Effect of phosphorus nutrition on growth and mycorrhizal dependency of *Coriaria nepalensis* seedlings

Kiran Bargali and S.S. Bargali*
Department of Botany, DSB Campus, Kumaun University, Nainital-263002, Uttaranchal,
kiranbargali@yahoo.co.in
* Department of Forestry, College of Agriculture, IGAU, Raipur

Abstract: *Coriaria nepalensis* Wall. is a common native shrub species of the Central Himalayan region between 1200 to 2500 m elevation. In present experiment growth and mycorrhizal dependency of *C.nepalensis* seedlings was observed under varying levels of phosphorus availability. For this, seedlings of *C. nepalensis* were raised from seeds of current year crop and kept under two inoculation treatments i.e. (i) inoculation of seedlings with VAM fungi and (ii) uninoculated control. For each treatment, there were four sub- treatments i.e. 0, 30, 60 and 90-ppm P_2O_5 levels. Plants were uprooted after 6 and 12 months after germination. Mycorrhizal development was determined by estimating the percentage of roots that were mycorrhizal. Height, dry mass and phosphorus content in different component of seedling were determined for each treatment.The results showed that VAM fungal inoculation resulted in 50-70% mycorrhizal colonization in *C. nepalensis* roots. The growth of inoculated as well uninoculated seedlings increased with increasing phosphorus level. The effect of VAM inoculation was maximum at 30-ppm phosphorus and towards higher P level the effect became insignificant. This indicates that the extent to which the *C. nepalensis* depended on VAM fungi for dry matter production decreased as the levels of soil P increased. [Nature and Science. 2009;7(6):19-24]. (ISSN: 1545-0740).

Key words: Phosphorus, inoculated, uninoiculated, dry mass, colonization

Introduction

The genus *Coriaria nepalensis* Wall. is an important plant having ability to grow and form monospecific stands on severely eroded hill slopes. It is an actinorhizal shrub having dual symbiotic association with nitrogen –fixing actinomycete *Frankia* and arbuscular mycorrhizal fungi (Tewari et al 2003). Being a nitrogen fixing species it depend on an adequate supply of phosphorus to achieve the potential for relatively rapid growth. Presence of mycorrhizal fungi can indirectly affect the carbon balance of the host through their effect on the phosphorus nutrition of the seedlings. They are well known to enhance the P-uptake of seedlings growing under P-poor conditions (Bowen 1973), this altered p nutrition alone have a substantial impact on the host carbon balance.

It is likely that the formation of VAM will be an important factor in the successful establishment of *C. nepalensis* and other mycorrhiza dependent plants (Jasper et al 1989) in the nutrient deficient soil of the degraded lands. Mycorrhizas are commonly associated with increased plant uptake of P from soil, though the response is determined, in part, by the P status of the soil relative to the physiological requirements of the plant. In present study efforts were made to evaluate the growth responses as well as its dependence on VAM fungi to attain maximum growth under varying levels of soil P.

Materials and Methods

Seedlings of *C. nepalensis* were raised from the seeds of the current year crop collected from a stand of *C. nepalensis* located at 1840 m (south-east aspect) 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 (autoclaved twice at 120^{o}C for 1 h, 2 days). After germination seedlings were transplanted in polybags containing 2 kg of sterilized soil and sand mixture. After establishment of seedlings there were two inoculation treatments i.e. (i) inoculation of seedlings with *Frankia* only and (ii) inoculation of seedlings with VAM fungi and *Frankia*. For each treatment, there were four sub- treatments i.e. 0, 30, 60 and 90-ppm P_2O_5 levels.

Mycorrhizal development was determined by estimating the percentage of short roots that were mycorrhizal as described by Reid et al (1983). Five seedlings were harvested just prior to the initiation of the treatment phase. Additionally five seedlings were harvested from each treatment at six and twelve month's interval. Immediately following harvesting, each seedling was separated into foliage, stem and roots. The foliage and stem were oven-dried for 48 h at 80 ^{o} C and weighed. The roots were thoroughly rinsed with tap water to remove adhering soil particles and then nodules and roots were separately oven dried for 48 h at 80oC and weighed. Plant material was grounded and phosphorus concentration in different component was determined with a spectrophotometer following Jackson (1958).The growth rate (RGR) was determined using the classical growth- analysis equation:

$$RGR = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

where, t is time and W is plant dry weight at time 1 and 2 (Causton and Venus 1981).

Results

Mycorrhizal colonization

The results showed that the VAM fungi inoculum caused mycorrhizal colonization in *C. nepalensis* seedlings in all treatments (Fig.1). The colonization by VAM (MCP) tended to decrease with increasing levels of soil phosphorus (Table 1).

Plant growth

The increase in height and drymass in VAM-inoculated plants compared to uninoculated plants was maximum at P_1 level (16.7% in terms of height and 52% in terms of drymass) followed by plants at P_0 levels (14.8% in terms of height and 33.3% in terms of drymass).Irrespective of inoculation, the height and dry mass of seedlings increased with increasing P level (Table 1). However, the positive effect of inoculation decreased with increasing P level.

Morphological and physiological traits

Leaf weight ratio (LWR) increased with increasing P level in both inoculated and uninoculated plants while root: shoot ratio decreased with increasing P level ((Table 2). At each P level LWR increased in inoculated plants while root: shoot ratio decreased. Leaf number and leaf area per seedling increased with increasing P level and at P level both increased in inoculated plants. While Specific leaf area (SLA; cm^2 g^{-1}) and Leaf area ratio (LAR cm^2 g^{-1}) decreased with increasing P level and at each P level SLA and LAR decreased in inoculated plants (Table 2).

Mycorrhizal dependency

The mycorrhizal dependency(the extent to which a plant benefits from the presence of VAM fungi) of *C. nepalensis* on VAM inoculation increased with the soil P concentration upto P1 level and exhibited the lowest dependence at the highest soil P level (Table 1). The maximum dependence on the VAM inoculation was 34.2 % at P1 level and decreased more than 50% at highest P level.

P content and P utilization efficiency

Significant improvement in P uptake with increasing soil P levels was observed for roots and shoots of both inoculated and uninoculated plants (Fig. 2). The significant increase in p content in inoculated than uninoculated plants was observed at P_0 and P_1 levels and towards higher P levels (P_2 and P_3 levels) the increase was insignificant (Fig. 3). However, the P utilization efficiency was higher in uninoculated plants than their inoculated counter parts, with maximum for uninoculated (1159.51) and inoculated (1053.49) *C. nepalensis* seedlings at P_0 levels (Fig. 4).

Discussion

The response of actinorrhizal *C. nepalensis* inoculated with VAM indicated that this species is able to associate and from a tri-partite symbiotic association. Similar results have been reported for several other leguminous and non-leguminous nitrogen fixing plants (Xie et al 1995). Colonisation by VAM developed in all inoculated seedlings in all P treatments. The taproots as well as laterals adjacent to the taproot were colonised. Only hyphae and vesicles were present and arbuscules were absent. The growth of *C. nepalensis* was substantially increased by addition of phosphorus and by inoculation with VAM fungi. These results suggested that this species depend on VAM for uptake of phosphorus when grown in a nutrient-deficient soil. An increase in the total dry mass yield with increasing soil P level indicates the considerable demands for P by *C. nepalensis*. At each P level mycorrhizal inoculation resulted in an appreciable increase in the growth of seedlings. An increase in growth response due to VAM inoculation has been earlier reported by several worker using different test plants (see Becard and Piche 1989)

The mean concentration of P in the seedlings of *C. nepalensis* at each P treatment in the presence of VAM appeared to be higher than in the seedlings grown in the absence of the VAM. However, differences were not statistically significant towards higher P levels. It is known that phosphorus is a highly immobile element in soil and its demand is much higher than its mobility. Mycorrhizal plants can explore great volume of soil resulting in the increased flow of phosphorus from soil resulting in the increased flow of phosphorus from soil to plants (Webber 1992). Non- mycorrhizal plants growing in low to moderately fertile soils frequently require a supply of soluble phosphate in order to improve their P nutrition and growth. Such improvement in phosphorus uptake by roots occurs through improving physical exploration of the soil.

Root: shoot ratio decrease with increasing P level and at each P level root: shoot ratios were lower in inoculated plants as compared to uninoculated plants. At low P level seedlings plants enhance light interception by means of a high biomass allocation to leaves and the formation of thin leaves with high SLA leading to a high LAR. With increasing P levels leaf thickness increased this increasing photosynthetic capacity of leaf as indicated by decreased SLA and LAR. At each P level VAM inoculation resulted in decreased SLA and LAR.

In order to examine how VAM fungi can benefit *C. nepalensis* and what condition are required for their development, it is essential to understand that plant inoculated with VAM fungi differ markedly in their growth responses (Daft and Nicolson 1969). This growth stimulating effect, referred to as mycorrhizal dependency (Gerdemann 1975) is highly variable (Sanders et al 1977) and influenced by several factors such as plant species (Gerdemann 1968), and soil fertilization (Hayman and Mosse 1971). Mycorrhizal dependency of *C. nepalensis* seedling was maximum at P_1 level and decreased towards higher phosphorus level.

Table 1. Effect of VAM inoculation on growth of *C.nepalensis* seedlings at different P levels. Values in parenthesis indicate MIE (% mycorrhizal inoculation effect)

Treatments	Root colonization (%)	Height (cm seedling^{-1})	Root weight (g seedling^{-1})	Stem weight (g seedling^{-1})	Leaf weight (g seedling^{-1})	Nodule weight (g seedling^{-1})	Total weight (g seedling^{-1})	Relative growth rate (g g^{-1}d^{-1})
P_o	70	27	0.55	0.43	0.86	0.05	1.89	0.0055
P_o + VAM	67	31 (12.9%)	0.65 (15.4%)	0.58 (25.8%)	1.20 (28.3%)	0.13 (61.5%)	2.56 (26.2%)	0.0063
P_1	70	30	0.70	0.50	1.25	0.08	2.53	0.0063
P_1 + VAM	63	35 (14.3)	1.05 (33.3%)	0.85 (41.2%)	1.94 (35.6%)	0.15 (46.7%)	3.89 (34.9 %)	0.0075
P_2	61	32	0.75	0.75	1.67	0.12	3.29	0.0070
P_2 + VAM	58	36 (11.1%)	0.86 (12.8%)	0.88 (14.7%)	2.18 (23.39%)	0.18 (33.3%)	4.10 (19.75)	0.0076
P_3	55	35	0.98	0.76	2.04	0.15	3.39	0.0075
P_3 + VAM	50	39 (10.2%)	1.07 (8.4%)	0.85 (10.5%)	2.37 (13.9%)	0.20 (25%)	4.49 (12.47%)	0.0079

Table 2. Effect of VAM inoculation on various morphological and physiological traits of *C.nepalensis* seedlings at different P levels.

Treatments	LWR	R:S	Leaf (no seedling^{-1})	Leaf area (cm^2 seedling^{-1})	LAR (cm^{2}g^{-1})	SLA (cm^{2}g^{-1})
P_o	0.455	0.426	78	246	130.16	286.04
P_o + VAM	0.469	0.365	84	254	99.22	211.67
P_1	0.494	0.401	80	252	99.60	201.6
P_1 + VAM	0.498	0.390	86	260	66.83	134.02
P_2	0.507	0.309	84	266	80.85	159.28
P_2 + VAM	0.536	0.281	90	272	66.34	119.29
P_3	0.519	0.350	85	268	68.19	131.37
P_3 + VAM	0.528	0.332	90	280	62.36	118.14

LWR= Leaf weight ratio; R:S = Root: shoot ratio; LAR = Leaf area ratio; SLA= Specific leaf area

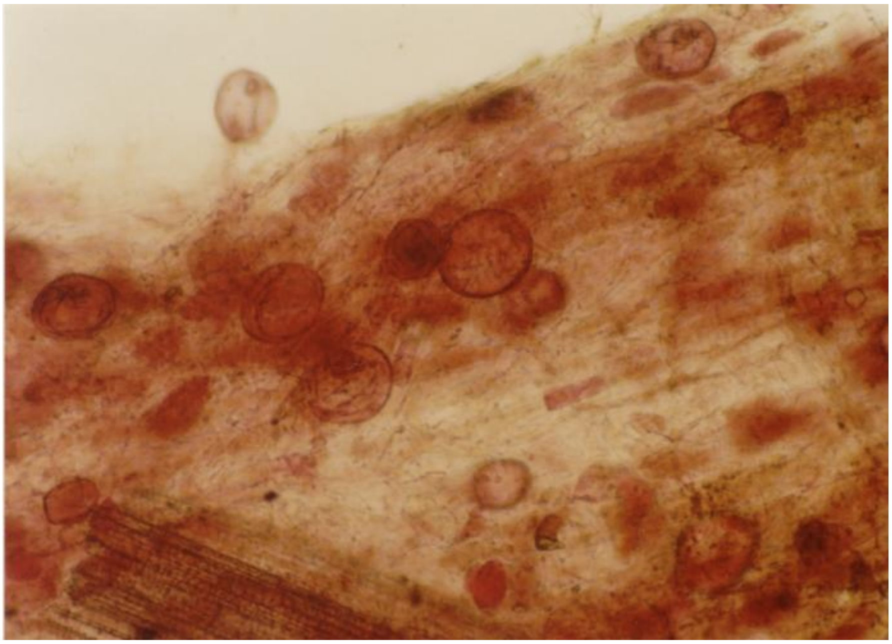

Fig. 1. VAM colonization in *Coriaria nepalensis* roots.

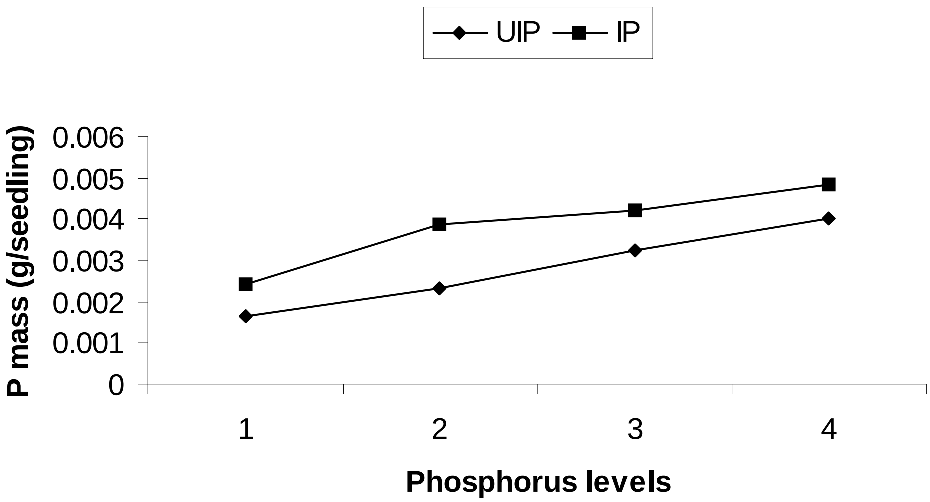

Fig.3. Phosphorus mass of inoculated and uninoculated seedlings of *C. nepalensis* seedlings at different P levels. UIP= Uninoculated plants; IP= Inoculated plants

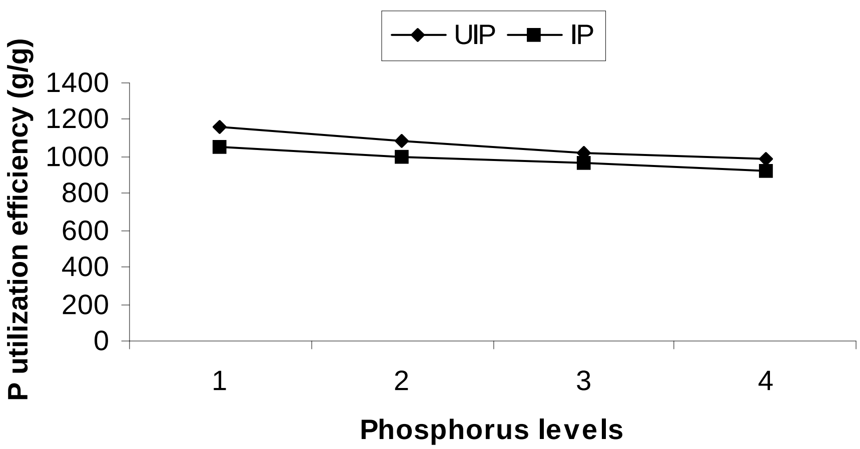

Fig. 4. Phosphorus utilization efficiency of C. nepalensis seedlings as affected by VAM inoculation and P levels. UIP = Uninoculated plants; IP= Inoculated plants

Conclusion

Our results underline the importance of considering VAM as an essential for optimising fertilizer efficiency in the growth increment of N_{2}- fixing plants like *C. nepalensis* in nutrient-poor soils. Use of VAM as a biofertilizer is a low cost technique and can be helpful in the establishment of plants in degraded lands.

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4/4/2009