

Response of *Grindelia camporum* to P-amendment and VAM Inoculation in a Gomti Entisol

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Abstract: *Grindelia camporum* plants were grown at 5 graded levels of applied P, with and without *Glomus fasciculatum* and *Scutellopora callospora* inoculation, in a Gomti entisol. VAM improved plant growth in terms of height, average leaf area and the shoot biomass. At 50 mg kg⁻¹ P₂O₅, VAM largely annulled its adverse effect on capitula yield, which has maximum grindelane diterpenoids; it increased the total yield of grindelane diterpenoids, specially in its ethyl acetate extractable hydrocarbon fraction. The beneficial effects of VAM could be attributed to its effect on root growth, uptake and translocation of P in the plant system and on the resulting biomass.

Key words: *Grindelia camporum*, grindelane diterpenoids, P-amendments, petrocrop, VAM.

Grindelia camporum Greene (family Asteraceae), commonly known as gum plant, has the yield potential of 11 t biomass or 12.7 bbl hydrocarbon ha⁻¹ yr⁻¹ (Hoffmann, 1983). Besides grindelane diterpenoids as the major component of hydrocarbons, the plant has flavenoids, acetin, kumafekenin, quercetin and grindelic acids (Timmermann *et al.*, 1983). High resin content makes the plant suitable for many applications like adhesives and protective coatings. Grindelane acids also have aphid-feeding-deterrent activity (Rose *et al.*, 1981).

Vesicular Arbuscular Mycorrhiza (VAM) association with roots of tree and shrub species, including agricultural plants, is known to improve plant growth through enhanced P availability and uptake (Harley, 1989). The association of *Glomus* sp. with a shrubby resiniferous petrocrop *Grindelia camporum* Greene (Sidhu *et al.*, 1990) is known. The present study was conducted

to know the P-nutritional behaviour of such an association with respect to growth and productivity of *G. camporum*.

Materials and Methods

A pot-culture study was made in a loamy-sand (coarse sand 0.5%, fine sand 71%, silt 24.5% and clay 4%) Gomti entisol, having pH (1:2.5) 6.7, CaCO₃ 1.22%, organic matter 0.67% and electrical conductivity (1:2) 0.4 dS m⁻¹. The soil was low in Olsen's available P (3.4 mg kg⁻¹) and marginal in DTPA extractable Zn (0.75 mg kg⁻¹). The soil was sun-dried for 7 days with maximum day-time air temperature ranging between 42° and 44°C. The dried soil was sieved through 45 µm sieve to get soil relatively free of VAM spores. Such soil had an average of 60 VAM spores per 100 g soil.

The bulk soil was amended with N-25, K-22, Cu-2, Zn-2.5, Mo-0.5 and B-0.5 mg kg⁻¹ (w/w) as NH₄NO₃, K₂SO₄·5H₂O, ZnSO₄·7H₂O, (NH₄)₂ MoO₇·24H₂O and H₃BO₃, respectively, and was divided into 5 lots. To these soil lots, P was applied at 5 graded P₂O₅ levels (0, 5, 12.5, 25 and 50 mg kg⁻¹) as Na₂HPO₄·2H₂O. The supply of Na at each level of P was equated through Na₂SO₄.

Each P level soil, thus prepared, was divided into two lots. While one lot was left as such (-V), the other lot was inoculated with VAM (+V). The soil VAM inoculum had two VAM species - *Glomus fasciculatum* [(Thax. sensu Gerd.) Gerd. Trappe Gerd.)] and *Scutellospora callospora* (Nicol. Walker and Sand.). VAM inoculum was obtained from Tree Biology Division, NBRI, Lucknow, and multiplied on *Grindella camporum* Greene roots in pot culture using sterilised sand-vermiculite (1:1). 40 g VAM inoculum from such 5-month-old culture containing approximately 120 chlamodospores of *Glomus fasciculatum* and 80 azygospores of *Scutellospora callospora*, along with infected root bits (hyphal inoculum), was inoculated to the +VAM treatment soil series by the method of Sidhu and Behl (1992) who used 50 and 100 VAM spores as inoculum. The inoculum was placed 20 mm below the soil surface at the time of transplanting of the one-month-old *G. camporum* seedlings in soil pots.

Grindella camporum seedlings were initially raised in purified sand. Thirty-day-old seedlings were transplanted in both +V and -V series of graded P amended soils filled in clay pots (25 cm diam) having a drainage hole and with their inside surfaces painted

twice with bitumen and lined with alkathene. The plants were periodically irrigated upto field capacity with de-ionised water.

At harvest, after determining fresh biomass yield (of tops and roots, separately), fresh plant samples were thoroughly washed and oven-dried at 70°C for 48 h and estimated for biomass. Portions of oven-dried plant material were acid digested (Piper, 1942) and estimated for P colorimetrically by the method of Nicholas as described by Wallace (1961).

Soil samples, drawn at harvest, were analysed for Olsen's available P colorimetrically (Watanabe and Olsen, 1965). A mycorrhizal dependency factor was determined for each soil (Kitt *et al.*, 1988) as under:

$$\left[\frac{(\text{d. wt. of plants in inoculated soil}) - (\text{d. wt. of plants in non-inoculated soil})}{(\text{d. wt. of plants in inoculated soil})} \right] \times 100.$$

A portion of the above-ground plant parts dried at 45°C was ground in a Wiley mill and utilised for the sequential soxhlet extraction and estimation of ethylacetate and methanol extractable hydrocarbons (Timmermann *et al.*, 1983).

VAM infection was studied as described by Phillips and Hayman (1970). The spores of VAM fungi were isolated from the soil surrounding the roots by wet sieving and decantation following the method of Gerdemann and Nicolson (1963) and their numbers recorded g⁻¹ soil.

The correlation coefficients were computed to determine the linear relationship among the characters.

Table 1. Effect of VAM inoculation along with graded levels of P application to a Gomti entisol on root colonization, available soil P, growth attributes and biomass in *G. camporum* (N = 3)

Treatment (mg kg ⁻¹ P ₂ O ₅)	VAM inocul- ation (+ or (-))	Root infection (%)	Av. soil P (mg kg ⁻¹)	Height (cm)	Leaf area (cm ²)	Dry matter (g plant ⁻¹)		
						Capitula	Stover	Roots
0	-V	-	4.0	39.5	7.8	2.5	11.7	1.3
	+V	71.32	3.8	44.0	9.7	3.2	12.8	4.0
5	-V	-	5.8	47.0	9.0	3.4	12.1	2.6
	+V	70.81	5.2	55.8	12.1	4.4	14.6	4.6
12.5	-V	-	9.0	59.8	13.2	4.9	13.6	3.9
	+V	72.63	7.8	61.5	14.1	4.5	16.5	6.1
25	-V	-	11.8	55.5	9.4	4.0	12.7	3.1
	+V	62.13	9.4	60.0	9.7	4.7	19.4	5.6
50	-V	-	15.2	50.0	8.3	2.9	9.0	2.3
	+V	61.61	13.6	49.8	8.4	4.1	12.9	2.8
LSD P 0.05			0.9	4.2	0.6	0.1	0.3	0.4

Results and Discussion

The detailed examination of roots of *G. camporum* under the compound microscope confirmed the good establishment of the root colonization by introduced VAM (Table 1). It was in the range of 62-72%, being relatively lower (around 62%) at higher (25 and 50 mg kg⁻¹) P₂O₅ levels. After harvest, the fungal spore population of *Glomus fasciculatum* far exceeded that of *Scutellopora callospora*. This indicated that the former can be used as an efficient VAM inoculant, not reported earlier, for *G. camporum* plants. Association of other species of *Glomus* (*G. diamorphicum* and *G. australe*) has, however, been reported for this plant (Sidhu *et al.*, 1990).

Application of phosphate from 5-50 mg kg⁻¹ soil on P-responsive soil increased the available soil P from 4 in control to a range of 5.8 to 15.2 mg kg⁻¹ in the -V and 5.2 to 13.6 mg kg⁻¹ in the +V series

(Table 1). As both mycorrhizal and non-mycorrhizal roots absorb P from the same soil source (Tinker, 1980), the present observation could be due to the additional uptake of P by the VAM fungi. Similar decrease in total soil N by VAM inoculation has been reported in *Hedysarum boreale* (Carpenter and Allen, 1988). Concentration of soil P was generally related to mycorrhizal dependency. The presently used P-deficient soil displayed a high mycorrhizal dependency at nil and 5 mg kg⁻¹ P₂O₅, specially for capitula and total biomass (Table 1). Available P at these levels ranged between 3.8 and 5.8 mg kg⁻¹.

Though higher mycorrhizal dependency in P-deficient soils is known (Kitt *et al.*, 1988), a further high mycorrhizal dependency (34-27) was also presently observed in soils applied higher P (25 and 50 mg kg⁻¹ P₂O₅, respectively). Pfeiffer and Bloss (1988), however, did not find any marked

Table 2. Effect of VAM inoculation along with graded levels of P application to a Gomti entisol on mycorrhizal dependency, growth and biomass attributes and tissue P concentrations in *G. camporum* (N = 3)

Plant part	VAM* inoculation	Applied soil P levels (mg kg ⁻¹ P ₂ O ₅)					LSD P 0.05
		0	5	12.5	25	50	
Mycorrhizal dependency							
Capitula		21.9	22.7	8.8	14.9	29.3	
Rest tops		8.6	17.1	17.6	34.5	30.2	
Total shoots		11.2	18.0	11.9	30.4	28.5	
Total plant (shoots+roots)		22.5	23.4	17.3	33.1	26.8	
Fruit number (plant⁻¹)							
	-V	27.0	41.8	46.0	46.5	32.5	
	+V	46.5	49.0	53.0	66.3	38.3	9.0
100 Capitula weight (g)							
	-V	9.2	8.0	10.7	8.7	8.8	
	+V	6.9	9.0	8.6	7.1	10.7	1.0
Top/Root ratio (biomass)							
	-V	11.4	6.1	5.1	5.9	5.8	
	+V	4.1	4.1	3.5	4.4	6.0	3.1
Tissue P (% dry matter)							
Capitula	-V	0.32	0.40	0.42	0.53	0.65	
	+V	0.47	0.58	0.54	0.57	0.62	0.08
Stover	-V	0.12	0.22	0.26	0.31	0.36	
	+V	0.16	0.25	0.23	0.23	0.45	0.04
Roots	-V	0.10	0.17	0.28	0.30	0.30	
	+V	0.28	0.29	0.31	0.32	0.31	0.05

* *Glomus fasciculatus* and *Scutellospora callospora*.

effect of the soil application of 100 mg kg⁻¹ P on VAM colonisation of guayule roots by *Glomus intradies*.

Growth of plants (height and average leaf area) increased with an increase in the level of applied P (Table 1), the effect being maximum at 12.5 mg kg⁻¹ P₂O₅ in -V, and at 12.5 to 25 mg kg⁻¹ P₂O₅ for height and at 12.5 mg kg⁻¹ P₂O₅ for average leaf area in +V. Thus, VAM inoculation improved the growth of plants at low levels

of P application, generally upto 12.5 mg kg⁻¹ P₂O₅. Maximum increase in biomass of stover (total top-capitula) was also found at 12.5 mg kg⁻¹ P₂O₅ in -V series and at 25 mg kg⁻¹ P₂O₅ in +V series. P application upto 12.5 mg kg⁻¹ P₂O₅ in -V and upto 25 mg kg⁻¹ P₂O₅ in the +V series increased the fruit (capitula) yield mainly due to an increase in the number of capitula per plant (Table 1). Under conditions of P-deficiency, the beneficial effect of VAM on the growth and productivity is known for a variety

Table 3. Effect of VAM inoculation along with graded levels of P application to a Gomti entisol on P uptake and hydrocarbon yield in *G. camporum* (N = 3)

Treatment (mg kg ⁻¹ P ₂ O ₅)	V A M inocul- ation (+) or (-)	P uptake (mg plant ⁻¹)			Hydrocarbon yield (g plant ⁻¹)					
		Capitula	Stover	Roots	Eth. acet. extr.		Meth. extr.		Total extr.	
					Cap	Stov.	Cap.	Stov.	Cap.	Stov.
0	-V	8.1	13.6	1.3	0.26	0.89	0.16	0.78	0.43	1.60
	+V	14.9	19.8	10.1	0.40	0.97	0.20	0.83	0.60	1.89
5	-V	13.4	27.0	4.5	0.37	0.98	0.18	0.66	0.54	1.65
	+V	25.3	35.7	13.4	0.50	1.15	0.27	0.85	0.78	2.00
12.5	-V	20.7	35.6	11.0	0.53	0.97	0.26	0.90	0.79	1.84
	+V	24.3	37.8	18.8	0.51	1.43	0.25	1.03	0.76	2.46
25	-V	21.4	39.4	9.4	0.42	0.90	0.24	0.73	0.66	1.63
	+V	26.4	45.0	17.4	0.50	1.30	0.27	1.58	0.78	2.88
50	-V	18.4	32.4	6.0	0.20	0.64	0.14	0.53	0.34	1.18
	+V	25.1	60.2	8.5	0.43	0.75	0.22	1.03	0.65	1.78
LSD P 0.05			3.0	0.41	0.06	0.30	0.05	0.2	0.16	0.5

of plants - subterranean clover (Pairunan *et al.*, 1980), onion (Manjunath *et al.*, 1981), and *Cymbopogon winterianus* (Kothari and Singh, 1996). However, VAM was also found to significantly negate the adverse effect of supra-optimal P on the capitula, stover and root biomass of *G. camporum*. Though such VAM modulation of the supra-optimal P effect is not well understood, it could be through the accumulation, as polyphosphates, of the excess P taken up by VAM in its arbuscules (Marschner, 1986; Harley, 1989).

Shoot/root ratio of the biomass tended to decrease significantly upto 50 mg kg⁻¹ P₂O₅ level (46.55%) in -V series (Table 2). A remarkable decrease in shoot/root ratio in +V as compared to -V series was observed at no addition of P (64%) and 5 mg kg⁻¹ P₂O₅ level (3%).

Tissue concentration, as well as total content of P in different plant parts gradually increased with an increase in the level of

P application. In all plant parts, the magnitude of such an effect was relatively less pronounced in the +V as compared to -V series, mainly due to the marked increase in the tissue concentration, as well as uptake of P in plants due to VAM when no P was applied. At low doses of applied P, VAM also improved translocation of P to different above-ground plant parts (Tables 2 and 3). Application of P, as well as VAM, decreased top/root ratio of tissue P, the effect of VAM being most marked at low P levels (upto 5 mg kg⁻¹ P₂O₅). The present results could be explained in terms of the beneficial effect of VAM through adequate P-nutrition mediated increased root growth, specially under conditions of reduced or insoluble P supply (Pairunan *et al.*, 1980; Hoffman and Mitchell, 1986; Marschner, 1986; Harley, 1989).

Under conditions of low P-stress, bulk of the P taken up was translocated to the above-ground plant parts (Table 3), but

at higher levels of applied P, there was a tendency of P being retained in the roots. VAM inoculation tended to reverse both the trends. The results thus indicated a beneficial role of VAM on the P-nutrition of plants, including its reallocation to different plant parts.

An increase in the level of applied P, generally upto $12.5 \text{ mg kg}^{-1} \text{ P}_2\text{O}_5$ in the -V and upto $25 \text{ mg kg}^{-1} \text{ P}_2\text{O}_5$ in the +V series, increased the hydrocarbon yield (Table 3) of plant-shoots, including that of fruits (capitula). Magnitude of such an effect was relatively more marked in the +V series, specially at lower P levels. Higher level of P application significantly decreased the hydrocarbon yield of both the extractives in the -V and only of ethyl acetate extractive in the +V series. VAM inoculation, thus, greatly ameliorated the adverse effect of supra-optimal P on the hydrocarbon, specially methanol extractive, yield of plants (Table 3). VAM also slightly increased the total hydrocarbon content of plants at all the levels of applied P. Bloss and Pfeiffer (1984) also observed an increase in the hydrocarbon content in mycorrhizal guayule (*Parthenium argentatum*); however, Kothari and Singh (1996) did not find any influence of VAM on the quality of Citronella oil (diterpenoids).

The present observed beneficial effect of applied P at its lower doses, as well as that of VAM inoculation on the hydrocarbon yield of plants could be explained in terms of their effect on root growth, uptake and translocation of P in the plant system and the resulting biomass (Harley, 1989). Besides, the beneficial effect of P on hydrocarbon yield could also be due

to the specific role of P at several steps in the secondary metabolites biosynthesis, particularly for diterpenoids, the important hydrocarbon constituent of *Grindelia camporum* Greene in the form of Grindelane diterpenoids (Timmermann *et al.*, 1983). P is a known constituent of isopentenyl pyrophosphate in the mevalonic acid pathway of secondary metabolism (Harwood and Russel, 1984).

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References

- Bloss, H.E. and Pfeiffer, C.M. 1984. Latex content and biomass increase in mycorrhizal guayule (*Parthenium argentatum*) under field condition. *Annals of Applied Biology* 104: 175-183.
- Carpenter, A.T. and Allen M.F. 1988. Responses of *Hedysarum boreale* Nutt. to mycorrhizae and *Rhizobium*: Plant and soil nutrient changes in a disturbed shrub steppe. *New Phytologist* 109: 125-132.
- Gerdemann, J.W. and Nicolson, T.H. 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. *Transactions of the British Mycology Society* 46: 235-244.
- Harley, J.L. 1989. The significance of mycorrhiza. *Mycological Research* 92: 129-139.
- Harwood, J.L. and Russel, N.J. 1984. *Lipid in Plants and Microbes*. George Allen and Unwin, London.
- Hoffmann, J.J. 1983. Arid land plants as feed stocks for fuel and chemicals. *Critical Reviews in Plant Science* 1: 95-116.
- Hoffman, M.T. and Mitchell, D.T. 1986. The root morphology of some legume species in south western cape (South Africa) and the relationship of vesicular - arbuscular mycorrhizae with dry-

- mass and phosphorus content of *Acacia saligna* seedling. *South Africa Journal of Botany* 52: 316-320.
- Kitt, G., Hertick, B.A.D. and Wilson, C.W.T. 1988. Relationship of soil fertility to suppression of the growth response of mycorrhizal big blue stem in non-sterile soil. *New Phytologist* 109: 473-482.
- Kothari, S.K. and Singh, U.B. 1996. Response of *Citronella java* (*Cymbopogon winterianus* Jowitt.) to VA mycorrhizal fungi and soil compaction in relation to P supply. *Plant and Soil* 178: 231-237.
- Manjunath, A., Mohan, R. and Bagyaraj, D.J. 1981. Interaction between *Beijerinckia mobilis*, *Aspergillus niger* and *Glomus fasciculatum* and their effects on growth of onion. *New Phytologist* 89: 723-727.
- Marschner, H. 1986. The soil-root interface (Rhizosphere) in relation to mineral nutrition. In *Mineral Nutrition of Higher Plants*, pp. 137-143. Academic Press, Harcourt Brace Jovanovich, Publishers, London.
- Pairunan, A.K., Robson, A.D. and Abott, L.K. 1980. The effectiveness of vesicular-arbuscular mycorrhizae in increasing growth and P uptake of subterranean clover from P sources of different solubilities. *New Phytologist* 84: 327-338.
- Pfeiffer, C.M. and Bloss, H.E. 1988. Growth and nutrition of guayule (*Parthenium argentatum*) in a saline soil as influenced by vesicular-arbuscular mycorrhiza and P fertilization. *New Phytologist* 108: 315-321.
- Phillips, J.M. and Hayman, D.S. 1970. Improved procedure for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycology Society* 55: 158-161.
- Piper, C.S. 1942. *Soil and Plant Analysis*. Waite Agriculture Research Institute. The University of Adelaide, Adelaide.
- Rose, A.F., Jones, K.C., Haddon, W.F. and David, D.L. 1981. Grindelane diterpenoid acids from *Grindelia humilies*: Feeding deterrence of diterpene acids towards aphids. *Phytochemistry* 20: 2249-2253.
- Sidhu, O.P. and Behl., H.M. 1992. Infectivity and effectiveness of VAM fungi in *Casuarina obes* for alkaline soil sites. *Nitrogen Fixing Tree Research Reports* 10: 150-154.
- Sidhu, O.P., Behl, H.M. and Mehrotra, N.K. 1990. Occurrence of vesicular-arbuscular mycorrhiza (Endogonaceae) in *Grindelia camporum* Greene. *Journal of the Indian Botanical Society* 69: 55-58.
- Timmermann, B.N., Luzletal, D.J., Hoffmann, J.J., Jolad, S.D., Schram, K.H., Bates, R.B. and Klench, R.E. 1983. Grindelane diterpenoids from *Grindelia camporum* and *Chrysothamnus paniculatus*. *Phytochemistry* 22: 523-525.
- Tinker, P.B. 1980. Role of rhizosphere micro-organism in phosphorus uptake by plants. *Proceedings of the Symposium on Role of Phosphorus in Agriculture*, pp. 617-654.
- Wallace, T. 1961. *The Diagnosis of Mineral Deficiencies in Plants*. A Colour Atlas and Guide, 3rd edn., HMSO, London.
- Watanabe, F.S. and Olsen, S.R. 1965. Test of an ascorbic acid method for determining phosphorus in water and NaHCO₃ extracts from soil. *Proceedings of the Soil Science Society of America* 29: 677-678.