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Effect of Vesicular-arbuscular Mycorrhiza (vam) Inoculation on Growth Performance of *Senna spectabilis*

Kung'u, J.B.

School of Pure and Applied Sciences, Kenyatta University, P.O. Box 43844 Nairobi Kenya
Email: kungu@avu.org

Abstract

The influence of vesicular-arbuscular mycorrhiza (VAM) fungi inoculation on growth performance of *Senna spectabilis* was studied in a screen house experiment. The results obtained indicated the dependence of *Senna spectabilis* on mycorhizal symbiosis. Inoculation with vesicular-arbuscular mycorrhiza significantly improved the growth performance of *Senna spectabilis*. The height growth increased significantly by 85% after only three months while the root collar diameter increased by 71%. Shoot production increased by 213% while root biomass increased by 241%. Inoculation with vesicular-arbuscular mycorrhiza increased plant tissue phosphorus, nitrogen and potassium content. The better growth response of mycorrhizal plants were attributed to improvement in nutrient uptake, especially phosphorous, nitrogen and potassium. Vesicular-arbuscular mycorrhiza inoculation has a high potential in agroforestry as a bio-fertilizer.

Introduction

The intense exploitation of tropical forests has led to degradation of once stable ecosystems. There has been changes in abiotic and biotic soil properties, which hampers the re-establishment of proper vegetation cover (Miller, 1987). Soils in these areas are very infertile and are acidic in nature. The soils are characterized by low effective cation exchange capacity, low available water and nutrient reserve, low soil pH, low organic matter and phosphorus content and are highly susceptible to soil erosion. The deforested and degraded areas no longer regenerate into woody perennials due to lack of mycorrhiza propagules for recolonization but rather into the so-called "derived savannas" which now occupy million of hectares in Africa in form of *Imperata cylindrica* and *Themeda triandra* grasslands (Janos, 1980a). This is because grasses are the most independent of mycotrophic plants and they can tolerate low soil fertility inspite of their low ineffectiveness (Baylis, 1975).

Agroforestry, a land-use system and technology in which trees are deliberately planted on the same units of land with agricultural crop and /or animals, has been recognized as one of the most promising strategy for rehabilitating the already degraded areas. The benefits of agroforestry includes the amelioration of soil chemical and physical properties, the reduction of soil erosion, improved weed control and increased availability of fuel wood and /or fodder (Young, 1997; Chin and Huxley, 1996). The degree to which an agroforestry system can provide the above benefits partially depends on the quantity of biomass an agroforestry tree species can produce.

Acid soils are known particularly to be unfavorable for legumes due to iron, aluminum and /or manganese toxicities, as well as molybdenum, calcium, and/or magnesium deficiencies. Molybdenum is an essential nutrient in nitrogen fixation, while calcium requirements in legumes are high and therefore deficiencies of either of these elements can cause low biomass yields in an agroforestry leguminous tree species.

Mycorrhizal fungi are known to affect growth of most plant species through various ways. They increase phosphorus uptake, enhance uptake of other plant nutrients by root system and are beneficial in the biological nitrogen fixation of *Rhizobium*, biological control of root pathogens and drought resistance (Harley and Smith, 1983; Sieverding, 1991; Dela Cruz, 1987; Janos, 1980b). The potential benefit of mycorrhizal fungi in rehabilitation of degraded areas by use of agroforestry system is more apparent than ever before. The need to increase food, fibre, and fuel wood production to keep pace with the fast growing population in Africa is crucial. The low biomass production of agroforestry tree species in degraded areas can, therefore, be circumvented by the use of mycorrhizal fungi. Unfortunately, there seems to be very little research in using mycorrhizal fungi in an agroforestry setting. This paper reports a green house experiment that tested the effect of vesicular-arbuscular mycorrhiza inoculation on growth performance of *Senna spectabilis*. The plant is an important agroforestry tree species, which has passed the tests of practicability and acceptability in the eyes of researchers and farmers. The tree is widely recommended as an agroforestry tree species for degraded areas in many parts of the tropics but its main problem lies in slow growth rate in acidic soils.

Materials and Methods

The experiment was conducted in a screen house in the University of the Philippines Los Baños. The experiment was laid out in a randomized complete block (RCB) design, with four replicates and four treatments. Each treatment consisted of five 20cm clay pots. A total of eighty clay pots were used and a total of 240 plants were planted. Top soil (0-15cm) was collected from a degraded grassland area that was dominated by *Imperata cylindrica*. The soil was air dried, pulverized and passed through a 2mm sieve. The soil was then sterilized with hot air at 100 °C for 48 hours. The soil had an initial pH of 5.14 (Potentiometric Method), organic matter content of 1.67% (Walkley-Black Method), total nitrogen 0.18% (Modified Kjedahl Method), potassium 4.11me/100g (Flame Photometer Method) and available phosphorus 70.18 ppm (Bray No.2 Method).

The soil was then put into the 20cm top diameter clay pots. The VA-mycorrhizal fungi inoculants consisting of spores, mycorrhizal root fragments and infected soil was collected from pot cultures of trap plants (*Pensacola bahia*) grass which had been grown for five months after being inoculated with mycorrhiza fungus species of *Glomus tunicatum* and *Glomus macrocarpum*. The inoculants were added to some pots, at the rate of one table spoon per pot which consisted of 23 spores per gram of soil added. The rate of spores per gram of soil was determined by wet sieving and decanting, surface sterilized in 2% sodium hypochlorite and then washed. The non vesicular arbuscular control pots were left uninoculated. Seeds of *Senna spectabilis* were pre-treated with hot water for three minutes. The seeds were then germinated in sterilized river sand. After the seedlings had developed two leaves each, three seedlings were transplanted to each clay pot containing the sterilized soil, plus or minus the vesicular arbuscular mycorrhiza inoculum. Seedlings were then watered twice a day for the first week and then once a day in the following weeks.

To determine the effect of vesicular arbuscular mycorrhiza inoculation on growth performance of *Senna spectabilis*, inoculated and non-inoculated plants were raised in a screen house for three months. Height growth was measured after every 15 days, except during the first months. Root collar diameter was measured at the end of three months. After four months, 50% of the plants per block were harvested using destructive sampling and vesicular arbuscular mycorrhiza colonization above and below ground biomass production, root number and root length were determined. At the end of fifth month, some plants were harvested randomly per treatment and vesicular arbuscular mycorrhiza infection level was assessed by clearing the roots for 2 hours at 90°C in 10% KOH, neutralizing them in lactoglycerol for 20 minutes. Infection was determined by the grid-line intersect method (Giovanetti and Mosse, 1980). Biomass increment due to mycorrhiza inoculation was computed as dry weight of inoculated plants minus dry weight of non-inoculated plants divided by dry weight of non-inoculated plants multiplied by 100%.

For the plant tissue nutrient content, above ground biomass was harvested and was oven dried at 70 °C. The plant tissue were then analyzed for total nitrogen (Micro-kjedahl method), Total phosphorus (Vanadomolybdate method) and Potassium (Flame photometer method). The numbers and length of primary roots per plants were assessed and determined. The measured plants parameters were analysed using IRRISTAT version 92-1 computer software. Analysis of variance was used to describe the data.

Results and discussion

Plant Height

The results obtained indicated the dependence of *Senna spectabilis* on mycorrhiza symbiosis. The effect of vesicular-arbuscular mycorrhiza inoculation on the height increment was obvious on visual comparison at the end of 90 days. As Table 31.1 shows, a significant height increment in inoculated *Senna spectabilis* was recorded after only 60 days. The enhanced height increment in *Senna spectabilis* could be attributed to the vesicular arbuscular mycorrhiza colonization. Mycorrhiza infection is known to enhance plant growth by increasing nutrients uptake (Marschner *et al.*, 1994). Nye *et al.* (1977) reported that the uptake of nitrogen, phosphorus and potassium is limited by the rate of diffusion of each nutrient through the soil. 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 (Brandon and Shelton, 1993).

Table 31.1: Effects of VA mycorrhizal fungi inoculation on shoot height (cm) of *Senna spectabilis* after 90 days in the screen house.

Days after planting	*Senna spectabilis*		
	Treatment with vesicular arbuscular mycorrhiza	Treatment without vesicular arbuscular mycorrhiza	Difference
30	7.06[e]	6.51[c]	0.55[ns]
45	9.90[d]	8.20[bc]	1.70[ns]
60	14.21[c]	8.29[bc]	6.08**
75	17.16[b]	9.03[ab]	8.13**
90	19.80[a]	10.72[a]	9.08**

Means in columns followed by the same letter are not significantly different at 5% level based on DMRT test.

** = significant at 1% level
ns = not significant

At the end of ninety days, height growth of inoculated *Senna. spectabilis* 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 (Cooper, 1984) and greater rates of photosynthesis (Allen *et al.*, 1981). 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 (Huang *et al.*, 1985; Ellis *et al.*, 1985). Sander *et al.* (1983), reported that the inflows of phosphorus to mycorrhiza roots can be greater than inflows to comparable non-mycorrhiza roots by up to 2-5 times.

Shoot Biomass

Inoculating *Senna spectabilis* with vesicular-arbuscular mycorrhizal fungi, increased significantly the shoot biomass yield. As shown in Table 31.2, the shoot biomass production increased by 213% and was highly significant. The highly significant shoot biomass production by the inoculated plants, could be attributed to enhanced inorganic nutrition absorption and greater rates of photosynthesis in inoculated plants (Allen *et al.*, 1981; Cooper, 1984). Vesicular-arbuscular mycorrhiza have been said to affect both the uptake and accumulation of nutrients. Chulan and Martin (1992), reported a significant shoot dry weight increment when *Theobroma cacao* was inoculated with VA-mycorrhiza. Aggangan and Dela Cruz (1991), reported a dry matter yield increment of up to 631% when *L. leucocephala* was inoculated with vesicular-arbuscular mycorrhiza. Zajicek *et al.* (1987) reported a significant increment in dry matter yield when two forbs were inoculated with vesicular-arbuscular mycorrhizal fungi. Vesicular-arbuscular mycorrhizal fungi are reported to enhance plant growth rate through an increase in nutrient uptake, especially phosphorus which is relatively immobile in soils (Kormanik *et al.*, 1981, 1982; Dela Cruz, 1987; Janos, 1980a). Vesicular-arbuscular mycorrhiza inoculation could have enhanced *Senna spectabilis* to absorb more nutrients via an increase in the absorbing surface area. Similar observation has been reported by Marschner and Dell (1994).

The movement of nutrients to plant roots and the rate of absorption of nutrients by roots, especially nitrogen, phosphorus and potassium, is known to be limited by the rate of diffusion of each nutrient through the soil and not by the ability of the root to absorb the nutrient from low concentration in the soil solutions (Abbott and Robson, 1982). In the present study, since the soil used was not very fertile, inoculation with vesicular-arbuscular mycorrhiza could have resulted in an increase in nutrient uptake by merely shortening the distance that the nutrients had to diffuse from the soil to the roots. This in turn, could have enhanced a higher shoot biomass production in the inoculated *Senna spectabilis* .

Root Biomass

As Table 31.2 shows, inoculating *Senna spectabilis* with vesicular-arbuscular mycorrhiza significantly increased the root biomass production. Vesicular abuscular mycorrhiza infection has been reported to increase both the uptake of nutrients by the roots and the concentration of nutrients in the plant tissues (Smith *et al.*, 1979). An increase in nutrient uptake, especially phosphorus in the infertile soil used, could have resulted in relief of nutrients stress and an increase in photosynthetic rate, which obviously could have given rise to an increase in plant growth. Research has shown that when root exploration is restricted, up to 80% of the plant phosphorus can be delivered by the external vesicular-arbuscular mycorrhizal hyphae to the host plant over a distance of more than 10 cm from the root surface (Li *et al.*, 1991) .

Hattingh *et al.* (1973) found that vesicular arbuscular mycorrhizal hyphae, could intercept labelled phosphorus, placed 27mm from a mycorrhizal root, whereas it remained unavailable to non-mycorrhizal roots. This confirms that vesicular-arbuscular mycorrhizal hyphae could have increased the volume of soil available to the *Senna specabilis* for nutrient uptake.

Table 31.2: Effect of vesicular-arbuscular mycorrhiza inoculation on growth performance of *Senna spectabilis* after 90 days in a screen house.

Growth Parameter	Treatment VAM Innoculation	Measurement	Increment percentage
Shoot dry weight	Non inoculated	2.82	213
	Inoculated	8.83**	
Root dry weight (g pot^{-1})	Non inoculated	1.66	241
	Inoculated	5.66**	
Total shoot length (cm)	Non inoculated	10.72	85
	Inoculated	19.80**	
Root collar diameter (cm)	Non inoculated	0.21	71
	Inoculated	0.36**	
Leaf number	Non inoculated	4.30	105
	Inoculated	8.80**	
Root/shoot ratio (R/S)	Non inoculated	0.60	8
	Inoculated	0.65^{ns}	
Root length (cm)	Not inoculated	24.33	25
	Inoculated	30.41**	
Root Number/plant	Non inoculated	10.0	7.5
	Inoculated	10.75^{ns}	
Roots colonized (%age)	Non inoculated	0	67.8
	Inoculated	67.75**	

** = significant at 1% level,
ns = not significant

Mycorrhizal roots have been known to absorb phosphorus faster per gram of root than non-mycorrhizal plants (Jakobsen *et al.*, 1992). This may relate to the greater surface area per gram of mycorrhiza roots. It therefore follows that mycorrhiza were able to enhance the absorption of nutrients from the soil, which could have moved to the roots principally by mass flow, in addition to those which could have diffused through the soil slowly. This could have resulted in a higher root biomass in inoculated plants.

Root collar diameter

Vesicular-arbuscular mycorrhiza inoculation increased the root collar diameter of *Senna spectabilis* by 74%. As shown in Table 31.2, the increment of the root collar diameter of the vesicular-arbuscular mycorrhiza inoculated, plants was highly significant. The higher diameter increment of the inoculated plants could be attributed to enhanced inorganic nutrition absorption and greater rates of photosynthesis of inoculated plants (Allen *et al.*, 1981; Cooper, 1984). Vesicular- arbuscular mycorrhiza have been said to affect both the uptake and accumulation of nutrients. 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 (Huang *et al.*, 1985; Sieverding, 1991).

Many authors have reported a significant increment in root collar diameter, after inoculating the plants with vesicular-arbuscular mycorrhiza. Reid *et al.* (1988), reported an increment in root collar diameter when sugar maple seedlings were inoculated with vesicular-arbuscular mycorrhiza. Osonubi *et al.* (1989), while working with inoculated *Gmelina* seedlings, reported a significant biomass increment. Huang *et al.* (1985) while working with inoculated *Leucaena leucocephala*, reported a significant increment in plant growth parameters. Aggangan and Dela Cruz (1991), while working with *Acacia auriculiformis* and *Leucaena leucocephala*, reported a diameter increment of between 18% to 123% when the two plants were inoculated with different types of vesicular-arbuscular mycorrhizal fungi. Castillo (1993), while working with *Pterocarpus indicus*, reported a significant diameter increment when the plants were inoculated with vesicular-arbuscular mycorrhizal fungi. Kormanik *et al.* (1981) reported a significant increment in root collar diameter when sweetgum seedlings were inoculated with vesicular-arbuscular mycorrhizal fungi. He reported that inoculation with vesicular-arbuscular mycorrhiza increased the root collar diameter by 268%.

Root to Shoot Ratio

As shown in Table 31.2, the difference between the root to shoot ratio of inoculated and non-inoculated *Senna spectabilis*, was not statistically significant at 5% level though the inoculated *Senna spectabilis* had a higher root to shoot ratio as compared to non inoculated plants. The higher root to shoot ratio of the inoculated plants could be attributed to the effect of mycorrhiza infection, which could have increased nutrients absorption, giving rise to a higher root and shoot biomass increment with a uniform growth. Clapperton and Reid (1992) while researching on the relationship between plant growth and increasing vesicular-arbuscular mycorrhizal inoculum density, reported that as the colonization by vesicular-arbuscular mycorrhizal fungi increased, so did root to shoot ratios. They concluded that this was due to the vesicular-arbuscular mycorrhizal plants being able to translocate more carbon to the roots than non-mycorrhiza plants. The same has been reported by Kucey and Paul (1982); Douds et al. (1988) and Wang *et al.* (1989). Tree seedlings with higher root to shoot ratios are able to have a higher survival percentage when planted in the field.

Root number and length

As Table 31.2 shows, inoculating *Senna spectabilis* with vesicular-arbuscular mycorhiza fungi, significantly increased the root length. The inoculation with VAM increased the root length by 25%. Huang *et al.* (1985) reported a root length increment of up to 80% when *Leucaena leucocephala* was inoculated with vesicular-arbuscular mycorrhiza. Levy and Syvertsen (1983) 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 (Marschner and Dell, 1994), mycorrhiza colonization could have protected roots from soil pathogen (Perrin, 1990), and therefore increased root growth and nutrients acquisition of *Senna spectabilis.* 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 (Marschner and Dell, 1994). 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 (Perrin, 1990) and, therefore could have lead to an increase in not only the root growth and nutrient acquisition of the host roots, but also the number of surviving roots.

Root Colonization Percentage

As shown in Table 31.2, inoculating *Senna spectabilis* with vesicular-arbuscular mycorrhiza fungi resulted into a 67.8% colonization. There was no vesicular-arbuscular mycorrhiza contamination as evident in the non inoculated plants (control) which showed a 0% colonization.

Mycorrhiza colonization is normally attributed to the tree species and environmental factors. Smith *et al.* (1979) reported that the extent to which typical vesicular-arbuscular mycorrhiza fungi colonize root systems varies with species of plant. It has also been noted that there are differences in the extent of infection between genotypes of the same species. The extent of mycorrhiza infection in root systems is also known to be influenced by environmental conditions; the most important being the age of the plants, the level of phosphate (P) in the soil relative to the requirements of the plant and the capacity of the population of mycorrhiza propagules in the soil to form mycorrhiza. *Senna spectabilis* is a non nodulating legume (Ladha *et al.*, 1993) and rhizobium bacteria could not have posed any threat in competing with mycorrhiza fungi for carbohydrates. The time period of the seedlings (five months) could have been too short to record a higher colonization percentage since the root system infected normally increases with time sigmoidally. Seasonal patterns in the formation of mycorrhiza have also been said to vary considerably from year to year (Allen *et al.*, 1989).

Plant Tissue Nutrients Concentration

Inoculating *Senna spectabilis* with vesicular-arbuscular mycorrhiza, increased plant tissue nutrients concentration. As Table 31.3 shows, plant tissue phosphorus, nitrogen and potassium concentration was much higher in the inoculated plants than non inoculated ones. The higher phosphorus concentration in the inoculated plants could be attributed to a higher nutrients absorption rate by mycorrhiza plants.

Table 31.3: Effect of vesicular-arbuscular mycorrhiza inoculation on nutrient concentration (NPK) in shoot of *Senna spectabilis* after 90 days in a screen house

	Plant Concentration	Tissue	Nutrient
VAM Innoculation	Phosphorus (%)	Nitrogen (%)	Potassium (%)
Inoculated	0.46**	3.05^{ns}	1.64^{ns}
non-inoculated	0.19	2.99	1.53

** = significant at 1% level,
ns = not significant

Several authors have reported that mycorrhizal roots are able to absorb several times more phosphate than non inoculated roots from soils and from solutions (Pearson and Gianinazzi, 1983; Michelsen and

Rosendahl, 1990; Fitter, 1988; Dela Cruz *et al.*, 1988; Nielsen, 1983). Increased efficiency of phosphorus uptake by mycorrhizal plants could have led to higher concentrations of P in the plant tissues. The greater phosphate absorption by vesicular-arbuscular mycorrhizae has been suggested to have arisen due to superior efficiency of uptake from labile forms of soil phosphate, which is not attributable to a capacity to mobilize phosphate sources unavailable to non mycorrhizal roots (Pearson and Gianinaazzi, 1983). Under certain conditions, mycorrhiza is known to absorb fixed phosphate and even to stimulate root phytase activities (Pearson and Gianinazzi, 1983). Mycorrhizal roots are known to have not only a considerably greater phosphate inflow rates, but also to possess a pathway of phosphate uptake with a much higher affinity for phosphate than non mycorrhizal roots.

The higher plant tissue nitrogen content in inoculated plants could be attributed to hyphae uptake. It has been reported that the existence of extra-radical hyphal bridges between individual plants permits transfer of nutrients such as nitrogen (Marschner and Dell, 1994). The two have reported that about 24% of the total nitrogen uptake in mycorrhizal plants could be atributed to uptake and delivery by the external hyphae. There is also evidence that nitrogen is taken up by vesicular-arbuscular mycorrhiza hyphae from inorganic sources of ammonium (Ames *et al.*, 1983) and therefore, the higher nitrogen concentration in mycorrhizal plants could be attributed to the hyphae uptake. The same could be said of the higher potassium concentration in inoculated plants. In a compartment pots experiment, Li *et al.* (1991), demonstrated that about 10% of the total potassium uptake in mycorrhizal coach grass was due to hyphal uptake and transport.

Conclusion

The current study had shown that inoculating *Senna spectabilis* with vesicular-arbuscular mycorrhyza enhances growth performance. The inoculation resulted in an increment in height growth by 85% and root collar diameter by 71% within three months. Shoot biomass increased significantly by 213% while root biomass increased by 241%. Inoculated plants subequently 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 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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