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Competition of *Rhizobium* with the root rot pathogen (*Sclerotium rolfsii*) on groundnut rhizoplane

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In vivo studies conducted in pots by incubating the host plants with both the pathogen and the symbiont, revealed that the pathogen was dominent over the corresponding strains of *Rhizobium* in presence of its respective host. Simultaneous inoculation with the pathogen and the *Rhizobium* have given lower nodulation than that of the *Rhizobium* inoculated plants. The percent intensity of disease was more on plants inoculated with the pathogen than with the pathogen and symbiont. This may be due to the competition of the symbiont with the pathogen or a posssible inhibitory action of the symbiont on the pathogen.

Field trials were conducted in two consequtive years. The crop grown from seed inoculated with *Rhizobium* produced significant yield. However, this increase in yield was lower in plants inoculated with both the pathogen and symbiont in different combinations. Double inoculation, however, resulted in higher yield compared to plants inoculated only with pathogen. It is of considerable interest that besides increasing yields by inreasing nodulation, *Rhizobium* inoculation simultaneously restrained collar rot intensity.

Key words : *Rhizobium*, groundnut, nitrogen fixation, *S. rolfsii*

INTRODUCTION

Leguminous plants grow in nature in symbiotic relationship with nitrogen fixing bacteria belonging to the genus *Rhizobium*. Like other crops legumes also suffer from diseases caused by a large number of pathogens resulting in symptom expression, mortality and yield reduction. The interaction between a pathogen and a legume host or the *Rhizobium* with the legume host have been studied seperately by different workers (Garren and Wilson, 1951; Nagraj Rao, 1974; Bhargaba *et al.*, 1979). The present study was aimed at understanding the effects of the pathogen *S. rolfsii* and the symbiont e.g. Jcg-9 strain of cowpea group *Rhizobium* on the groundnut host.

MATERIALS AND METHODS

The interactions of the pathogenic fungi and the strains of cowpea *Rhizobium* were studied *in vivo* in unsterilised soil in earthen pots of 10 cm diameter. *Rhizobium* cells were grown in Yeast Mannital Agar (YMA) medium containing (g/l) of Mannitol 10.09, Dipotassium monohydrogenphosphate 0.59, Magnesium sulphate 0.29, sodium chloride 0.1, Yeast extract 1.09, calcium carbonate 0.5, Distilled water 1.0 L, Agar 15.09, pH 6.8-7.0, (Vincent, 1970). Yeast Mannitol Broth (YMB) was prepared without addition

agar of agar. The pathogen was grown in sand-maize medium (SM) containing 75 g of washd sand, 25 g of maize-meal and requisite quantity of distilled water to moisten the mdium. Three days old YM-broth cultures of the symbiont and 15 days old SM cultures of the pathogn were used for inoculation of the plants.

Seeds of variety JL-24 of groundnut plants inoculated with the JCG 9 strain of the symbiont at a strength of 69.71 x 10^6 cells/ml in the 1st year and 70.11 x 10^6 cells/ml in the 2nd year were sown in the soil. The plants were inoculated with the pathogen immediately before (pre-sowing) and 30 days after sowing of seeds (post-sowing) @ 20 g of SM culture inoculum per pot after its dilution with dry soil in 1:1 ratio. Three treatments namely inoculation with *S. rolfsii*, with *Rhizobium* and with both of them were included in the study. In the field trial nine treatments consisting of different combinations of pathogen and symbiont inoculation and uninoculated control were replicated four times in a randomised block design with microplots of 2 sq. m net plot size. The groundnut plants were inoculated with the corresponding symbiont namely JCG -9 strain of cowpea group (i) by seed inoculation and (ii) inoculation at 30 days of age of the plants by drenching with *Rhizobium* suspension at a strength of 32.66 x 10^6 and 35.66 x 10^6 cell/ml in two years. The soil was inoculated with the SM inoculum of the pathogen on 30 and 45 days after sowing. The inoculum was diluted with soil in 1:1 ratio and used @ 500 g/2 sq m plot. Nitrogen was estimated following the method of Jackson (1967). Necessary replicates and uninoculated control were maintained. The experiments were repeated in two successive years. Observations were made on 30, 45, 60 and 75 days old plants after sowing. Results obtained are classified in three groups namely -

i) the effect of growth characteristics of the host plant.
ii) nitrogen estimation of plant samples *in vivo* and
iii) the disease, and nodule development and yield of groundnut and presented.

The disease intensity and severity were recorded as percent disease index at 30,45,60 and 75 days after sowing following the standard scale of 0-9 (Mayee and Dalvi, 1986).

RESULTS AND DISCUSSION

a) Effect of inoculation on plant growth characteristics

Results presented in Table 1 showed that the root length of the plant inoculated with the symbiont was higher compared to uninoculated control plants. Lower root length compared to control were recorded in plants inoculated only with the pathogen. In case of mixed inoculation the root length was observed to be lower compared to symbiont treated plants but higher compared to uninoculated control. The dry weight of the plants inoculated with the pathogen was lower compared to the symbiont inoculated and uninoculated plants consistently in both the years. Plants inoculated simultaneously with *Rhizobium* sp. and *Sclerotium* sp. recorded higher dry weight throughout the growth of the plants compared to the plants inoculated with *Sclerotium* alone.

b) Nitrogen estimation of plant samples in vivo

Result presented in Table 2 revealed that *Rhizobium* inoculated plants recorded higher percentage of nitrogen both in pre- and post-sowing inoculation compared to uninoculated

control. In case of double inoculation i.e. where the plants were inoculated with both *Rhizobium* sp. and *S. rolfsii*, the percentage of total nitrogen decreases, but the percentage is more compared to only pathogen inoculated and uninoculated control plants.

c) Disease development, nodulation and yield

As regards disease intensity the plants inoculated with the pathogen showed significantly higher disease intensity. The plants inoculated with both *Rhizobium* and *Sclerotium* also showed disease but lower compared to pathogen inoculated plants (Tables 3 and 5). Disease intensity on *Rhizobium* inoculated and uninoculated plants were recorded to be nil as expected. This trend were recorded both in pot and field trials.

The nodulation (i.e. the number and volume of nodule) of the plant inoculated with the symbiont were recorded to be significantly higher compared to double inoculation. The number of nodules in *Sclerotium* treated plants *in vivo* was recorded to be nil. This trend was continued upto 75 DAS in the 1st year excepting on 60 DAS where few nodules were recorded (Table 4).

Yields of groundnut inoculated with the pathogen at one month age was lower by 35.09% in the 1st year and 26.14% in the 2nd year over the respective uninoculated control plants. The yield of plants inoculated with the pathogen at 45 days after sowing were also lower by 28.36% in the 1st year and 37.22% in the 2nd year over the respective uninoculated control plants. The plants grown from seeds inoculated with *Rhizobium* produced significant yield. The increase was by 109.54% in the 1st year and 115.25% in the 2nd year. However, this increase in yield was lower in plants inoculated with both the pathogen and symbiont in different combinations. Double inoculation resulted in higher yields compared to plants inoculated only with the pathogen and also over uninoculated control where *Rhizobium* was treated at seed and the pathogen were treated at 30 DAS. This increase was to the extent of 28.07% in the 1st year and 11.09% in the 2nd year over the respective uninoculated control (Table 5).

Inoculation with *Rhizobium* increased nodulation (Bajpai *et al.*, 1974; Thomas *et al.*, 1986; Bhattacharyya and Mukherjee, 1990) and inoculation with *Sclerotium* increased the extent of collar rotting (Khan and Main, 1973; Bhattacharyya and Mukherjee, 1990) was already known. The present work has confirmed them. In the present work nodulation appeared to be more affected by presowing inoculation with pathogen than its post-sowing inoculation. The report on the effect of *Rhizobium* sp. in reducing the disease intensities and thereby maintaining a level of nodulation was indicated earlier (Bhargava *et al.*, 1979; Purakayastha *et al.*, 1981; Hussain *et al.*, 1990; Ehteshamul *et al*, 1995). This has been confirmed with the present experiment. Simultaneous inoculation with the pathogen and *Rhizobium* have given lower nodulation than that of the *Rhizobium* inoculation plants (Patil, 1985). Nevertheless, nodulation was higher than that of the plants inoculated with the pathogen only. But this restraining effect of the symbiont on the pathogen was not of longer duration, particularly under *in vivo* condition.

In field trial, similar results were actually achieved like pot trials. The plant grown from seeds inoculated with *Rhizobium* produced significant yield. Double inoculation, however, resulted in higher yield compared to plants inoculated only with pathogen. The present

Table 1 : Effect of inoculation on groundnut with *Sclerotium rolfsii* and *Rhizobium* sp. on length and weight of the plant root *in vivo*

		Root characters/plant							
		Pre-sowing				Post-sowing			
		Length (cm)		Dry weight (g)		Length (cm)		Dry weight (g)	
Inoculation	Year	30DAS	45 DAS	30 DAS	45 DAS	60 DAS	75 DAS	60 DAS	75 DAS
Sclerotium sp.	1st	3.03	19.11	0.18	0.78	9.15	7.06	2.91	1.83
	2nd	9.00	0	1.10	0	6.23	3.21	1.93	1.63
Rhizobium sp.	1st	6.39	25.43	1.29	1.71	18.93	16.27	6.96	7.97
	2nd	13.80	23.52	1.28	1.50	18.93	19.95	7.18	7.93
Sclerotium &	1st	5.42	22.70	11.08	1.49	11.25	10.73	3.39	2.13
Rhizobium spp.	2nd	12.93	20.83	1.27	1.41	11.42	7.87	4.16	2.97
Uninoculated	1st	5.30	18.16	0.38	1.40	11.93	12.11	6.71	7.19
control	2nd	9.56	13.58	1.19	1.28	16.31	16.43	6.05	6.28
S.Em ±	1st	2.91	4.23	0.27	0.69	0.54	0.57	0.19	0.45
	2nd	0.37	0.40	0.26	0.90	0.24	0.36	0.58	0.61
C.D. at 5%	1st	6.01	8.73	0.50	1.42	1.34	1.39	0.46	1.10
	2nd	0.75	0.83	0.54	1.85	0.60	0.88	1.43	1.49

* DAS - Days after sowing

data are therefore encouraging. It is of considerable interest that besides increasing yields by increasing nodulation, *Rhizobium* inoculation simultaneously restrain collar rot intensity.

A challenge of the pathogen by the *Rhizobium* has been expressed. The possibility of using a high *Rhizobium* inoculum for lowering the pressure of the root-rot disease of groundnut due to *S. rolfsii* is clearly expressed from the present work. This has opened up a new area of possible use of *Rhizobium* culture not only for inducing nodulation but also for favouring the *Rhizobium* in a competetion in soil against the plant pathogen to lower the disease pressure on the groundnut crop.

Table 2 : Estimation of total nitrogen on interaction of *Sclerotium rolfsii and Rhizobium sp.* on groundnut

Treatment	Pre-sowing (% of N)	Post-sowing (% of N)
Sclerotium sp.	0.025	0.036
Rhizobium sp.	0.106	0.109
Sclerotium and Rhizobium	0.082	0.091
Uninoculated control	0.073	0.073

Table 3 : Effects of inoculation of groundnut with *Sclerotium rolfsii and Rhizobium* sp. on disease intensity *in vivo*

		Percent disease intensity/plant			
		Pre-sowing		Post-sowing	
Inoculation	Year	30 DAS*	45 DAS	60 DAS	75 DAS
Sclerotium sp.	1st	60.66	88.66	77.33	100.00
	2nd	55.00	88.66	80.00	100.00
Rhizobium sp.	1st	0	0	0	0
	2nd	0	0	0	0
Sclerotium & *Rhizobium* spp.	1st	58.00	73.33	73.66	93.00
	2nd	44.00	77.33	77.33	88.66
Uninoculated control	1st	0	0	0	0
	2nd	0	0	0	0
S.Em	1st	1.75	1.93	1.73	2.45
	2nd	2.20	3.67	1.87	3.54
C.D. at 5% level	1st	4.28	4.72	4.23	6.00
	2nd	5.39	8.99	4.58	8.67

* DAS - Days after sowing

Table 4 : Effect of inoculation of groundnut with *Sclerotium rolfsii* and *Rhizobium* sp. on nodulation *in vivo*

Inoculation	Year	Nodule characters / Plant							
		Pre-sowing				Post-sowing			
		Number		Volume in CC		Number		Volume in CC	
		30DAS	45 DAS	30 DAS	45 DAS	60 DAS	75 DAS	60 DAS	75 DAS
Sclerotium sp.	1st	0	0	0	0	2.11	0	0.06	0
	2nd	5.77	4.90	0.10	0.08	2.49	0	0.05	0
Rhizobium sp.	1st	7.44	9.33	0.14	0.19	12.53	15.77	0.28	0.31
	2nd	10.77	13.53	0.16	0.23	15.12	18.46	0.36	0.39
Sclerotium &	1st	4.96	3.66	0.11	0.06	3.84	0	0.05	0
Rhizobium spp.	2nd	8.66	3.36	0.14	0.08	5.05	2.50	0.07	0.03
Uninoculated	1st	3.88	5.17	0.10	0.13	5.21	6.00	0.08	0.10
control	2nd	7.66	8.16	0.12	0.14	7.01	8.34	0.10	0.14
S.Em ±	1st	0.52	0.60	0.05	0.01	0.93	0.54	0.01	0.01
	2nd	1.02	0.50	0.01	0.06	0.33	0.50	0.12	0.01
C.D. at 5%	1st	1.27	1.48	0.13	0.03	2.29	1.33	0.02	0.34
	2nd	2.49	1.22	0.03	0.15	0.80	1.23	0.30	0.34

* DAS - Days after sowing

Table 5 : Interaction of *Rhizobium* with *Sclerotium rolfsii* on disease intensity and yield of groundnut in field condition

Treatments	Mean disease intensity (%) per plant		Mean yield in q/ha	
	1st Year	2nd Year	1st Year	2nd Year
Control	Nil	Nil	4.98	5.05
Sclerotium at 30 days	41.30	40.07	3.24	3.73
Sclerotium at 45 days	40.70	41.55	3.58	3.17
Rhizobium at seed	Nil	Nil	10.45	10.87
Rhizobium at 30 days	Nil	Nil	7.65	8.43
Rhizobium at seed and *Sclerotium* at 30 days	45.25	37.22	6.39	5.61
Rhizobium at seed and *Sclerotium* at 45 days	32.30	31.22	4.88	3.76
Rhizobium at 30 days and *Sclerotium* at 30 days	31.95	30.00	4.09	4.78
Rhizobium at 30 days and *Sclerotium* at 45 days	28.50	29.87	4.41	3.17
C.D. at 5% level	6.67	10.05	0.81	0.25

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REFERENCES

Bajpai. P.D., Lehri, L.K. and Pathak, A.N. (1974). Effect of seed inoculation with *Rhizobium* strains on the yield of Leguminous crops. *Proc. India. Natl. Sci. Acad. Part-B,* **40**(5) : 571-575.

Bhargava, S.N., Shukla, D.N. and Singh, N. (1979). Correlation between the number of root nodules and incidence of root rot and wilt disease of pulses. *Proc. Natl. Acad. Sci. India B,* **49**(1) : 56.

Bhattacharyya, P. and Mukherjee, N. (1990). *Rhizobium* challenges the root rot pathogen (*Sclerotium rolfsii*) on groundnut surfaces. *Indian Agriclturist,* **34**(1) : 63-71.

Ehtheshmul-Haque, S.; Abid, M.; Ghaffar, A. (1995). Efficacy of *Bradyrhizobium* sp. and *Paecilomyces lilacinus* with oil cakes in the control of root rot of mungbean. *Tropical Sciences.* **35** \(3) : 294-299.