MYCORRHIZAL INOCULATION EFFECT ON GROWTH RESPONSES AND DRY MASS PRODUCTION OF *MIMOSA HIMALAYANA* GAMBLE SEEDLINGS

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Abstract: *Mimosa himalayana* is a nitrogen fixing shrub of Central Himalayan region. In the present study, effect of mycorrhizal inoculation was observed on the seedlings of *M. himalayana.* For this, seedlings of *M. himalayana* were raised in polyethylene bags containing sterilized mixture of soil and commercial sand. The seedlings of *M. himalayana* became colonized when inoculated with vesicular-arbuscular mycorrhizal fungi. When compared with uninoculated seedlings, inoculated seedlings showed increased root and shoot length with 48% to 58% mycorrhizal dependency for total seedling biomass. Present study suggested that the vesicular arbuscular mycorrhizal fungi act as an important biological factor that contributes to the efficiency of nutrient uptake and use.

Keywords: Colonization, Inoculation, *Mimosa himalayana*, Mycorrhiza, Production

INTRODUCTION

Degradation of land subsequent to deforestation is one of the major ecological problems in the developing world, especially in mountain areas (Echolm 1975). Growing demand for fuel, fodder, wood and food has extensively depleted protective plant cover and exposed soil surface to processes of degradation, resulting in partial to complete loss of soil productivity (National Wasteland Development Board 1987). Mycorrhizal fungi are likely to be most beneficial in diverse (degraded) ecosystems where the production of plants able to form mycorrhiza is high and nutrient deficiencies are an important limitation to plant growth. These fungi can make an important contribution to the rehabilitation of degraded lands, as mycorrhiza can increase the apparent size of the pool of soil nutrients (Barea *et al*.1990). It indicates that mycorrhizal plants can either use available N forms more efficiently, or derive nutrients from sources less available to non-mycorrhizal plants.

The *Mimosa himalayana* Gamble (Family Mimosacae) is a large straggling very prickly shrub with terminal elongate clusters of many tiny pink flowers in globular heads and with twice-pinnate spiny leaves. It is a nitrogen fixing leguminous plant that form root nodules with *Rhizobium* sp. (Singh and Pokhriyal 1997) and grow upto 1600 m asl along the water courses and scrub jungles. Its ability to fix atmospheric nitrogen through biological nitrogen fixation makes it useful in afforestation and reclamation of degraded and nutrient-poor lands. In the present study the influence of VAM fungi on growth of *M. himalayana* seedlings was analyzed. Main objectives of the present experiment were: (i) to determine the potential of *M. himalayana* seedlings to be colonized by VAM fungi; (ii) to quantify the effect of colonization by VAM fungi on seedlings of *M. himalayana.*

MATERIAL AND METHOD

Seedlings of *M. himalayana* were raised from the seeds of the current year crop collected from a forest stand near Nainital town (29°22' N lat. and 79°25' E long.) in the Kumaun region of the Central Himalaya. Seeds were rinsed and surface sterilized (30% H_2O_2 w/v for 20 min). These seeds were sown in polyethylene bags containing 1 kg commercial sand and sieved forest soil in 3:1 ratio (autoclaved twice at 120^{o}C for 1 h, 2 days). VAM inoculation was done with 1 g of surface sterilized infected fine *M. himalayana* root fragments following Tewari *et al* (2003). The inoculum was added to bags while sowing just below the seeds. In addition, uninoculated plants were also maintained as control. For *Rhizobium* inoculation healthy *M. himalayana* nodules were collected from the field. Fresh young nodule lobes were cleansed of soil and homogenized with distilled water in a blender. Inoculum was applied on the surface of soil as liquid suspension when seeds were sown

After establishment one plant per bags was maintained and bags were randomly arranged in block in the glasshouse. Five plants from each sub treatment were uprooted for observation at 6 and 12 months interval. Plant height, root length were recorded. Weight of stems, leaves and roots were estimated by drying them in an oven at 80^{o}C for 24 hour.

AM colonization was observed following root clearing method (Phillips and Haymann 1970). The colonization (%) was calculated by using Nicolson's (1975) formula:

Colonization (%) =

$$\frac{\text{Root segments colonized with VAM}}{\text{Total number of root segments observed}} \times 100$$

The mycorrhizal inoculation effect (MIE) was calculated using the formula given by Bagyaraj (1992):

$$MIE = \frac{\text{Dry weight of inoculated plant-dry weight uniculated plant}}{\text{Dry weight of inoculated plant}} \times 100$$

RESULT

Effect of inoculation on colonization

Colonization developed in all seedlings of *M. himalayana* at each harvest except in the control without inoculation. Hyphae and vesicles were present but no arbuscules were observed. The inoculated seedlings showed 70% colonization by VAM fungi.

Effect of inoculation on seedling growth

Inoculation with VAM fungi significantly increased growth of *M. himalayana* seedlings at each harvest (Table 1). In one-year old seedlings of *M. himalayana*, increment in root and shoot length was 1.9 and 3.1 cm, respectively

The dry mass of *M. himalayana* was significantly greater in plants inoculated with VAM fungi (Table 2). The ANOVA calculated for dry mass yield clearly indicated significant difference in respect to harvest, inoculation and their interaction (Table 3). Mycorrhizal inoculation effect (MIE) was greater on leaf at first harvest and on stem at second harvest (Table 2). In terms of total dry mass MIE increased from I^{st} harvest (48%) to second harvest (58%). Root: shoot ratio decreased in inoculated plants while leaf weight ratio and stem weight ratio increased.

DISCUSSION

Roots of *M. himalayana* were colonized by VAM fungi in nature and the seedlings of this species were colonized in the glass house experiment when inoculated. Colonization consisted of huphae and vesicles. No arbuscles of VAM fungi were present in the roots of *M. himalayana*. Successful establishment of most woody legumes depends on their ability to form symbiotic association between their roots and beneficial microorganisms like rhizobia and mycorrhizas (Barea et al 1990). Different types of mycorrhizal fungi form associations with plant roots, but the arbuscular mycorrhiza are by far the most widespread type of mycorrhiza in nature and are also the most commonly occurring on nodulated nitrogen fixing plants (Barea *et al*., 1992; Roskoski *et al*., 1986).

VAM fungi have potential to increase growth of host plants (Carey *et al* 1992, Bryla and Koide 1990) thus, inoculation with VAM fungi resulted on an average 93% increase at first harvest and 140% increase at second harvest in total seedling dry mass of *M. himalayana* seedlings. Similar results were reported for other leguminous plants viz. *Gliricidia sepium* (Twum-Ampofo K. *2008*), *Albiziz saman* (Rahman *et al* 2004), *Acacia nilotica* (Sharma *et al* 1996), *Indigofera heterantha* (Bargali, 2006). The presence of mycorrhizal fungi in the rhizosphere greatly affect the nutrient mobilization by producing enzymes and low molecular weight organic acids that interact with soil compound resulting in the increased nutrient availability and uptake for symbiotic plants and improve the growth of the plants. Root: shoot ratio decreased in inoculated plants while Leaf weight ratio and Stem weight ratio increased. Bargali (2006) observed that the mycorrhizal fungi increased the absorption surface of the plants and resulted in increased allocation of biomass to the aboveground parts.

Woody legumes are useful for revegetation of water deficient ecosystems that have low availability of N, P and other nutrients. The scarcity of available phosphorus and the imbalance of trace elements in degraded ecosystems actually limit legume establishment and N_2- fixation. But when associated with mycorrhiza it is reported to increase the establishment of legume (Barea *et al* 1992). In addition, woody legumes exhibit a considerable degree of dependence of mycorrhizae to thrive in stressed situation (Osonubi *et al* 1991). In this study, *M. himalayana* seedlings showed 48 % mycorrhizal dependency at first harvest and 58% at second harvest, respectively. In the Himalayan region some leguminous plants like *M. himalayana* grow on nutrient poor soils such as eroded lands, rocky surfaces etc. Though, these plants themselves do not yield economic goods, but they can improve fertility of soil as well as growth of economically important non nitrogen fixing plants. Thus, VAM associated *M. himalayana* seedling can play a crucial role in restoration of degraded wastelands and can be used to stabilize degraded areas all over Himalaya.

Table 1. Effect of AM inoculation on *M. himalayana* seedlings.

Months after germination	**Parameters**	**Treatments**		**Increment**
		Inoculated	Uninoculated	
6	Root length(cm)	20.3 ± 0. 858	18.5 ± 0.685	1.8
	Shoot length (cm)	15.0± 0.068	10.8 ± 0.244	4.2
12	Root length(cm)	22.1 ± 0.904	20.2 ± 0.668	1.9
	Shoot length (cm)	15.2 ± 0.085	12.1 ± 0.287	3.1

Table 2. Effect of AM inoculation on growth performance of *M.himalayana* seedlings in a glass house experiment (Mean ± SE).

Parameters	**I st Harvest**			**II nd Harvest**		
	+ VAM	**-VAM**	**MIE(%)**	**+ VAM**	**-VAM**	**MIE(%)**
Leaf dry mass (g/seedling)	0.205±0.0008	0.058±0.0007	71.70	0.451±0.0007	0.152±0.0007	66.29
Stem dry mass (g/seedling)	0.212±0.0007	0.085±0.0006	59.90	0.475±0.0006	0.155±0.0005	67.36
Root dry mass (g/seedling)	0.195±0.0007	0.174±0.0005	10.76	0.380±0.0004	0.236±0.0004	37.89
Total seedling dry mass (g/ seedling)	0.612±0.0008	0.317±0.0036	48.20	1.306±0.0018	0.543±0.0013	58.42
Root: shoot ratio	0.467	1.216	-	0.410	0.769	-
Leaf weight ratio	0.335	0.182	-	0.345	0.279	-
Stem height: Stem dry wight ratio	0.346	0.268	-	0.363	0.285	-
Relative growth rate ($g\ g^{-1}\ d^{-1}$)	-	-	-	0.0041	0.0029	-

Table 3. Analysis of variance for total seedling biomass.

Source of variation	Degree of freedom	Sum of square	Mean Square	F value	Significance level
Replicate (R)	4	0.008	0.002	3.03	P< 0.05
Harvest (H)	1	1.05	1.05	1606.06	P< 0.001
Inoculation(I)	1	1.39	1.39	2106.06	P< 0.001
Interaction (H x I)	1	0.283	0.283	428.78	P<0.001
Error	12	0.008	0.00066		
Total	19	2.739	0.144		

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