with forceps or a fine-tipped instrument such as a dissecting needle or a paint brush.

Surface sterilisation of spores of AMF [7]: A solution containing the strong oxidizing agent, chloramine T, and a surfactant (e.g., Tween 20) is widely used to sterilize AM fungal spores. Incubation for 10 s in 96% ethanol, followed by 10 min in a solution of 2% Chloramine T, 0.02% streptomycin, 0.01% gentamycin and Tween 20, and then 6 min in 6% calcium hypochlorite. To maintain spore dormancy, all steps from spore isolation to rinsing should be done on ice. If spores are not to be used immediately, they should be stored at 4°C, either in distilled water, on water agar, or on 0.1%MgSO4·7H2O solidified with 0.4% gellan gum.

Mass multiplication of AMF in pot cultures [8]: The culture of VAM fungi on plants in disinfested soil using spores are used as inocula for increasing propagule numbers.

Selection of host plant: The host plant selected for mass multiplication was *Sorghum bicolor*. The seeds of *Sorghum bicolor* are disinfested with 0.525% NaOCl for 5 to15 mins followed with five washes of water. Washing with water may also remove fungicides and other agrichemicals which may adversely affect VAM fungi.

Disinfestation of potting material: The potting material used were vermicompost, soil: sand (1:1) mixture and coir pith compost. Disinfestations is done twice by means of autoclaving at 121 degree 15psi for 20 mins. The treatment details is given below (Table 1).

Initiation of pot culture: Pot culture is initiated by placing a layer of inoculums 1 to 2 cm below the seeds. Inoculum consisted of 200 spores of *Glomus intraradices* which were healthy and uniform.

Light, moisture, and temperature: Good quality light and high irradiance are necessary for maximum inoculum production. Where natural light conditions are poor, high intensity lamps are used. Moisture content of the potting material affects VAM sporulation, with nonsaturated and nonstressed water conditions provide maximum sporulation. Excessive moisture may encourage problems with hyperparasites in the culture. The best strategy is to apply water to well- drained soil. Temperature is also important for pot cultures. Sporulation is positively correlated with temperature from 15 to near 0 degree for many VAM fungi; however, at higher temperatures sporulation may decrease.

Results and Discussion

Mycorrhizas play a significant role in plant growth. The performance of the plants in most circumstances depends upon the establishment of mycorrhizal associations that serve many purposes, apart from just facilitating the uptake of nitrogen, phosphorous and water, enhancing the tolerance / resistance to root pathogens, toxic heavy metals, pH fluctuations and even forming a protective barrier against certain edaphic factors. It is believed that the fungal mycelium which extends from the mycorrhizal roots form a three dimensional network which links the roots and the soil environment. It constitutes an efficient system for nutrient uptake and scavenging in nutrient poor conditions [9]. It also contributes to the formation of water stable aggregates necessary for good soil tilth [9]. Thus, mycorrhizal associations are multi-functional.

In the present study, forty five soil samples were collected from different places of Idukki district, Kerala. By means of wet sieving and decanting method four different AMF spores were extracted (Figure 1). Based on the colour, shape and size, the extracted AMF spores were identified as *Glomus mossae*, *Glomus fasiculatum*, *Glomus intraradices* and *Glomus microcarpum* with the help of stereo dissecting microscope and the spore count of each species was taken (Figure 2). As the spore count was more in *Glomus intraradices*, this was taken for further study.

Table 1:

Treatment No.	Treatment details
T1	Vermicompost + AMF
T2	Vermicompost alone
T3	Soil: Sand + AMF
T4	Soil: Sand alone
T5	Coir pith compost + AMF
T6	Coir pith compost alone

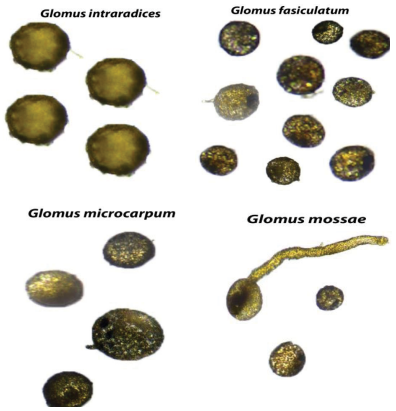

Figure 1: Microscopical Observation of AMF Spores.

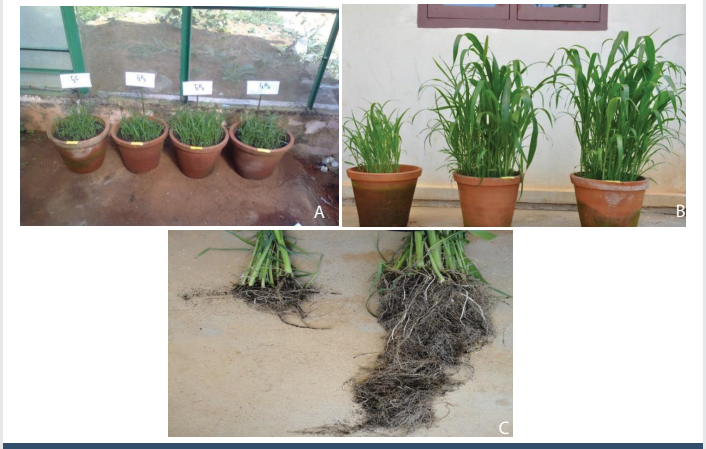

Figure 2: Mass Multiplication of AMF in Vermicompost. **a:** Pre treatment **b:** Post treatment. **c:** Effect of AMF on root growth (T_1 & T_2).

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Glomus intraradices was surface sterilized in 53 µm sieve using 96% alcohol, 2% Chloramine T reagent, Tween 20, 0.02% streptomycin sulphate, 0.01% gentamycin and 6% Calcium hypochlorite solution. The surface sterilized AMF spores were washed with distilled water and collected in a beaker. Pot culture is initiated by placing a layer of inoculums 1 to 2 cm below the seeds. Proper light, moisture and temperature were maintained.

There was found to be an increase in shoot length and root length of AMF inoculated plants when compared to non-inoculated plants. AMF colonization was found to be higher in case of vermicompost whereas it was less in soil: sand. No colonization was observed in coir pith compost (Table 1).

From these, it is understood that, the inoculation of AMF in vermicompost positively influenced the growth of sorghum seedlings (Figures 3,4).

As figure shows, inoculating sorghum plants with *Glomus intraradices* significantly increased the root length. The inoculation with VAM increased the root length by 25%. Huang et al., reported a root length increment of up to 80% when *Leucaena leucocephala* was inoculated with vesicular-arbuscular mycorrhiza. Levy and Syvertsen, while working on the effect of drought stress on citrus, reported that, although plant to plant variations obscured significant differences, vesicular-arbuscular mycorrhiza plants did tend to have greater total feeder root length per plant than control plants. In addition to the mycorrhiza inoculation enhancing the plants absorption of more nutrients, especially phosphorus, via an increase in the absorbing surface area [10], mycorrhiza colonization could have protected roots from soil pathogen [11], and therefore increased root growth and nutrients acquisition of sorghum plants. Inoculated plants had higher number of roots than non-inoculated ones, though the increment was not significant at 5% level. Mycorrhiza inoculation is known to enhance the plants absorption of more nutrients especially phosphorus via an increase in the absorbing surface area [10]. This in turn could have enhanced a higher plant growth rate resulting to more roots per plant. Mycorrhiza colonization also protect the roots from the soil pathogens [11] and, therefore could have led to an increase in not only the root growth and nutrient acquisition of the host plant, but also the number of surviving roots.

Figure 3: Mass Multiplication of AMF in Soil: Sand. **a:** Pre treatment **b:** Post treatment.

Figure 4: Mass Multiplication of AMF in Coir Pith Compost. a: Pre treatment b: Post treatment.

The effect of *Glomus intraradices* inoculation on the height increment was obvious on visual comparison at the end of 60days. The enhanced height increment in *sorghum plants* could be attributed to the *Glomus intraradices* colonization. Mycorrhiza infection is known to enhance plant growth by increasing nutrients uptake [10]. The uptake of nitrogen, phosphorus and potassium is limited by the rate of diffusion of each nutrient through the soil [12]. It seems likely that vesicular arbuscular mycorrhiza in this study increased nutrient uptake by shortening the distance nutrients diffused through the soil to the roots. During the first 45 days, there was no significant difference in height increment between inoculated and non-inoculated plants, although the height increment in inoculated plants was higher. This could be due to the "lag phase" effect of mycorrhiza inoculation. Many studies have shown that there is a lag phase between mycorrhiza inoculation and the time period when its effect is manifested in the plant.

At the end of sixty days, height growth of inoculated *Sorghum bicolor* was highly significant as compared to the non-inoculated plants. The higher height increment registered with inoculated plants could be as a result of enhanced inorganic nutrient absorption [13] and greater rates of photosynthesis [14]. Vesicular arbuscular mycorrhiza are known to affect both the uptake and accumulation of nutrients and therefore, act as an important biological factor that contribute to efficiency of both nutrient uptake and use. Researchers have demonstrated that vesicular-arbuscular mycorrhiza fungi, not only increases phosphorus uptake, but also plays an important role in the uptake of other plant nutrients and water [15,16]. The inflows of phosphorus to mycorrhiza roots can be greater than inflows to comparable non-mycorrhiza roots by up to 2-5 times [17].

Menge et al., and Jaizme-Vega and Azcón, considered inoculation with AMF a good strategy for successful plant transplantation because of improved water and nutrient absorption. In the study conducted it was proved that inoculation with AMF increased the growth of sorghum plants and this may benefit rates of photosynthesis and also nutrient transport by mass flow.

Conclusion

The current study had shown that inoculating *Sorghum bicolor*awith vesicular-arbuscular mycorrhiza in vermicompost enhances growth performance. The inoculation resulted in an increment in height growth as well as root length. Inoculated plants subsequently produced more leaves per plant, which could have increased the rate of photosynthesis. Inoculated plants produced also more roots per plant which were longer than in the non-inoculated plants. This improvement in plant growth could be attributed to the enhancement of the plant

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to absorb more nutrients, via an increase in the absorbing surface area. Vesicular–arbuscular mycorrhiza colonization also protects roots from soil pathogens and thereby increase root growth and nutrients acquisition of the host plants.

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