EFFECT OF VESICULAR ARBUSCULAR MYCORRHIZA (VAM) FUNGI INOCULATION ON COPPICING ABILITY AND DROUGHT RESISTANCE OF SENNA SPECTABILIS

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Abstract

The influence of Vesicular arbuscular mycorrhiza fungi inoculation on coppicing ability and drought resistance of *Senna spectabilis* was studied in a screen house experiment. The result obtained indicates the dependence of *Senna spectabilis* on mycorrhizal symbiosis. Under well watered conditions, arbuscular mycorrhiza inoculation increased coppicing biomass production of *Senna spectabilis* by 269% while under water stressed conditions, coppice biomass production increased by 317%. Analysis of variance revealed that interaction between the mycorrhizal fungi and water stress was highly significant. Inoculating *Senna spectabilis* with VAM improved its drought resistance. Under drought conditions, inoculating *Senna spectabilis* increased total shoot length by 100% root collar diameter by 74% shoot dry weight by 435% root dry weight by 397% and plant leaves number by 105%. Inoculated plants had more leaf water content than non inoculated plants. Inoculated *Senna spectabilis* plants took more days to show signs of drought stress (total leaf folding, loss of shoot and leaf turgor and, wilting of lower leaves). The better growth responses of mycorrhizal plants were attributed to higher nutrients uptake and higher moisture absorption. Arbuscular mycorrhiza inoculation has a high potential in water stressed environment in maintaining water relationship.

Introduction

There has been intense exploitation of tropical forests leading to change in abiotic and biotic solid properties which hampers the re-establishment of proper vegetation cover (Miller, 1987). Soils in these areas are characterized by low 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 degraded areas lack mycorrhiza propagules and only regenerate in the so-called "derived savannas" which now occupy million of hectares in Africa by *Imperata cylindrica* and *Themeda triandra* grasslands (Janos, 1980). This is because grasses are the most independent of mycotrophic plants and they can tolerate low soil fertility in spite 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 induction of soil erosion, improved weed control and increased availability of fuel wood and /or fodder (Young, 1997; Chin Ong & P. 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.

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Acid soils are known particularly to be unfavourable for legumes due to iron, aluminium 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 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 & 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 developing countries in the tropics 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 experiments were conducted in screen house of the University of the Philippines, Los Banos. The experiments were laid out in a randomized complete block (RCB) design with four replicates and four treatments. Each treatment consisted of five 20 cm clay pots. A total of 80 clay pots were used and a total of 240 plants were planted. Top soil (0-15 cm) was collected from a degraded grassland area. The soil was air dried, pulverized and passed through a 2 mm sieve. The soil was then sterilized with hot air at 100°C for 48 h. The soil had an initial pH of 5.14 (Potentiometric Method), organic matter content of 1.67% (Walkley-Black Method), total nitrogen 0.18% (Kjedahl Method), potassium 4.11 me/100g (Flame Photometer Method) and available phosphorus 70.18 ppm (Bray Method).

The soil was then put into the 20cm top diameter clay pots. The VAM fungi inoculants consisting of spores, mycorrhizal fragments, and infested soil was collected from pot cultures of trap plants (*Pensacola bahia*) grass which had been grown for five months after being inoculated with *Glomus tunicatum* and *Glomus macrocarpum*. The inoculants were added to some pots at the rate of one tablespoon per pot, which consist 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 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 VAM inoculum.

To determine the effect of VAM inoculation on coppicing ability *Senna spectabilis*, inoculated and non-inoculated plants were raised for three months. After three months, 25 % of the plants were chopped off at 10 cm above the root collar and all the leaves were removed. The plants were then left to coppice. Watering was done after every two days. At the end of the first month, the coppice shoots were harvested and oven dried at 70° C for 48 h. The coppicing biomass due to VAM inoculation was then computed using the formula:

Percentage biomass increment =
$$\frac{D. W. (+M) - D.W. (-M)}{D.W. (-M)} X100$$

where D. W. (-1 M) is the dry weight of the sample inoculated with VAM and D. W. (-M) is the dry weight of the sample without VAM inoculation. To determine the effect of VAM inoculation on drought resistance, plants were well watered for three months. Afterwards watering was withheld from the plants until the first plants showed the signs of severe drought stress which was; total leaf folding, loss of shoot and leaf turgor and wilting of lower leaves. Bio-mass increment under drought stress due to VAM infection was then computed using the formula:

Percentage biomass increment in drought conditions = $\frac{D. W. (+M) - D.W. (-M)}{D.W. (M-)} X100$

where D.W. (+M) is the dry weight of the sample inoculated with mycorrhiza, and D.W. (-M) is the dry weight of the sample without mycorrhiza inoculation. For the determination of the effect of arbuscular mycorrhiza inoculation on leaf water content (LWC), four leaves from inoculated and non-inoculated plants were harvested, weighed and oven dried. The leaf water content (LWC) was then computed using the formula:

Leaf water content (LWC) = $\frac{\text{(Fresh weigh) leaf- Oven dry leaf weight)}}{\text{Ovens dry leaf weight}} X100$

At the end of the fifth month, the plants were harvested and the VAM infection level was assessed by clearing the roots for 2 h at 90°C in 10% KOH, neutralising them in 1% HCl and staining them with 0.05% trypan blue in lactoglycerol for 20 minutes. The infection was determined by the grid-line intcr.scft method (Phillips & Haymann, 1980). The data were analysed using IRRISTAT computer software version 92-1 (Gomez *et al.*, 1992). Treatment means were compared using partition of sum of squares. Analysis of variance was used to describe the data.

Results and Discussion

The results obtained indicate the dependence of *Senna spectabilis* on mycorrhizal symbiosis. As shown in Table 1, a significant height increment was observed in inoculated *Senna spectabilis* seedlings after 60 days. The enhanced height increment in *Senna spectabilis* was attributed to VAM colonization. VAM infection is known to enhance plant growth by increasing nutrients uptake (Marschiier *et al.*, 1994). Nye & Tinker, (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 that VAM inoculation in this study increased nutrients uptake by shortening the distance these 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 VAM 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 (Bnindon & Shelton, 1993).

	Senna spectabilis				
Days after planting	Treatment with (+) VAM	Treatment without (-) VAM	Difference		
30 days	7.06e	6.51c	0.55ns		
45 days	9.90d	8.20bc	1.70ns		
60 days	14.21c	8.2.9bc	6.08**		
75 days	17.16b	9.03ab	8.13**		
90 days	19.80a	10.72a	9.08**		

Table 1.	Effects	of VAM	fungi ino	culation	on shoot	height	(cm) of	
		Senna s	pectabilis	after 90	days.			

Means in columns followed by the same letter are not significantly different at 5 % level based on DMRT test. ** = Significant at 1 %,

ns = Not significant

Table 2. Effect of VAM fungi inoculation on coppicing ability (gm) of *Senna spectabilis* under watered and stressed conditions.

		Mycorrhizal inoculation			
Tree species	Water condition	+ VAM inoculants	- VAM inoculants	M-mean	Difference
Senna spectabilis	Watered	1.330	0.360	0.845	0.970**
	Water Stress	0.388	0.093	0.240	0.295*
Mean		0.491	0.168	0.329	0.324

** =Significant at 1%,

ns = Not significant.

Height growth of inoculated *Senna spectabilis* at the end of 90 days was highly significant as compared to the non-inoculated plants. The height increment registered with inoculated plants could be attributed to enhanced inorganic nutrient absorption (Cooper, 1984) and greater rates of photosynthesis (Alien *et al.*, 1981). VAM are known to affect both the plant uptake and accumulation of nutrients and therefore, acting as an important biological 'factor that contribute to efficiency of both nutrient uptake and use. Many researchers have reported 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).

Inoculating *Senna spectabilis* with VAM increased the coppice biomass production under water stressed conditions (Table 2). The coppice biomass increment of *Senna spectabilis* under water stressed conditions increased significantly by 317% as compared to under well watered conditions where it increased by 269%. The ability of a plant to coppice has been related to a number of factors, such as the size and age of donor trees and the quality of the site where the trees arc grown (Kijkar, 1991). In the present study, inoculating *Senna specubilis* with arbuscular mycorrhiza fungi could have played an important role in altering the rhizosphere environment by shortening the distance the nutrients had to diffuse through the soil to the roots and therefore, ircreasing the nutrients uptake, especially phosphorus.

Many researchers have reported a growth increment when plants are inoculated with VAM fungi (Silverding, 1991; Dela Cruz 1991; Chulan & Martin 1992; Abbort & Robson 1991; Jones 1980; Aggangan & Dela Cruz 1991). Inoculating plants with VAM have been reported to increase nutrients uptake in stressed plants, lower stomatal resistance, enable plants to use water more efficiently and increase root hydraulic conductivity (Busse & Elias, 1985; Stahl & Smith, 1984; Graham & Syvertson, 1984; Sieverding, 1983).

The higher coppice biomass production in inoculated *Senna spectabilis* could therefore, be due to not only higher phosphorus uptake, but also to higher moisture absorption by the inoculated plants. Kijkar (1991) reported that shoot production depends not only on the vigour of the tree, but also on the environmental factors such as site conditions, soil moisture and season. In the present study nutrient and moisture conditions could have played a major role. Under water stressed conditions, the higher coppice shoot biomass production in inoculated *Senna spectabilis* could be attributed to the higher nutrients uptake, better water usage efficiency and an increase in root hydraulic conductivity.

Colonisation by VAM fungi improves the drought resistance of plants (Puppi & Bras, 1990, Ellis *et al.*, 1985; Huang *et al.*, 1985). Michelsen (1990) reported that seedlings *of Acacia nilotica* were more drought resistant after they were inoculated with arbuscular mycorrhiza. Alien *et al.*, (1981), reported that VAM increases transpiration and that whole-plant resistance to water transport was reduced by 50%. It can then be concluded that VAM provides an advantage to the host plant in times of moisture stress.

In greenhouse experiments, mycorrhizas| have been shown to increase the drought resistance of cultivated crops, such as wheat, onion and pepper (Ellis *et al.*, 1985; Nelson & Safir, 1982; Waterer & Coltman, 1989). The improved plants nulritional status by mycorrhiza during periods of soil water deficit enhances drought resistance (Nyc & Tinker, 1977; Nelson & Safir 1982; Fitter, 1988). Other factors that could be associated with mycorrhiza colonisation are improved leaf water, turgor potentials, maintenance of stomatal opening and transpiration and increased rooting length and depth (Ellis *et al.*, 1985; Kothali *et al.*, 1990; Auge *et al.*, 1987).

As shown in Table 3, inoculating *Senna spectabilis* with VAM improved its drought resistance. Inoculation with VAM significantly increased total shoot length, root collar diameter, shoot dry weight, leaf number, number of days before wilting and leaf water content.

Under water stressed conditions, inoculating *Senna spectabilis* with VAM increased the total shoot height by almost 100% while the root collar diameter increased by 25%. The leaf number increased by 84% while the shoot biomass and root dry matter production increased by 436% and 401% respectively. These findings are in agreement with other authors who have shown that VAM fungi translocate more carbon to the roots than non-mycorrhizal plants (Kucey & Paul, 1982; Vang *et al.*, 1989; Wright & Millner, 1994). The improved nutrient absorption by mycorrhiza plants especially phosphorus under drought conditions have had a direct impact on growth parameters.

120 days old Schuld Speedabilis Seedings						
Crowth nonomator	Treatment VAM	Senna	Percentage			
Growin parameter	inoculation	spectabilis	increment			
Total shoot length (cm)	-M	12.33				
_	+M	24.63**	99.79			
Root collar diameter (cm)	-M	0.27				
	$+\mathbf{M}$	0.47**	74.63			
Shoot dry weight (g/pot)	-M	1.33	435.99			
	$+\mathbf{M}$	7.12**	104.65			
	$+\mathbf{M}$	8.8**				
Root/shoot ratio	-M	0.53				
	+M	0.54 ^{ns}	2.37			
Number or days before wilting	-M	8				
	$+\mathbf{M}$	12**	50			
Leaf water content	-M	29.09				
	$+\mathbf{M}$	38.22*	31.4			

 Table 3. The effect of VAM inoculation on drought resistance of

 120 days old Senna spectabilis seedlings.

** = Significant at 1%, ns = Not significant.

Many studies have shown that VAM may be relatively more important to plant growth under dry conditions than when soil moisture is plentiful (Fitter, 1985; Nelson & Salir, 1982; Michclsen & Rosendahl, 1990). Colonisation by VAM fungi has been reported to improve the drought resistance of plants (Ellis *et al.*, 1985; Huang *et al.*, 1985; Puppi & Bras, 1990). Vesicular arbuscular mycorrhiza fungi are said to improve plant growth by taking up the relatively more immobile nutrients such as phosphate, increasing drought tolerance and interact with other rhizosphere organisms (Nelsen, 1986; Brundrett *et al.*, 1994). This could explain the reason for the inoculated plants having higher growth rate and being more drought tolerance.

There was more leaf water content in the inoculated *Senna spectabilis*, plants than in non-inoculated (Table 3). Inoculating *Senna spectabilis* with vesicular arbuscular mycorrhiza increased the leaf water content by 31%. These findings are in agreement with other authors who have reported that inoculated plants sustain less drought strain as compared to non-inoculated ones (Auge *et al.*, 1987). In the current study, the inoculated plants took more days to show signs of drought stress (total leaf folding, loss of shoot and leaf turgor, wilting of lower leaves) than the non-inoculated plants. The inoculated plants took also less time to recover from water stress as compared to non-inoculated plants. The association of VAM with plants have exerted an influence on water status of the host plants, both in well watered and under water deficit stress situations.

The increase in transpiration, leaf conductance and root hydraulic conductivity as well as decreases in leaf water potential have been observed in plants inoculated with VAM. Various mechanisms have been proposed to account for these effects. These mechanisms includes; enhanced phosphorus nutrition of mycorrhizal hosts (Safir & Nelsen, 1985), altered hormonal relations (Alien *et al.*, 1981) and increased water uptake and transport provided by external hyphae. It has been reported that since the diffusion coefficient for phosphate in soil is linearly related to soil moisture content, mycorrhizal phosphorus supplies are likely to be very much more advantageous to plants in dry than in wet soils. In the current study, inoculated plants could have had higher phosphorus content, which could have influenced stomatal resistance. Inoculating *Senna spectabilis* with VAM could have increased the nutrient uptake of the stressed plants (Busse & Ellis,

1985), lowered stomatal resistance (Stahl & Smith, 19M), enabled the plants to use water more efficiently (Sieverding, 1983), and increased root hydraulic conductivity (Graham & Ssyvertson, 1984). Potassium uptake could have also been enhanced (Sieverding & Toro, 1988; Huang *et at.*, 1985) making the plants to be more drought resistance (Nye & Tinker. 1977; Nelson & Safir, 1982 Bolgiano *et al.*, 1983; Filter, 1988). It is well known fact that under drought conditions, the growth of feeder roots is usually affected and the diffusion of elemental nutrients decreases with drought. Since VAM hyphae are more resistant to drought than nutrient-absorbing feeder root of plants the inoculated *Senna spectabilis* could have resulted into higher growth rate.

Conclusion

The study has shown that inoculating *Senna spectabilis* with VAM increases biomass production significantly under well watered and water stressed conditions. Under well watered conditions, arbuscular mycorrhiza inoculation increased coppicing biomass production of *Senna spectabilis* by 269% while under water stressed conditions, coppice biomass production increased by 317%. Analysis of variance revealed that interaction between the VAM and water stress was highly significant. Under drought conditions, inoculated plants improved their drought resistance leading to an increase in total shoot length by 100%, root collar diameter by 74%, shoot dry weight by 435%, root dry weight by 397% and plant leaves number by 105%. Inoculated plants had more leaf water content as compared to non-inoculated plants. Inoculated *Senna spectabilis* plants took more days to show signs of drought stress (total leaf folding loss of shoot and leaf turgor and wilting of lower leaves) as compared to non-inoculated plants. The better growth responses of mycorrhizal plants are attributed to higher nutrients uptake and higher moisture absorption. Arbuscular mycorrhiza inoculation has a high potential as a biofertiliser in water stressed environment.

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