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## EFFECT OF *RHIZOBIUM* ON THE INTERACTION OF VESICULAR - ARBUSCULAR MYCORRHIZAE AND ROOT-KNOT NEMATODE ON BLACKGRAM (*VIGNA MUNGO*)

by

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**Summary**. The effect of *Rhizobium* on the interaction of the vesicular-arbuscular mycorrhizae (*Glomus fasciculatum* and *G. mosseae*) and the root-knot nematode *Meloidogyne incognita* on blackgram was investigated in a pot experiment. The interaction of *Rhizobium* with VAM and the nematode resulted in a significant increase in the growth parameters of blackgram. The highest root nodulation was recorded in plants inoculated with *Rhizobium* and *G. mosseae*. Inoculation of *M. incognita* reduced the number of nodules per plant whereas the combination of *Rhizobium* + *G. mosseae* + *M. incognita* resulted in increased nodules and was similar to the *Rhizobium* + *G. mosseae* treatment. The combination of *Rhizobium* and VAM resulted in the least nematode population density and in the maximum spore, mycorrhizal colonization, total nitrogen and phosphorus content of blackgram.

The association of rhizobia with legume hosts has a beneficial effect on plant nutrition and growth unlike the plant-nematode relationship which has an adverse effect on plant growth. As a result of nematode infection, nodulation and nitrogen fixation has been reported to be suppressed or stimulated or remain unaffected. In contrast, inoculation of rhizobia generally has an adverse effect on nematode reproduction (Siddiqui and Mahmood, 1994) although in a few studies inoculation has been reported to stimulate nematode reproduction (Dalal and Bhatti, 1989). The vesicular arbuscular mycorrhizae (VAM) and Rhizobium interaction improves the plant growth, nodulation and nitrogen fixation in most of the legumes. Further, the VAM association is known to suppress

the nematode infestation or nullify the damage caused by the nematodes (Sankaranarayanan and Rajeswari Sundarababu, 1994). A study was therefore undertaken on the effect of *Rhizobium* on the interaction of two VA-Mycorrhizal fungi *Glomus fasciculatum* (Thaxter *sensu* Gerd) Gerd *et* Trappe and *G. mosseae* (Nicol and Gerd) Gerd and Trappe with root-knot nematode *Meloidogyne incognita* (Kofoid *et* White) Chitw. on blackgram, *Vigna mungo* L.

## Materials and methods

Two kg capacity earthen pots were filled with a sterilized mixture of red soil: sand: farm-yard manure (2:2:1). An efficient strain of *Rbi*-

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zobium (CO Be 7) maintained in the culture collection, Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore was used in the investigation. About 50 grams of rhizobial culture were mixed thoroughly with rice gruel to produce a slurry. Blackgram CV. CO 5 seeds were dipped in the slurry and dried in the shade for 30 minutes before using them in the experiment.

The mycorrhizal fungi *Glomus fasciculatum* and *G. mosseae* were inoculated in separate pots, at the rate of 20 g inoculum (containing fungus hyphae and spores) per pots. The inoculum was mixed thoroughly in the pots and covered with a thin layer of sterilized soil. Rhizobial treated and untreated blackgram seeds were sown as per the treatment at the rate of two seeds/pot and after germination thinned to one seedling/pot. Ten days after germination second stage juveniles of *M. incognita* were inoculated at the rate of one nematode/cc of soil. The experiment had 12 treatments (Table I) each with three replications.

Data on plant growth, nematode reproduction, gall index (Heald *et al.*, 1989), spore count and per cent mycorrhizal colonization were recorded after 70 days. VAM root infection levels were assessed from randomly selected root material after cutting the entire system into 1 cm pieces. Roots were cleared in KOH and stained in tryphan blue (Phillips and Hayman, 1970). Percent root colonization was determined by the method of Giovannetti and Mosse (1980). Total nitrogen content and total P content (Jackson, 1973) in plant material was estimated.

## Results and discussion

The dual inoculation of *Rhizobium* and VAM to the blackgram plants increased all the growth parameters with respect to the individual inoculation of either VAM or *Rhizobium*. Lowest shoot length was recorded in the nematode alone treatment (Table I). The combination of

Rhizobium, G. mosseae and M. incognita increased shoot length to the same degree as the plants inoculated with Rhizobium and G. mosseae. The same trend occurred with root length, shoot weight and root weight also (Table I). Rhizobium inoculation, either alone or in combination with G. mosseae or G. fasciculatum or with M. incognita, or all together increased the nodule number significantly compared with the uninoculated control. The greater nodulation (56.3/plant) was recorded in plants inoculated with Rhizobium and G. mosseae and was significantly different from Rhizobium alone treatment. Inoculation of nematode to Rhizobial plants reduced the number of nodules/plant (34.3), while the combination of Rhizobium, G. mosseae and M. incognita was associated with a high nodule count (56/plant) which was similar to the Rhizobium + G. mosseae treatment. Combined or individual inoculations of Rhizobium or VAM increased the pod vield. The combination of Rhizobium and G. mosseae resulted in the highest pod yield (7.8 g/plant), and was followed by G. mosseae alone (7.2 g/plant). Inoculation of nematodes to blackgram resulted in the least pod yield (1.3 g/plant). The combined inoculation of G. mosseae and M. incognita together with Rhizobium produced a higher yield (7.1 g/plant) than Rhizobium and G. fasciculatum either as individual or dual inoculations with M. incognita. In general, dual inoculation of VAM + Rhizobium significantly increased nodulation and growth over Rhizobium alone. There was a reduction of growth and nodulation when nematodes were inoculated to Rhizobium plants, when compared to Rhizobium alone treated plants. Nodulation and N fixation were good in all plants inoculated with VAM + Rhizobium showing a synergistic effect or additive interaction between VAM and Rhizobium with consequences on nodulation, N fixation and P uptake.

The maximum gall index (5) was recorded in the nematode alone treatment. Inoculation of VAM or *Rhizobium* led to a reduction in gall in-

Table I - Effect of Rhizobium on the interaction of VAM and Meloidogyne incognita on blackgram.

Treatment	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)	No. Nodule . (plant)	Pod yield (g)	Gall <sup>1</sup> index	Nematode population (200 g soil)	Spore population (50 g soil)	Mycorrhizal coloniza- tion (%)
G. fasciculatum alone	30.4	26.2	26.8	11.8	14.3	6.4	1.0	0.0	168.0	60.0
G. mosseae alone	35.4	32.4	31.4	14.4	15.7	7.2	1.0	0.0	170.0	67.0
Rhizobium alone	31.4	25.2	26.4	12.1	44.3	6.1	1.0	0.0	0.0	0.0
G. fasciculatum + Rhizobium	33.4	19.4	28.8	13.2	52.7	6.8	1.0	0.0	160.0	61.0
G. mosseae + Rhizobium	35.9	33.8	32.0	18.6	56.3	7.8	1.0	0.0	178.0	69.0
Rhizobium + M. incognita	30.3	20.1	24.8	14.3	34.3	5.9	3.8	470.0	0.0	0.0
G. fasciculatum + M. incognita	29.6	20.8	25.3	13.8	12.7	6.1	3.5	430.0	118.7	38.0
G. mosseae + M. incognita	32.1	28.3	27.1	14.1	16.3	6.3	3.3	380.0	120.3	48.0
Rhizobium + G. fasciculatum + M. incognita	32.8	31.4	28.3	15.4	47.0	6.4	3.4	440.0	140.3	55.0
Rhizobium + G. mosseae +	ū		ū						ū	
M. incognita	34.1	29.8	30.3	16.3	56.0	7.1	3.1	370.0	155.0	58.3
M. incognita alone	16.4	10.3	10.8	3.8	8.3	1.3	5.0	580.0	0.0	0.0
Uninoculated control	21.6	24.6	15.8	2.6	11.3	3.3	1.0	0.0	0.0	0.0
CD (P=0.05)	2.6	3.1	3.0	1.8	5.9	1.4	NA	NA	NA	NA

<sup>&</sup>lt;sup>1</sup> Gall index: 1 = no galls, 2=1-25 galls/root system, 3=26-50 galls/root system, 4=51-75 galls/root system, 5=>75 galls/root system.

dex from 3.8 to 2.1. Among the treatments, plants inoculated with *Rhizobium* + *G. mosseae* and *M. incognita* had the least gall index (3.1) followed by *Rhizobium* + *G. fasciculatum* + *M. incognita* (3.4) inoculated plants. Also a similar trend occurred with nematode populations with the highest population (580) being recorded in the nematode alone treatment.

Dual inoculation of VAM with *Rhizobium* increased the spore population and mycorrhizal colonization more than with VAM alone. Increases in N and P content of blackgram were recorded in plants inoculated with both VAM and *Rhizobium* compared with individual inoculation (Table II). Inoculation with nematodes

reduced both N and P content but when the nematodes were inoculated along with *Rhizobium* or VAM, or both, the N and P contents increased and were almost on a par with *Rhizobium* or VAM alone or combined inoculation of *Rhizobium* and VAM treatments. Mycorrhizal and non mycorrhizal plants differ in their biochemical constitution. Thus the specific components of root exudates in mycorrhizal legumes may act as chemotaxic attractants to rhizobia (Gitte *et al.*, 1979). Thus VAM penetration and spread within the roots somehow predisposes the legume host to form more nodules and results in higher N fixation. High phosphorus content in VAM plants is associated with a re-

Table II - Effect of Rhizobium, VAM and M. incognita on total P and N content of blackgram.

Treatment	Totạl P co	N content/plant		
Treatment	Shoot	Root	(mg)	
G. fasciculatum alone	0.38	0.28	0.1920	
G. mosseae alone	0.41	0.31	0.2013	
Rhizobium alone	0.40	0.29	0.2613	
G. fasciculatum + Rhizobium	0.51	0.37	0.2631	
G. mosseae + Rhizobium	0.55	0.37	0.2643	
Rhizobium + M. incognita	0.31	0.23	0.2028	
G. fasciculatum + M. incognita	0.31	0.24	0.1981	
G. Mosseae + M. incognita	0.39	0.25	0.2010	
Rhizobium + G. fasciculatum + M. incognita	0.48	0.31	0.2432	
Rhizobium + G. mosseae + M. incognita	0.56	0.38	0.2456	
M. incognita alone	0.20	0.18	0.1920	
Uninoculated control	0.23	0.19	0.2423	
CD (P=0.05)	0.04	0.20	0.0098	

duced nematode population; this might be related to the high mycorrhizal colonization in the VAM infected plants.

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## Literature cited

Dalal M. R. and Bhatti D. S., 1989. Pathogenicity of *Heterodera cajani* in mungbean and cluster bean as affected by presence or absence of *Rhizobium*. *Indian J. Nematol.*, 19: 153-158.

GITTE R., RAI P. and PATIL R. V., 1979. Chemotaxis of Rhi-

zobium spp. towards root exudates of *Cicer arietinum*. *Plant Soil.*, *50*: 553-556.

GIOVANNETTI M. and Mosseae B., 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytol.*, 84: 489-500.

HEALD C. M., BRUTON B. D. and DAVIS R. M., 1989. Influence of *Glomus intraradices* and soil phosphorus on *Meloidogyne incognita* infecting *Cucumis melo. J. Nematol.*, 21: 69-73.

JACKSON K. L., 1973. Soil chemical analysis. Prentice Hall of India Pvt. Ltd., New Delhi.

PHILLIPS J. M. and HAYMAN D. S., 1970. Improved procedures for clearing roots and obtain parasitic and vesicular-arbuscular myccorrhizal fungi for rapid assessment of infection. *Trans. Brit. Mycol. Soc.*, *15*: 158-161.

Sankaranarayanan C. and Rajeshwari Sudarababu, 1994. Interaction of *Glomus fasciculatum* with *Meloidogyne incognita* inoculated at different timings on blackgram (*Vigna mungo*). *Nematol. medit.*, 22: 35-36.

SIDDIQUI Z. A. and MAHMOOD I., 1994. Effect of *Heterodera* cajani on growth, chlorophyll content and activity of some enzymes in pigeonpea. *Nematropica*, 24: 103-111.

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