

## Response of Three *Glomus* Species on Growth of *Prosopis juliflora* Swartz at High pH Levels

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### Abstract

Relative tolerance of three *Glomus* species: *G. mosseae*, *G. fasciculatum*, and *G. macrocarpum* to graded pH levels (7.8 to 10.5), and their influence on productivity and P uptake in *Prosopis juliflora* Swartz seedlings was investigated. Increase in pH adversely effected seedling growth, their biomass and P concentration. Chlamydospore formation in the rhizosphere soil by all the three *Glomus* species decreased with increase in pH. Application of vesicular-arbuscular mycorrhizal (VAM) resulted in a significant increase in root-shoot length, collar diameter and seedling biomass even at high pH levels. Mycorrhizal plants grown at pH 10.5 reached a level of biomass equivalent to that of non-inoculated ones grown at soil pH of 7.8. P concentration in both root and shoot tissues increased significantly in VAM inoculated seedlings. Root P concentration increased by 68% in VAM inoculated seedlings at pH 7.8, however the increase was only 40% at 10.5 pH. Increase in growth of VAM inoculated seedlings can be attributed to enhanced P uptake. Variations between three *Glomus* species were insignificant, yet *G. fasciculatum* originally isolated from high pH soil site (pH 9.2) had relatively high tolerance to pH 8.5 to 10.5 compared to other species, as exhibited by higher degree of chlamydospore formation and root colonization. The study suggests that VAM isolates can tolerate high pH and assist in alleviating stress of high pH by promoting P uptake and seedling growth.

Keywords: *Prosopis juliflora*, *Glomus fasciculatum*, *G. mosseae*, *G. macrocarpum*, pH, Phosphorus

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## 1. Introduction

*Prosopis juliflora* Swartz (Leguminosae) is a promising tree species for it can meet wood-fuel and fodder requirements in arid and semi-arid conditions (Ibrahim, 1992). It is well adapted to a wide range of ecological conditions, however, its establishment and productivity is adversely affected on alkaline soil sites. Juniper and Abbott (1993) demonstrated that vesicular-arbuscular mycorrhizal (VAM) fungi assist in amelioration of saline soils leading to increased plant growth. There are several reports on increased stress tolerance of the host plants due to mycorrhizal colonization (Hartmond et al., 1987). Heijne et al. (1996) suggested that VAM infection decreased the stress caused by acidity (low pH). Earlier, Hirrel and Gerdemann (1980) and Ojala et al. (1983) reported that VAM fungi enhance salinity tolerance in host plants. On the other hand, soil acidity has been reported to restrict the ability to form mycorrhizae with *Glomus* species (Abbott and Robson, 1985).

The beneficial effects of mycorrhizae on plant growth have often been related to the increase in uptake of immobile nutrients, especially phosphorus (Bolan, 1991). Rate of P uptake in mycorrhizal plants is faster than in non-mycorrhizal plants (Smith, 1982). Azcon et al. (1976) reported effective utilization of rock phosphate in alkaline soils by plants inoculated with VAM fungi.

Wood-fuel plantations are usually raised on nutrient poor, degraded and salt-stress soil sites. The buffering capacity of these soils is low. There have been few investigations on response of VAM fungi to high pH stress, particularly with reference to wood-fuel and fodder tree species. The objective of this study was to assess the relative tolerance of three *Glomus* isolates to high pH; and their influence on productivity of *P. juliflora* seedlings.

## 2. Materials and Methods

### *Plant material*

Seeds of *Prosopis juliflora* were obtained from Biomass Research Centre (Banthra) of the National Botanical Research Institute, Lucknow. Seeds were washed with commercial bleach (0.5%) for 10 minutes and rinsed thoroughly with sterilized distilled water. Seedlings were raised in plastic trays containing sterilized sand and vermiculite (1:1).

### *Soil*

The sandy loam soil used in the experiment had pH of 7.8 and low electrical

conductivity ( $1.62 \text{ dS m}^{-1}$ ) of the saturation extract. Available P, total K and Na in the unamended soil were  $55.3 \mu\text{g}$ ,  $3.7 \text{ mg}$  and  $1.03 \text{ mg g}^{-1}$  soil, respectively. Total soil N and organic C were 0.09% and 0.24%, respectively with a C/N ratio of 2.66. The soil was air dried, sieved through 2 mm sieve and autoclaved twice over a three day period. Robust 20-days-old seedlings were transplanted in  $15 \times 15 \text{ cm}$  plastic pots containing 2 kg of dry soil.

### Treatments

The experimental design was a randomized complete factorial with four pH levels and four VAM treatments. Each treatment had eight replications for a total of 128 units. Soil with pH 7.8 was treated as control and designated as T1. Soil pH was modified using  $\text{Na}_2\text{CO}_3$  at the rate of 200 mg, 500 mg and 1200 mg  $\text{kg}^{-1}$  soil that resulted in pH values of 8.5, 9.5 and 10.5 designated as T2, T3 and T4, respectively. Four VAM treatments had a non-inoculated control and three isolates of VAM fungi. These isolates tested were: *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe (VAM1), obtained from Dr. K.K. Janardhanan of Central Institute of Medicinal and Aromatic Plants, Lucknow; a selected isolate of *Glomus fasciculatum* (Thaxter sensu Gerd.) Gerd. & Trappe (VAM2), initially isolated from *Casuarina equisetifolia* (Sidhu et al., 1990) and maintained at our laboratory for mass scale application; *G. macrocarpum* Tul. and Tul. (VAM3), obtained from Bhartiya Agro Industry Foundation, Pune. The VAM inoculum consisted of 10 g chopped roots and soil from 3-months-old pot cultures maintained on *Cenchrus ciliaris* grass roots in sand-vermiculite (1:1) medium. The non-inoculated treatment received 10 g of autoclaved inoculum. The inoculum was placed 20 mm below the soil surface at the time of transplanting in each treatment. The plants were grown for 12 weeks in a glass house with day/night regimes of 12 h, 30/21°C and 45/60% relative humidity and watered when necessary. pH of the soil media were periodically tested and maintained.

### Measurements

Plants were harvested 90 days after transplanting. Roots were measured for their length and biomass (dry weight) per plant. Similarly, data for shoot length, its diameter at collar (5 cm from the soil surface) and biomass per plant were recorded. The root samples were stained according to the method of Phillips and Hayman (1970). The degree of VAM infection was estimated in the stained roots by the grid-line intersection method of Giovannetti and Mosse (1980). Spores of VAM fungi from rhizosphere soil of experimental pots were isolated by wet sieving and decanting (Gerdemann and Nicolson, 1963). After

Kjeldahl digestion of dried plant material, phosphorus concentration of shoots and roots was determined spectrophotometrically using a Flow Injection Analyzer (Tecator, Model 5010, Sweden).

#### *Statistical analysis*

Root-shoot length and biomass, collar diameter and total seedling biomass were analyzed using a two-way ANOVA, completely randomized with mycorrhizal and pH treatments as the two main factors. All pair-wise comparisons were analyzed using Knewman-Keuls multiple range test to demonstrate differences between groups.

### **3. Results**

#### *Effect of pH on seedling growth and P uptake*

Increase in soil pH significantly reduced seedling growth. Root-shoot length and seedling biomass decreased with increase in soil pH (Table 1). Shoot length decreased from 63.4 cm in T1 to 34.4 cm in T4 with a gradual but significant decrease with increase in soil pH. Differences in collar diameter between T1-T2 and T2-T3 were not significant, however, collar diameter reduced significantly ( $P < 0.001$ ) in T4 as compared to the control. Seedling biomass in T4 was less than one-third that of T1, indicating the adverse effect high pH had on seedling growth. Increase in soil pH also restricted P uptake by the seedlings. Although differences in P concentration in roots of T1-T2, T2-T3 or T3-T4 were not significant, yet decrease between T1 and T4 was highly significant ( $P < 0.001$ ). Similar decrease in P uptake was observed in shoots (Table 1).

#### *Effect of pH on VAM colonization*

Percent root colonization in plants inoculated with VAM1, VAM2 and VAM3 at pH 7.8 was 67.6, 70 and 66.3%, respectively. It decreased to 33.5, 40.5 and 36.3% when the pH of soil was 10.5 (Fig. 1). Differences in percent root colonization between T1 and T2 were not significant, however the same dropped significantly at higher levels of pH. This response was nearly uniform for all the three VAM fungi. Ninety days after inoculation, VAM1, VAM2 and VAM3 produced 229, 235 and 233 spores per 100 g soil, respectively. Spore formation reduced with increase in soil pH. Spore formation in VAM1 and VAM3 treatments was 28 and 32% lower than in VAM2 at soil pH of 8.5 (Fig. 2).

Table 1. Effect of three VAM fungi on seedling growth and phosphorus uptake in four pH treatments

Variables	Treatments VAM	Soil pH			
		7.8	8.5	9.5	10.5
Shoot length, cm	Control	63.4	58.3	49.4	34.4
	VAM1	83.6	73.1	63.4	42.3
	VAM2	86.4	81.7	68.2	42.6
	VAM3	74.3	72.5	62.5	37.4
Root length, cm	Control	49.4	44.5	29.6	29.5
	VAM1	59.6	41.6	37.7	34.6
	VAM2	60.4	57.5	40.6	39.4
	VAM3	56.4	40.4	35.5	32.6
Collar diameter, cm	Control	2.62	2.51	2.49	1.84
	VAM1	3.75	3.65	3.37	2.23
	VAM2	3.98	3.69	3.51	2.29
	VAM3	3.68	3.58	3.31	2.17
Root biomass, g/plant	Control	0.63	0.59	0.52	0.16
	VAM1	1.04	1.04	0.93	0.39
	VAM2	1.12	0.97	0.95	0.47
	VAM3	0.95	0.89	0.87	0.45
Shoot biomass, g/plant	Control	3.34	3.09	2.40	1.00
	VAM1	5.62	5.07	4.58	2.68
	VAM2	5.71	5.56	5.34	2.65
	VAM3	5.36	5.18	4.75	2.08
Total biomass, g/plant	Control	3.97	3.68	2.92	1.16
	VAM1	6.66	6.11	5.51	3.07
	VAM2	6.83	6.53	6.29	3.12
	VAM3	6.31	6.07	5.62	2.53
Root phosphorus, mg/g dry wt	Control	1.04	0.96	0.92	0.80
	VAM1	1.78	1.52	1.16	1.12
	VAM2	1.65	1.28	1.28	1.07
	VAM3	1.59	1.20	1.12	1.08
Shoot phosphorus, mg/g dry wt	Control	2.75	2.60	2.42	1.92
	VAM1	4.00	3.68	3.36	2.52
	VAM2	4.04	3.03	2.88	2.38
	VAM3	3.75	3.20	2.81	2.44

VAM1 = *Glomus mosseae*; VAM2 = *G. fasciculatum*; VAM3 = *G. macrocarpum*.

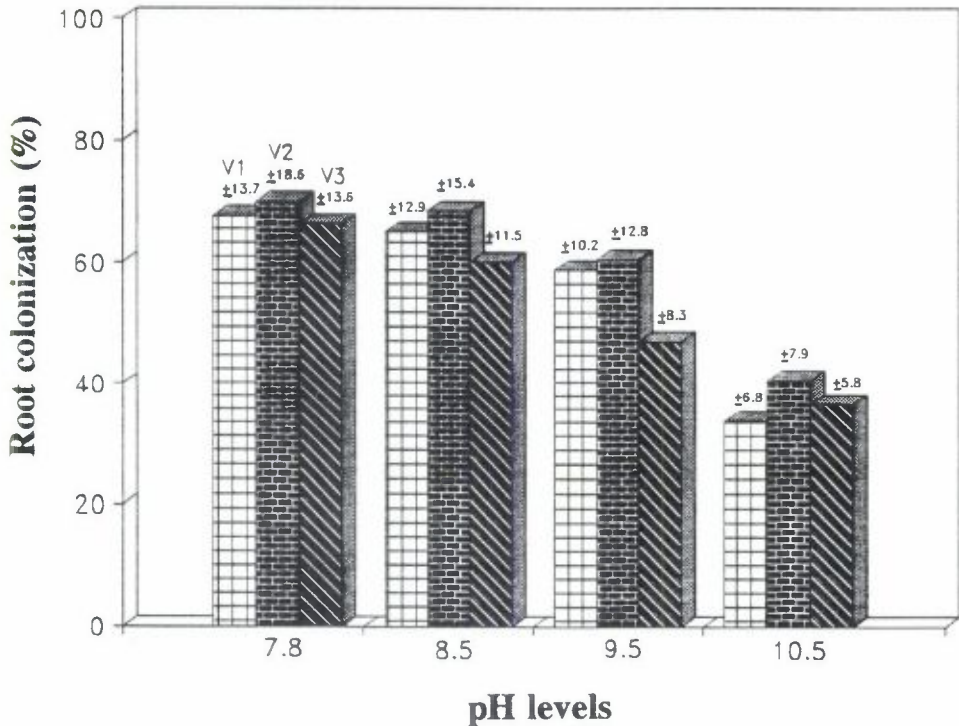


Figure 1. Percent root colonization in seedlings inoculated with *G. mosseae* (V1), *G. fasciculatum* (V2) and *G. macrocarpum* (V3) at four pH levels.  $\pm$  = standard error.

Inoculation with all the three isolates significantly ( $P < 0.001$ ) increased seedlings growth for all the growth parameters as compared to non-mycorrhizal controls.

#### *Interactions between VAM and pH treatments*

Non-inoculated plants were free of mycorrhizal colonization, while the roots of inoculated plants in all the three VAM treatments were adequately colonized. All plant growth parameters analyzed showed that mycorrhizal seedlings grew better than non-mycorrhizal ones at all the pH levels, however the three VAM isolates affected plant growth differently (Table 1). Mycorrhizal treatments significantly increased ( $P < 0.001$ ) root-shoot length and biomass and consequently seedling biomass as compared to non-mycorrhizal control. Even in extreme pH of 10.5, mycorrhizal seedlings had nearly 25% higher shoot and root length and 150% higher seedling biomass than control.

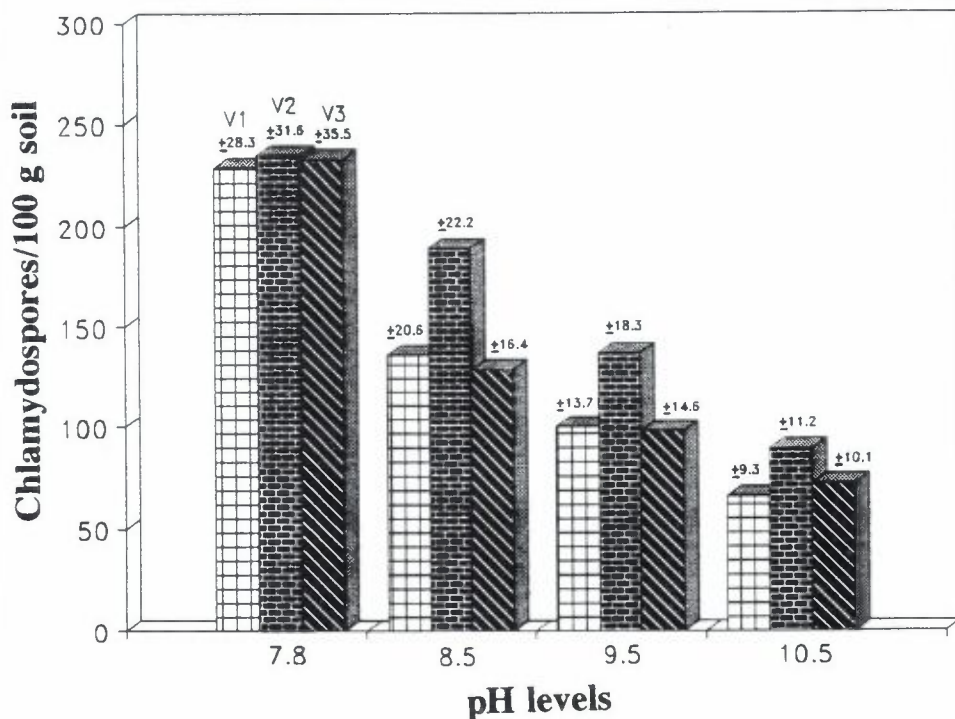


Figure 2. Number of chlamydospores per 100 g soil in seedlings inoculated with *G. mosseae* (V1), *G. fasciculatum* (V2) and *G. macrocarpum* (V3) at four pH levels.  $\pm$  = standard error.

Total biomass of VAM colonized seedlings was 6.3 to 6.8 g/plant in T1, 6.1 to 6.5 g/plant in T2, 5.5 to 6.3 g/plant in T3 and 2.5 to 3.1 g/plant in T4. The increase in seedling biomass varied between 40% to 150% as compared to non-inoculated control at different soil pH levels. In contrast to shoot dry weight, root dry weight was less affected by various VAM treatments leading to lower root/shoot dry weight ratios in mycorrhizal plants. At pH 9.5, shoot dry weight in mycorrhizal plants increased by an average of 103% as compared to 80% increase in root biomass over non-inoculated treatment. A similar response was observed in T1 and T2.

At all soil pH levels, concentration of P in shoot and root of mycorrhizal seedlings was significantly ( $P < 0.001$ ) higher as compared to non-mycorrhizal ones (Table 1). The mycorrhizal plants grown at a soil pH of 10.5 reached a level of shoot or root P concentration equivalent to that of non-mycorrhizal plants grown at a soil pH of 7.8. Irrespective of soil pH, the total shoot and root

P concentration per unit length of the mycorrhizal seedlings was 25 to 40% higher than that of non-mycorrhizal seedlings.

Comparison between VAM fungi and pH treatments are shown in Table 2. The differences in growth parameters in pH  $\times$  VAM treatments were significant at  $P < 0.001$ , while variations in P concentration of root and shoots were significant at  $P < 0.01$ . Response of three *Glomus* species in different pH treatments showed variable results for different parameters studied. For example, variation in collar diameter were not significant for VAM1 vs VAM2 or VAM2 vs VAM3. However, differences in shoot length, root length and seedling dry weight between VAM1, VAM2 and VAM3 were significant at all the pH levels.

Table 2. Source of variations (presented as F ratio) in growth and phosphorus concentration of seedlings grown at pH 7.8, 8.5, 9.5 and 10.5 with or without VAM fungi

	Source of variance		
	Soil pH treatments	VAM treatments	Interactions (pH $\times$ VAM)
Shoot length, cm	1094.4***	244.8***	11.5***
Root length, cm	530.4***	112.9***	14.5***
Collar diameter, cm	368.7***	199.4***	8.2***
Root biomass, g/plant	1066.9***	554.4***	11.6***
Shoot biomass, g/plant	1224.5***	800.1***	14.9***
Total biomass, g/plant	2056.4***	1277.8***	20.6***
Root phosphorus, mg/g dry wt.	102.7***	93.6***	6.7**
Shoot phosphorus, mg/g dry wt.	198.9***	108.2***	7.0**

Results are of two-way ANOVA (pH, VAM treatments, and interactions of pH  $\times$  VAM treatments) = 9, total = 79; \*\* =  $P < 0.01$ , \*\*\* =  $P < 0.001$ .

Differences in root and shoot P concentration between VAM1-VAM2 and VAM2-VAM3 were significant upto pH 9.5. However, these differences were insignificant at pH 10.5. Similarly no significant variation could be observed between VAM1 vs VAM3 at high pH suggesting that at pH 10.5 all the three *Glomus* species behaved nearly similarly with respect to P uptake.



#### 4. Discussion

Higher levels of pH substantially reduced seedling growth and P uptake in *P. juliflora* seedlings. Seedling biomass at pH 10.5 reduced to nearly one-third that of control plants. Inhibition of growth in long term exposure (days) to salt stress can result from osmotic effects on water availability, net CO<sub>2</sub> assimilation, specific ion effects or ion imbalance due to interference with uptake of essential nutrient ions (Lauchli, 1986; Munns and Termaat, 1986). The primary effects of salt stress are reduction in leaf growth rate, leaf emergence rate and overall shoot development (Aslam et al., 1986). We observed that root and shoot length, collar diameter, seedling biomass and P concentration reduced significantly with increase in soil pH.

Growth of seedlings and its P concentration was significantly increased by mycorrhizal inoculation as compared to non-inoculated control at all the pH levels analyzed. These findings are supported by others who have reported similar responses with increase in salinity (Allen and Cunningham, 1983; Pond et al., 1984; Poss et al., 1985) and alkalinity (Azcon et al., 1976). Mycorrhizal plants grown at pH 10.5 reached a level of biomass equivalent to that of non-inoculated plants grown at soil pH of 7.8. Seedling biomass, which is a reliable parameter of plant growth, increased significantly in VAM inoculated plants from pH 7.8 to 10.5.

Root colonization percentage by VAM fungi decreased in high pH soils, yet 42 to 50.4% root colonization was observed even at 10.5 pH. Root colonization and chlamydospore formation by the three *Glomus* species decreased with the increasing levels of soil pH. Ho (1987) surveyed alkaline desert soils and found VAM colonization of the roots of salt tolerant grasses and reported that the number of spores of VAM fungi was inversely correlated with sodium concentration of soil but were not related to pH, conductivity and concentration of a range of other cations. However, Hirrel (1981) and Juniper and Abbott (1991) have reported that increasing concentrations of NaCl inhibit VAM spore germination.

*G. fasciculatum* produced relatively higher number of chlamydospores at all pH levels as compared to other two isolates suggesting its tolerance to higher pH levels. The present study supports the view that isolates selected from high pH soil sites would have better performance on similar sites since *G. fasciculatum* isolate was originally isolated from high pH soil site (pH 9.2). Considerable physiological variations among isolates of *G. mosseae* was demonstrated by Stahl and Christensen (1991). Earlier Green et al. (1976) and Hepper (1984) showed that VAM spores isolated from soil of a particular pH germinated best at that pH. The concept that VA mycorrhizal fungi are adapted to edaphic conditions characterized in part by soil pH is also

supported by studies of Hayman and Tavares (1985) and Abbott and Robson (1985).

When available phosphorus levels in the soils are low, VAM fungi stimulate significant increase in P uptake resulting in a dramatic increase in plant growth (Mosse, 1973). Relative distribution of inorganic and organic P and availability of these forms depends mainly on soil pH (Sample et al., 1980). Hedley et al. (1982) suggested that changes in pH of the rhizosphere may change the availability of absorbed P to plants. Enhanced P concentration of roots and shoots of VAM inoculated seedlings suggests that the three *Glomus* species had a high tolerance limit for soil pH. P concentration of mycorrhizal seedlings at pH 10.5 was significantly higher than non-inoculated control. Apparently, enhanced P uptake facilitated increase in seedling growth in high pH stress conditions. Earlier, increase in tolerance to salinity by mycorrhizal plants have been attributed to enhanced mineral nutrition, particularly phosphorus by Graham (1986). Poss et al. (1985) reported that the effects of mycorrhizal fungi on onion and tomato plants in a saline environment could be duplicated by P fertilization.

With better P nutrition, plants can invest relatively more photosynthates (Cokmak et al., 1994). In high pH soils P usually remains chemically non-available to plants. There is no experimental evidence in the present study for enhanced release of organic acids or  $H^+$  by VAM fungi which could have mobilized soil P, however increased P concentration in both root and shoot in mycorrhizal seedlings does indicate better utilization of soil P as compared to non-mycorrhizal plants. Mycorrhizal colonization was effective in enhancing P uptake and plant growth even in high pH soils. VAM fungal amendment as bio-fertilizer application can be recommended for high pH-alkaline soil sites where available P is limited due to alkalinity. Healthy seedlings thus produced will have better chances of establishment in hostile environment.

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