EFFECT OF *HETERODERA CAJANI* ON GROWTH, CHLOROPHYLL CONTENT AND ACTIVITY OF SOME ENZYMES IN PIGEONPEA.

Zaki A. Siddiqui and Irshad Mahmood

Department of Botany, Aligarh Muslim University, Aligarh-202002, INDIA.

ABSTRACT

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

Pigeonpea was inoculated with 250, 500, 1 000, 2 000 and 4 000 *Heterodera cajani* second-stage juveniles (J2) in a glasshouse test. Pigeonpea growth and rhizobia nodulation progressively decreased as the inoculum level increased. Significant reduction in plant growth occurred with an inoculum of 500 or more J2 of *H. cajani* in both *Bradyrhizobium japonicum*-inoculated and noninoculated plants. Peroxidase activity was higher in *H. cajani*-inoculated plants with the highest level at an inoculum density of 500 J2. Nitrate reductase, catalase and chlorophyll content were reduced as inoculum levels of *H. cajani* increased. Chlorophyll content and enzyme activities were higher in *B. japonicum*-inoculated plants than in plants without *B. japonicum*.

Key words: Bradyrhizobium japonicum, catalase, chlorophyll, *Heterodera cajani*, nitrate reductase, peroxidase, pigeonpea.

RESUMEN

Siddiqui, Z. A. y I. Mahmood. 1994. Efecto de *Heterodera cajani* sobre el crecimiento, contenido clorofilíco y actividad de algunas enzimas en el frijol gandul. Nematrópica 24:103-111.

Plantas de gandul fueron inoculadas con los niveles de 250, 500, 1 000, 2 000 y 4 000 juveniles (J2) de *Heterodera cajani* en condiciones de invernadero. Tanto el crecimiento de plantas como la nodulación de rhizobium disminuyeron progresivamente conforme aumentaban los niveles de inóculo. La reducción significativa del crecimiento de plantas se obtuvo con el nivel de 500 o más J2 de *H. cajani* tanto en las inoculadas con *Bradyrhizobium japonicum* como en las no inoculadas. La actividad de las peroxidasas fue más alta en la plantas inoculadas con los niveles de inóculo mas grandes de *H. cajani* utilizado de 5 00 J2. Los contenidos de reductasa de nitrato, catalasa y clorofila se redujeron conforme aumentaron los niveles de inóculo de *H. cajani*. Los contenidos de clorofila y actividades enzimáticas fueron mayores en las plantas inoculadas con *B. japonicum* que en las no inoculadas.

Palabras clave: Bradyrhizobium japonicum, catalaza, clorofila, frijol gandul, *Heterodera cajani*, peroxidazas, reductaza de nitrato.

INTRODUCTION

Pigeonpea cyst nematode, *Heterodera cajani* Koshy, is an important pest of *Cajanus cajan* L. Millps. and is widely distributed throughout Uttar Pradesh (14) and other states of India (5,7,28). Plants infected with *H. cajani* are stunted with marked symptoms of chlorosis. Although *H. cajani* was described on pigeonpea in 1967, its pathogenicity has not been throughly investigated (37).

Root nodulation is a complex symbiotic process between host plant and rhizobia. Plant parasitic nematodes alter the establishment of rhizobia on or around roots of legumes (13). The association of rhizobia with plant parasitic nematodes in the rhizosphere is not always detrimental since it sometimes leads to stimulation of nodu-

lation (15). The presence of rhizobia in the rhizosphere also may protect the host roots from the damage caused by pathogens (8,29).

Several investigations of enzymes in nematode-infected plants have been conducted. Siddiqui and Mahmood (30) found a positive correlation between an increase in peroxidase activity and the damage threshold of *Meloidogyne incognita* race 3 on chickpea. It has been suggested that peroxidase is important in plant defense mechanisms (18,35). Peroxidase catalyzes the polymerization of phenolic compounds and forms cross-links between extensin, lignin and feruloylated polysaccharides (9,12,16,22). Peroxidases are important in the reinforcement of cell walls and probably play a role in plant resistance (36).

Nitrate reductase is a metalloflavoprotein that catalyzes the reduction of nitrate to nitrite. It is an inducible enzyme and the activity of nitrate reductase often controls the rate of protein synthesis in plants (24,31). Catalase is a soluble hemeprotein. It breaks down hydrogen peroxide to oxygen and water (10). It also acts as a detoxifying agent for enzymes and has important regulatory effects in controlling quantity of indole acetic acid in plants. Chlorophyll contents in plants are directly related to photosynthesis and decrease in their contents due to nematode parasitism may be correlated with the symptoms of chlorosis (34).

The present study was undertaken to determine the effect of different inoculum levels of *Heterodera cajani* on the growth of pigeonpea in the presence and absence of the nodulating bacterium, *Bradyrhizobium japonicum*. The effects of different infection intensities of *H. cajani* on chlorophyll, peroxidase, nitrate reductase and catalase also were studied.

MATERIALS AND METHODS

Heterodera cajani was collected from a pigeonpea field and multiplied on pigeonpea plants grown in sterile soil using second-stage juveniles (J2) obtained from a single cyst. The cysts from this population were later identified using vulval cones and larval morphometrics as described by Koshy *et al.* (19,20). Before experiment initiation, freshly hatched J2 were tested for infectivity. Cysts were collected and placed in pigeonpea root exudates for hatching. Twenty J2 were inoculated on each of 5 one-week-old seedlings of pigeonpea grown in small cups containing 20 g autoclaved soil. After 72 hours, plants were gently removed and the roots stained in cotton-blue (32). The J2 were found to have an infectivity rate of 70%.

Seed of pigeonpea (cv. UPAAS-120) were surface-sterilized with 0.1% mercuric chloride for 2 minutes and washed 3 times in distilled water. Half of the seed then were treated with the pigeonpea strain of *Bradyrhizobium japonicum* before sowing. The inoculated and noninoculated seed were sown seperately at 5 seed per pot in 15-cm-diam earthen pots. The pots contained 1 kg steam-sterilized sandy loam soil mixed with washed river sand and farm yard manure in the ratio of 3:2:1 (v/v), respectively. One week after germination, seedlings were inoculated with 0, 250, 500, 1 000, 2 000, or 4 000 J2 of *H. cajani*. Each treatment was replicated 10 times (5 for plant growth parameters and 5 for enzyme analysis). Pots were placed randomly on a glasshouse bench maintained at 28 + 2°C and watered as needed. The experiment was terminated 90 days after inoculation.

Data were recorded on plant height, fresh and dry weight, number of rhizobia nodules, and the number of cysts and J2 in the soil. Nematodes were extracted from

soil by Cobb's sieving and decanting technique followed by Baermann funnel while cysts were extracted with the Fenwick can (32). Females on the roots were counted after staining in cotton blue. Data were analysed as a two-way factorial and least significant differences (LSD) were calculated at $P \leq 0.05$. Peroxidase, catalase and nitrate reductase activities in leaves of control and inoculated plants were determined 8 days after inoculation and chlorophyll content after 90 days. Enzyme analyses were conducted from the first fully expanded leaves. Peroxidase and catalase activities were determined by the method of Chance and Maehly (3), and enzyme assays were conducted on the same plant tissues. A calibrated standard curve for peroxidase was plotted with graded concentrations of pure purpurogallin. The specific activity was calculated with purpurogallin formed per mg protein per minute. The protein estimation was done by the method of Lowry *et al.* (21). Catalase activity was assayed with the titrimetric method and expressed as μ moles of H_2O_2 disappearing per minute per g of fresh weight. Nitrate reductase activity was assayed by the method of Jaworski (17), which does not include nitrate in the assay medium. Activity of nitrate reductase was expressed as μ moles of NO_2 formed per g of fresh leaf per hour. Chlorophyll content of leaves was estimated by the method of Arnon (2).

RESULTS

The effects of *H. cajani* and *B. japonicum* on plant growth, rhizobia nodulation and nematode density were significant but no interaction was observed except for nodulation and J2 density (Table 1). Inoculation by *B. japonicum* resulted in an improvement of growth compared with uninoculated plants. The decrease in plant growth by *H. cajani* was inversely proportional to inoculum level, but damage was less in *B. japonicum*-inoculated than in noninoculated plants. Significant reductions in plant growth occurred at inolcum levels of 500 or more J2 of *H. cajani.* Greatest reduction in plant growth occurred in plants inoculated with the highest inoculum level of nematodes. Root nodulation in *B. japonicum*-inoculated plants decreased considerably at all J2 inoculum levels. Nodulation in noninoculated plants was not significantly different from the controls or those inoculated with *H. cajani.* Multiplication rate of *H. cajani* was highest at the lowest inoculum, and conversely, lowest at the highest inoculum level. *Bradyrhizobium japonicum* inoculation reduced nematode numbers at all inoculum levels.

The interaction of *H. cajani* and *B. japonicum* did not significantly affect peroxidase, nitrate reductase and catalase activities, but their individual effect was significant (Table 2). All J2 inoculum levels except the highest caused significant increases in peroxidase activity in both *B. japonicum*-inoculated and noninoculated plants. A significant increase in plant peroxidase activity occurred at the inoculum level of 250 J2 and greater. Peroxidase increased more in *B. japonicum*-inoculated plants than in noninoculated plants with or without nematodes. Nitrate reductase and catalase activities were reduced with increases in nematode inoculum but reductions in activities were not significantly different above the 250 J2 inoculum level. Nitrate reductase and catalase activities also were higher in *B. japonicum*-inoculated than in noninoculated plants.

The effects of *H. cajani* and *B. japonicum* on chlorophyll content were significant but no interaction was observed (Table 3). Chlorophyll content was

Table 1. Effect of *Heterodera cajani* on pigeonpea, *Cajanus cajan*, in the presence and absence of *Bradyrhizobium japonicum* (BJ).

Treatments[z] (n–5 replicate)	Plant height (cm)	Plant fresh wt. (g)	Plant dry wt. (g)	No. of nodules per root system	*H. cajani* cysts + juveniles	
Control	153.8	35.54	9.34	12	0	0
250	138.8*	32.50*	8.58*	9*	19	2 540
500	115.3	28.64	6.99	7*	37	4 560
1 000	89.6	23.18	5.59	5*	51	6 710
2 000	68.0	16.58	3.38	4*	72	8 935
4 000	50.8	12.66	2.38	5*	96	11 415
Control + BJ	171.4	39.66	10.29	87	0	0
250 + BJ	159.4*	36.72*	9.63*	79*	12	2 010
500 + BJ	143.4	32.47	8.36	62	28	3 750
1 000 + BJ	115.0	26.60	6.33	46	40	5 610
2 000 + BJ	87.6	21.32	4.76	38	58	7 360
4 000 + BJ	68.0	16.76	3.15	29	76	9 780
L.S.D. $P \leq 0.05$	16.5	3.86	0.92	8	8	242

[z]Numbers represent initial inoculum levels of *H. cajani*.

*Not significantly different from respective controls, all others significantly different at $P \leq 0.05$.

Table 2. Effect of *Herterodera cajani* on some enzymes of pigeonpea, *Cajanus cajan*, in the presence and absence of *Bradyrhizobium japonicum* (BJ).

	Peroxidase activity		Nitrate reductase activity		Catalase activity	
Treatments	Purpurogallin[y]	Percent reduction	μ moles NO_2/g/h	Percent reduction	H_2O_2 per/minute[z]	Percent reduction
Control	0.351	0	16.81	0	10.70	0
250	0.372	5.9	16.54*	1.6	10.45*	2.3
500	0.404	15.1	14.97	10.9	10.05	6.1
1 000	0.379	7.9	14.51	13.7	9.85	7.9
2 000	0.373	6.3	13.58	19.2	9.60	10.3
4 000	0.364*	3.7	11.83	29.6	9.40	12.1
Control + BJ	0.373	0	19.12	0	11.20	0
250 + BJ	0.394	5.6	18.57*	2.9	10.95*	2.2
500 + BJ	0.415	11.3	17.64	7.7	10.60	5.4
1 000 + BJ	0.403	8.0	16.63	13.0	10.45	6.7
2 000 + BJ	0.390	4.6	15.61	18.4	10.20	8.9
4 000 + BJ	0.384*	2.9	13.86	27.5	10.15	9.4
L.S.D. $P \leq 0.05$	0.017	–	0.63	–	0.46	–

[y]Purpurogallin formed per mg protein per minute.
[z]Expressed as μ moles H_2O_2 disappearing per minute.
*Not significantly different from respective controls.

Table 3. Effect of *Herterodera cajani* on chlorophyll content of pigeonpea, *Cajanus cajan*, in the presence and absence of *Bradyrhizobium japonicum* (BJ).

Treatments	Chlorophyll a		Chorophyll b		Total chlorophyll	
	mg per g leaf fresh wt.	Percent reduction	mg per g leaf fresh wt.	Percent reduction	mg per g leaf fresh wt.	Percent reduction
Control	1.486	0	0.866	0	2.352	0
250	1.440*	3.1	0.839*	3.1	2.279*	3.1
500	1.319	11.2	0.818*	3.5	2.137	9.1
1 000	1.215	18.2	0.721	16.7	1.936	17.7
2 000	1.076	27.6	0.664	25.6	1.720	26.9
4 000	0.947	36.3	0.509	41.2	1.456	38.1
Control + BJ	1.571	0	0.966	0	2.537	0
250 + BJ	1.508*	4.0	0.956*	1.0	2.464*	2.9
500 + BJ	1.415	9.9	0.913*	5.5	2.328	8.2
1 000 + BJ	1.351	14.0	0.806	16.6	2.157	14.9
2 000 + BJ	1.255	20.1	0.691	28.5	1.946	23.3
4 000 + BJ	1.155	26.5	0.615	36.3	1.770	30.2
L.S.D. $P \leq 0.05$	0.119	–	0.066	–	0.153	–

*Not significantly different from respective controls.

reduced as nematode inoculum increased but was not different from the control except at the 500 and 1 000 J2 inoculum levels. Reduction in chlorophyll was more pronounced in plants not inoculated with *B. japanicum* than in those that were inoculated. Nematode parasitism caused a greater reduction in chlorophyll b than in chlorophyll a.

DISCUSSION

The threshold for significant damage ($P \leq 0.05$) from *H. cajani* in this experiment was 500 J2 per kg of the soil, both in *B. japonicum*-inoculated and noninoculated plants. Zaki and Bhatti (37) reported significant reduction in growth paramaters of pigeonpea at 100 J2 per kg soil. The same threshold of *H. cajani* on mungbean and cluster bean was reported (4) while 130 J2 of *H. cajani* and 200 J2 per kg soil were the damaging levels on cowpea (1,26). Differences in these results may be attributed to differences in environmental conditions, soil mixture, hosts or cultivars. Damaging levels of *H. cajani* were the same in plants with or without *B. japonicum*; however, the damage caused by *H. cajani* was greater in the absence of *B. japonicum* than in its presence. *Bradyrhizobium japonicum* is reported to produce antipathogenic substances (6,8) and to reduce the damage caused by nematodes and other pathogens (8,23,29,33). Parasitism of *H. cajani* adversely affecting nodulation has been reported (27), results similar to those in this test.

The interaction of *Meloidogyne incognita* on cotton resulted in increased peroxidase activity, but this was believed to be the result of resistance mechanism not the cause (25). The increase in peroxidase activity up to the inoculum level of 500 J2 in our test indicated an increasing response to *H. cajani*. A decrease in nitrate reductase with the increase in the inoculum level of *H. cajani* may indicate an adverse effect of nematode parasitism on protein synthesis (24,31). Reduction in catalase activity may also indirectly affect resistance of the plant because catalases have an important regulatory effect on indole acetic acid. Moreover, an increase in indole acetic acid contents may stimulate nematode development and reproduction resulting in a susceptible response (11). Nematode parasitism adversely affected chlorophyll contents producing results similar to that found by others (34).

ACKNOWLEDGEMENT

The senior author is thankful to the Council of Scientific and Industrial Research (CSIR), New Delhi, for the award of a Research Associateship.

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Received: *13.X.1993*
Recibido:

Accepted for publication: *10.VI.1994*
Aceptado para publicación: