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INFLUENCE OF VESICULAR—ARBUSCULAR MYCORRHIZA ON GROWTH AND NITRATE REDUCTASE ACTIVITY OF BLACK PEPPER

S. Shivashankar and Rohini Iyer

Central Plantation Crops Research Institute
Kasaragod 670 124

Abstract : Supplementation of the pots with soil containing spores of *Glomus fasciculatum* (Thaxter *sensu* Gerdemann) Gerdemann and Trappe enhanced the root infection from 55 per cent in the control to 98 per cent at the end of five months in black pepper plants. The growth of plants varied directly in proportion with the percentage of root infection. The phosphate levels used in the study did not adversely affect the mycorrhizal population. Mycorrhizal plants showed statistically significant increase in the tissue N, P and leaf nitrate reductase activity over the control. The nitrate compensation point (NCP) was lower in mycorrhizal plants than in the control.

Keywords : Mycorrhiza, Nitrate reductase, Black pepper, *Piper nigrum*, *Glomus fasciculatum*

Mycorrhizae in association with the host plant improve the uptake of phosphorus by plant roots by acting as an auxiliary absorbing system (Sanders and Tinker, 1971). Mycorrhizae also provide other additional benefits to crops, such as tolerance to water stress (Allen, 1982; Menge *et al.* 1978) reduced transplantation shock, better survival in poorly fertilized soils and resistance to certain diseases (Schenck and Kellam, 1978).

Black pepper (*Piper nigrum* L.), a valuable spice crop of the tropics, is grown mainly as an intercrop in coconut and arecanut gardens on the West Coast of India. No report is available on the effect of VAM fungi on the growth of this economically important crop. In this study, therefore, data is presented to show the influence of pre-inoculation with VAM on infection, growth and nitrate reductase (NR) activity of pepper plants.

MATERIALS AND METHODS

Pepper plants (cv. Panniyur-I) raised from single node cuttings in the nursery were planted into pots filled with 3 kg of acid-washed sand and 600 g soil containing, on an averge, 31 spores per 100 g of air-dried soil, as counted by wet sieving and decanting described by Gerdemann and Nicolson (1963). Half the number of experimental seedlings were inoculated with the spores of *G. fasciculatum* after randomizing the pots and transferred to the glass house. Twenty-five plants each in both the inoculated and uninoculated (control) groups were supplemented with 30 mg P/kg soil. Superphosphate (soluble form) and 'Mussoorie phos' (insoluble form) were used for comparison. The pots were watered on alternate days with 100 ml of full-strength Hoagland's solution devoid of phosphorus.

Roots selected at random from each treatment were pooled together, stained with 1 per cent trypan blue, destained and mounted in lactophenol for microscopic examination. Infection per cent was calculated as the number of bits infected per 100 (1-cm bits) samples examined (Phillips and Hayman, 1970).

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Five replicates each of inoculated and control groups were harvested at monthly intervals. A portion of the third leaf was used for biochemical estimations. Leaf NR activity was assayed *in vivo* by infiltrating 200 mg leaf bits in buffered nitrate medium for 2 min and incubated in darkness for 1h. Nitrite produced was estimated colorimetrically following the method of Snell and Snell (1949).

For the experiments on nitrate uptake, five plants from each of the inoculated and control sets maintained in one-litre containers were pretreated for five days in aerated nutrient solution devoid of nitrogen. On the sixth day, the plants were transferred to medium containing 100 μM NO_3^-. The rate of disappearance of NO_3^- from the medium was monitored by measurement of UV absorbance of aliquots at 210 nm (Cawse, 1967) and returned to the containers quantitatively. The volume of solution in containers was maintained constant throughout the experiment by periodic addition of distilled water. The difference in NO_3^- concentration from the initial value was taken as the net NO_3^- uptake.

Total nitrogen was determined by the microkjeldahl procedure (AOAC, 1975). Total phosphorus in tissues was estimated by vanado-molybdate procedure (Jackson, 1967). The total leaf area of individual plants was measured using an electronic leaf area meter LI-3000 (Licor, USA). The root, stem and leaf parts were then dried in an electric oven and dry weights of all parts determined separately.

RESULTS AND DISCUSSION

Growth parameters

The effect of VAM infection on the growth of plants is presented in Fig. 1. The rate of increase in dry matter accumulation and leaf area of inoculated plants was higher than those of control plants. Large differences in leaf area and root weight were apparent only three months after the inoculation, which were further enhanced at the fourth month (Fig. 1). At this stage, statistically significant differences in total plant height, leaf area and root weight were observed between the inoculated and control plants (Table 1). However, the differences between the soluble and insoluble forms of phosphorus within both the groups were not signifioant (Table 1).

The large differences in leaf area were due to a higher rate of production of new leaves in inoculated plants. Further, the inoculated plants showed a faster rate of increase in shoot dry weight than the root dry weight. It may be noted that the enormous increase in leaf area of inoculated plants contributed to the increase in shoot dry weight. Thus, the root:shoot ratio of inoculated plants was reduced as compared to the control. Harley (1969) reported similar results and concluded that the low root:shoot ratio of inoculated plants was primarily due to their larger size. Enhanced growth and decreased root:shoot ratio was reported in VAM infected *Vaccinium macrocarpon* by Stribley and Read (1976) also.

The increase in leaf area and dry weight was found to be associated with a simultaneous increase in the root infection (Table 2). The root infection at the time of transplanting into pots in both the groups was 30 per cent. The infection rose to 60 per cent, one month after inoculation, and gradually increased to 98 per cent at the end

TABLE 1 : Growth parameters and NR activity

Source of variation / Months after inoculation	Height (cm)	Leaf area (cm^2)		Root weight (g)		Shoot weight (g)		NR activity (μ moles NO_2^- $h.^{-1}g^{-1}$fr.wt)	
	8	7	8	7	8	7	8	7	8
Control soluble P	139.0	798	920	1.20	2.26	8.7	11.3	1439.9	752.9
Control insoluble P	164.2	709	1015	0.86	1.80	7.9	10.9	1594.9	898.5
Inoculated soluble P	175.0	981	1206	1.95	2.22	9.2	13.0	1630.3	964.3
Inoculated insoluble P	179.8	1018	1321	1.37	2.34	9.6	13.2	1553.5	1530.3
Control	151.6	753	968	1.03	2.03	8.3	11.1	1517.8	825.7
Inoculated	177.4	1000	1264	1.66	2.28	9.6	13.1	1591.9	1447.2
Soluble P	157.0	889	1063	1.58	2.24	9.1	12.2	1535.1	858.6
Insoluble P	172.0	864	1168	1.12	2.07	8.8	12.1	1574.2	1214.4
S.E./Plot	25.3	166	232	5.30	8.36	2.9	3.5	504.8	303.1
Gen. Mean	164.5	877	1116	1.35	2.16	8.9	12.1	1554.7	1036.5
C.V. (per cent)	15.4	18.9	20.8	29.3	28.8	21.4	28.1	32.5	29.2
D. ($P = 0.05$)									
Control vs inoculated	23.9*	157.5†	220.6*	5.03*	—	—	—	—	287.36†
Soluble vs insoluble P	—	—	—	—	—	—	—	—	287.36*

*Significant at $P = 0.05$.
†Significant at $P = 0.01$.

TABLE 2 : Mycorrhizal infection (per cent) at various growth stages of black pepper

Months after inoculation	Plant age (months)	Control			Inoculated		
		Insoluble P	Soluble P	Z value	Insoluble P	Soluble P	Z value
1	5	30.0	30.0	0	60.0	60.0	0
2	6	28.0	40.0	1.79	80.0	85.0	0.93
3	7	37.0	55.0	2.51*	98.0	99.0	0.58
4	8	50.0	66.0	2.29*	98.0	98.0	0
Overall		36.25	—	—	84.0	—	13.79†
Period		—	47.7	—	—	85.5	11.32*

*Significant at $P = 0.05$.
†Significant at $P = 0.01$.

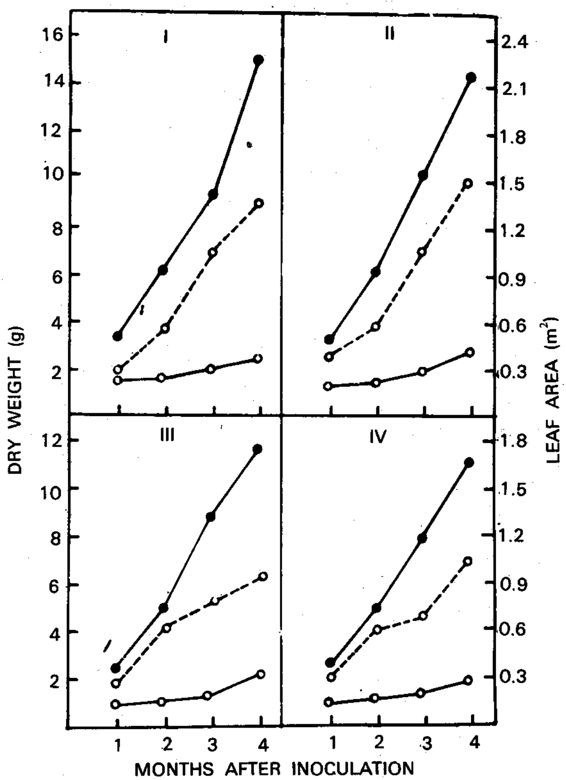

Fig. 1 : Pattern of increase in leaf area and dry weight of pepper plants.
I : Inoculated soluble P; II : Inoculated insoluble P; III : Control soluble P; IV : Control insoluble P
o——o Root dry wt; ●——● shoot dry wt; o·····o Leaf area

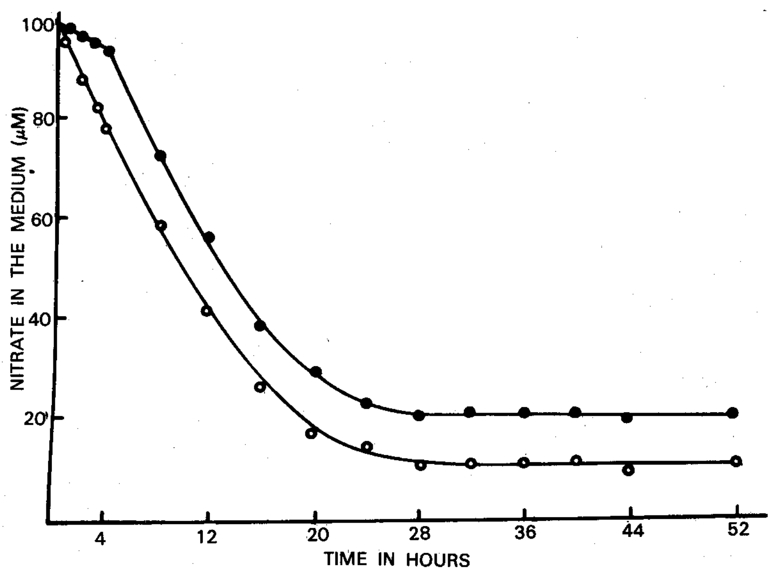

Fig. 2 : Showing the rate of uptake of NO_3^- by inoculated (o——o) and control (●——●) plants

TABLE 3 : Total nitrogen and phosphorus content in inoculated and control plants of black pepper (mg/g dry weight)

Months after inoculation	Plant age (months)	Treatment	Nitrogen			Phosphorus		
			Leaf	Stem	Root	Leaf	Stem	Root
3	7	Control	20.15	31.79	25.50	1.42	1.45	2.04
		Inoculated	22.74	33.40	33.83	1.80	1.84	2.28
		t Value	2.06	1.80	3.41†	4.48†	4.18†	2.48†
4	8	Control	31.95	19.77	31.68	1.44	1.52	2.38
		Inoculated	35.19	21.81	35.58	1.85	2.13	2.45
		t Value	5.05†	3.56†	3.99†	5.10†	4.93†	0.57†

All values represented as mean of 5 samples
*Significant at $P = 0.05$.
†Significant at $P = 0.01$.

of the experiment. The control plants recorded differences between the soluble and insoluble phosphate treatments. An initial increase from 30 to 40 per cent root infection after a month, reaching 66 per cent at the end was observed in the soluble P treatment, whereas the insoluble P group recorded an increase up to 50 per cent only (Table 2). This is in accordance with the results earlier reported by Mosse (1973). It is thus clear that the root infection was found to exert a direct effect on plant growth.

Nitrate reductase activity

The mean NR activity of inoculated plants was distinctly higher than control plants (Table 1). Tissue analysis showed that inoculated seedlings had significantly higher P and N concentration per unit dry weight of tissue than the control seedlings (Table 3). When these values are expressed on the basis of whole plant weight, the differences become far more significant, since the mycorrhizal plants exhibited higher rates of dry matter accumulation. Thus, we find that the enhanced NR activity of inoculated plants resulted in increased accumulation of N, supporting the hypothesis of Hageman *et al.* (1967) that NR is a valuable index of the reduced N status of the plant.

The data obtained from our study shows that the inoculated plants exhibited a faster rate of nitrate uptake and a much lower nitrate compensation point (NCP) of 10 μM as against 20 μM for the control plants (Fig. 1). NCP, that is, the minimum (NO_3^-) below which net uptake of nitrate ceases, has been used as a characteristic of nitrate uptake effectiveness by some workers (Edwards and Barber, 1976 a, b; Theorios *et al.*, 1979). Warncke and Barber (1974) found that plant species differed in the efficiency with which their roots removed nitrate from nutrient solution and reported NCPs ranging from 1.4 to 2.7 μM NO_3^- for some crops. The observed ability of inoculated plants to acquire nitrate at low ambient concentrations and a high rate of uptake even at saturating concentrations of nitrate in the medium are the two characteristics which are important factors in their response to nitrate supply. Thus, it is clear that the VAM infection increases the capacity of pepper roots to utilize the available soil nitrate more efficiently than the control plant roots.

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