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RESEARCH ARTICLE

EFFECTS OF MACRONUTRIENT CONTENTS OF RESISTANT AND SUSCEPTIBLE GREENGRAM CULTIVARS AS INFLUENCED BY ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*

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ABSTRACT

The present study aimed at determining the change in the leaf nutrient contents of different resistant and susceptible cultivars/varieties of greengram against root-knot nematode, *Meloidogyne incognita infection*. Four resistant cultivars, 24 ML-233, 7 GGG 10-14,17 IPM 9901-6 and 8 GM 04-02 and two susceptible cultivars 28 PM 10-12 and 29 PUSA 0672 were selected to assess for change in leaf. Nitrogen, phosphorus and potassium contents of both infected and healthy plants. Maximum N□ content of 196.61% was noted in variety 7 GGG 10-14 while minimum as 88.57% in variety 28 PM 10-12.Similarly P□O□ was maximum (46.49 %) noted in varieties 29 PUSA 0672 and minimum - 52.26% in varieties 28 PM 10-12. The crude protein content was maximum (-4.10 %) in the varieties 8 GM 04-02.

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INTRODUCTION

The root knot nematodes, *Meloidogyne* spp. are the most important nematode pests worldwide due to their great damage resulted in the very wide host range which include more than 3000 plant species. Nematode pests, in general, affect host plants quantitatively by reducing the total production causing about 20.6% worldwide yield loss, and qualitatively by devaluing the produced pods. Plant response to the parasite depends not only on the quantitative and qualitative composition of the nematode secretion and excretion but also on the chemical composition of the plants or the tissues attacked. Leaf nitrogen content decreased in the cultivar inoculated with root-knot nematode as compared to healthy plant (control). Phosphorus and Potassium content were also significantly decreased in plants infected by root-knot nematode as compared to uninoculated plants. The nematode parasitism adversely affects the nutrition uptake ability of the plants. Root-knot nematode infection also reduced photosynthetic activity of the plants by the reduction in chlorophyll contents of plants.

MATERIALS AND METHODS

A pot culture experiment was conducted in the department of nematology under green house condition.

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Pots containing sterilized soils were arranged on green house benches. Seeds collected from different sources were sown and sprinkled with water for germination purpose. To establish the basis of nematode resisitance four varieties namely 24 ML-233, 7 GGG 10-14 , 17 IPM 9901-6,8 GM 04-02,(resistant check),28 PM 10-12 and 29 PUSA 0672 (susceptible check) were grown in eartherm pots in green house, filled with sterilized soil and sand mixture (2:1:1) @1 kg /pot along with NPK @ 20:40:40 kg/ha. After two weeks of germination these plants were thinned to single plant per pot and inoculated with denematised nematodes @ 1J2/ cc soil in 1kg soil pot making wholes around the root zones of the potted plants and the experiment was terminated 45 days after nematode inoculation and processed for chemical analysis as described below.

Estimation of nitrogen, phosphorus, potassium & crude protein content of shoot

Estimation of Nitrogen &Crude Protein contents

Crude protein and nitrogen content of shoot were estimated by following the procedure of Mahadevan and Sridhar (1986). Two hundred mg of powdered plant parts were taken in 100 ml micro Kjeldahl digestion flasks. About 200 mg of digestion mixture (K_2SO_4: $CuSO_4$ = 5:1) and 4 ml of concentrated H_2SO_4 were added. These flasks were kept as such for about one hour and then heated slowly till frothing occurred. To check the frothing, two crystals of sodium thiosulphate were

added to each digestion flask. Thereafter, digestion was continued until the contents of the flask became completely clear blue syrupy liquid without any bubbling. The flask was cooled and content was diluted to 25 ml with distilled water. Then 10 ml of diluted sample extract was transferred into micro Kjeldahl distillation unit. Thereafter, 10 ml of 40 % NaOH was added and distillation was continued for 10 minutes. During distillation period, liberated ammonia was absorbed by 150 ml conical flask containing 2 drops of mixed indicator. After completion of distillation, distillate was titrated against 0.05 NH_2SO_4.

Calculation

Per cent N_2 in sample

$$= \frac{(\text{Sample titer - blank titer}) \times \text{N}_2 \text{ of } H_2SO_4 \times 14 \times 100 \times 2.5}{\text{Sample weight (g)} \times 1000}$$

Crude protein

Percentage of protein present in shoots was determined by multiplying the per cent N_2 with 6.25. This protein is called crude protein.

Estimation of phosphorus and potassium

Powdered plant samples (0.5 g) were taken in 100 ml conical flasks. To each flask 15 ml of concentrated HNO_3 was added. The flasks were kept as such overnight. Then the flasks containing samples were heated on a hot plate till brown fumes evolved. Five ml of di-acid mixture (HNO_3: $HclO_4$ (70 %) = 3:2) was added to each flasks. Again the flasks were heated till white fumes evolved reducing the volume of content to about 2 ml. Thereafter, conical flasks were taken out from hot plate and allowed to cool. One ml of 6N HCL was added and flasks were heated gently for one minute. Then 15 ml of warm distilled water was added to each flask. The content of the conical flask was transferred to a 50 ml volumetric flask followed by twice rinsing with distilled water. Then the volume was made up to 50 ml with distilled water and the aliquot was filtered through What man No.42 filter paper. The filtered extract was kept for the estimation of phosphorus and potassium.

Estimation of phosphorus present in plant samples

Standards of 0, 2.5, 5.0, 7.5 and 10.0 ml of 25 ppm phosphorus solution and 2 ml of digested sample extracts were taken in 25 ml volumetric flasks. Five ml of 2(N) HNO_3 solution was added to each flask. Then required amount of distilled water was added to each flask to make the final volume 15 ml. Thereafter, 2.5 ml molybdate van date solution was added. Final volume was made up to 25 ml with distilled water and flasks were shaken well. Absorbance was measured by a colorimeter at 420 nm after 20 minutes of shaking. The phosphorus content of plant samples was calculated in percentage by using the standard curve.

Estimation of potassium present in plant samples

One ml digested sample extract of shoot were taken in 25 ml volumetric flasks and the volume was adjusted to 25 ml with distilled water. Similarly 1, 2, 3, 4 and 5 ppm standard K solution (i.e. 0.1907 g KCL/lit) were taken in 100 ml volumetric flasks with water. The readings for standards and samples were taken in a digital flame photometer. As per the standard curve, the ppm of potassium present in extracting solution was calculated. Then the percentages of potassium present in shoot samples were calculated.

RESULTS AND DISCUSSIONS

Nitrogen contents of shoots influenced by the nematode

The total Nitrogen content was decreased in the shoot system of resistant varieties of greengram to the extent of 88.57 per cent in, 28 PM 10-12 and increased by 196.61 per cent in resistant variety, 7 GGG 10-14 over control. Whereas in the roots the Nitrogen content increases in all the varieties. The Nitrogen content (%) both in root and shoot were significantly higher in the resistant varieties as compared to susceptible variety. The present results of increase of total Nitrogen content of nematode infected root samples is in confirmation with the findings of the earlier workers (Zaki and Bhatti, 1986, Nayak,2006). The nutrient accumulation in infected roots maybe due to impaired translocation to the aerial parts or the mobilization of nutrition from shoot to root. The percentage increase in N2-content in the shoots of resistant varieties may be due to higher absorption capacity or higher requirement for the resistance mechanisms.

Effect of nematode infection on crude protein content in shoot

Due to infection of root-knot nematode, the crude protein content percentage was low (4.10 %) in variety 8 GM 04-02, 57.66% in 17 IPM 9901-6, 66.05% in 7 GGG 10-14,62.29% in 24 ML-233 than that of 29 PUSA 0672 (66.76 %) and 28 PM 10-12 (88.56 %) (Table 2 and Fig. 2). The crude protein of the infected shoot decreased in the similar trend like Nitrogen content of the infected shoot. The decrease in crude protein content in shoots of different varieties possibly due to action of proteolytic enzymes from host plant as well as the invading nematode pathogen. The changes in protein content during post infection period relates to defensive action of the host plant against nematode infection which is more pronounced in resistant varieties. The increased crude protein content in nematode infected plants was reported by various workers (Ganguly and Dasgupta, 1983; Devarajan and Rajendran, 2002; Vaitheeswaran *et al*., 2004, Nayak, 2006) (Table 2).

Phosphorus content in shoots influenced by the nematode

The phosphorus content (Table 3) of the shoot in the varieties, 28 PM 10-12,29 PUSA 0672 24ML-233,7 GGG 10-14,17 IPM 9901-6 and 8 GM 04-02 were 2.87, 1.57, 1.57, 2.70, 2.85, and 1.75 per cent on dry weight basis respectively. The variety 28 PM 10-12 contained highest percentage of phosphorus, but it was decreased to half after infection by the nematode and it was highest in case of resistant variety (Fig. 3). The results of the present investigation revealed that the infected plants had decreased percentage of phosphorus content in shoots of susceptible and resistant varieties. Similar trend was also observed by Hunter, 1958; Chakraborty and Mishra, 2002 in root-knot nematode infected plants. The aminoacid, protein, Sugar and absorbed N2, P2O5 and K2O and other elements accumulate in the roots, shoots and leaves of nematode infected plants and remain under-utilized by the plant system which ultimately find the path into the growth and production of nematode.

Table 1. Percentage increase /decrease in Nitrogen content in Healthy(H) and root-knot infected (I) plant

Sl. No.	Variety	Nitrogen content % on dry weight basis			
		Infected (I) Leaf	Healthy (H) Leaf	Mean Leaf	% increase(+)/ decrease(-) over control
1	28 PM 10-12	0.28	2.45	1.36	-88.57
2	29 PUSA 0672	0.35	1.05	0.70	-66.66
3	24 ML -233	0.59	1.57	1.08	-62.42
4	7 GGG 10-14	1.75	0.59	1.17	196.61
5	17 IPM 9901-6	1.26	2.9797	2.11	-57.57
6	8GM 04-02	3.25	3.39	3.32	-4.12
	SEM(±)	0.37	1.40		
	CD(0.05)	1.14	4.41		

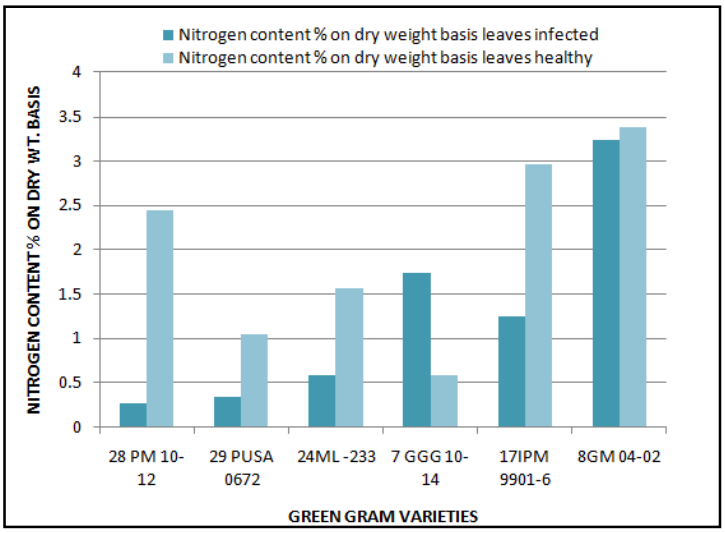

Fig. 1. Nitrogen content of control and infected plants

Table 2. Percentage increase /decrease in crude protein content in healthy (H) and root-knot infected (I) plant

Sl. No.	Variety	Crude Protein content % on dry weight basis			
		Infected(I) Leaf	Healthy(H) Leaf	Mean Leaf	% increase(+)/decrease(-)over control
1	28 PM 10-12	1.75	15.31	8.53	-88.56
2	29 PUSA 0672	2.18	6.56	4.37	-66.76
3	24 ML -233	3.71	9.84	6.78	-62.29
4	7 GGG 10-14	10.93	3.69	7.32	196.20
5	17 IPM 9901-6	7.87	18.59	13.23	-57.66
6	8GM 04-02	20.34	21.21	20.78	-4.10
	SEM(±)	1.79	3.01		
	CD(0.05)	5.64	9.48		

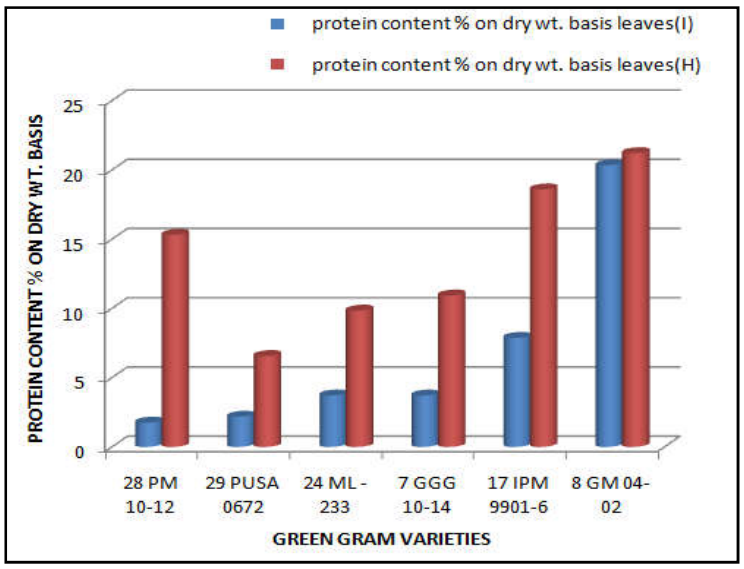

Fig. 2. Crude protein content of control and infected plants

Table 3. Percentage increase /decrease in Phosphorus content in Healthy (H) and root-knot infected (I) plant.

Sl. No	Variety	Phosphorus content % on dry weight basis			
		Infected(I) Leaf	Healthy(H) leaf	Mean Leaf	%increase(+)/decrease(-) over control
1	28 PM 10-12	1.37	2.87	2.12	-52.26
2	29PUSA 0672	2.30	1.57	1.94	46.49
3	24 ML -233	1.47	1.57	1.52	-6.36
4	7 GGG 10-14	2.67	2.70	2.69	-1.11
5	17 IPM 9901-6	1.65	2.85.5	2.25	-42.10
6	8GM 04-02	1.67	1.75	1.71	-4.57
	SEM(±)	0.42	0.39		
	CD(0.05)	1.33	1.21		

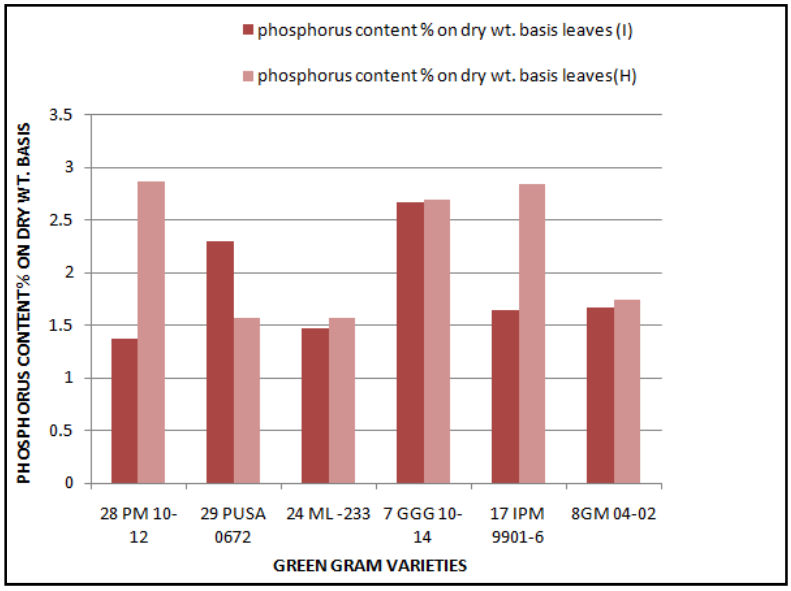

Fig. 3. Phosphorus content of infected and healthy shoots

Table 4. Percentage increase /decrease in potassium content in healthy (H) and root-knot infected (I) plant

Sl.No.	Variety	Potassium content % on dry weight basis			
		Infected(I) Leaf	Healthy(H) Leaf	Mean Leaf	%increase(+)/decrease(-) over control
1	28 PM 10-12	0.90%	0.84%	0.87%	7.14
2	29 PUSA 0672	0.45%	0.63%	0.54%	-28.57
3	24 ML -233	0.57%	0.59%	0.58%	-3.38
4	7 GGG 10-14	1.22%	1.09%	1.16%	11.9
5	17 IPM 9901-6	0.35%	0.65%.6	0.5%	-46.15
6	8GM 04-02	0.91%	0.66%	0.79%	37.87
	SEM(±)	0.25	0.27		
	CD(0.05)	0.80	0.85		

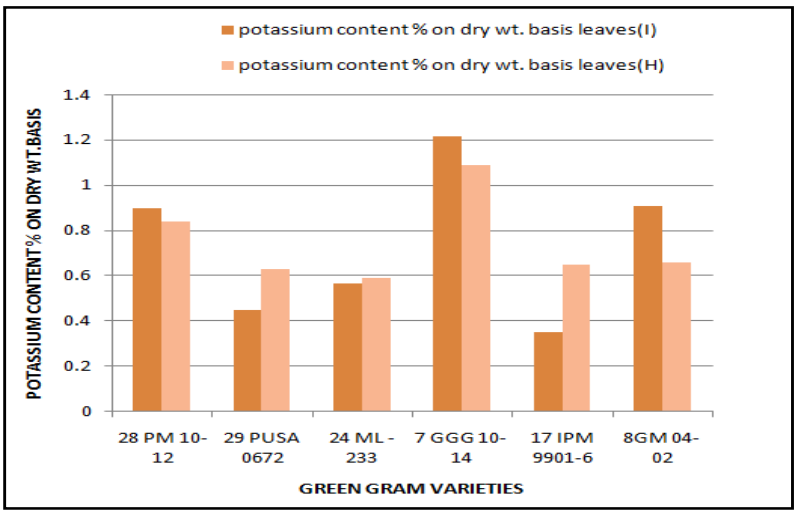

Fig. 4. Potassium content of infected and healthy plants

This may be the possible reasons for the accumulation of phosphorus in nematode infected plants (Table 3).

Influence of the nematode on potassium content in shoots

The root-knot nematode inoculated plants measured a reduction in K_2O value to the extent of 28.57, 3.38 and 46.15 per cent in 29 PUSA 0672 , 24 ML-233 and 17 IPM 9901-6 respectively while there was an increase in K□O content to the tune of 7.14,11.9 and 37.87 per cent in 28 PM 10-12, 7 GGG 10-14 and 8 GM 04-02 varieties, respectively. Table 4 & Fig-4 revealed that there was significant increase of potassium content of both susceptible and resistant infected plants which was more pronounced in shoots of resistant varieties as compared to the susceptible variety. Chakraborty and Mishra (2002) have also recorded decrease in potassium content due to infection of *Xiphinema americanum* and *M. incognita* in sour cherry and chickpea respectively. Potassium plays a significant role for maintaining turgor pressure of tissues of plants. In the nematode infected plant tissues various compounds relating to ion-exchange may be decreased by the reduction of 'K' content in nematode infected plant.

Conclusion

During investigation, it was also observed root gall index is directly proportional to the protein content of roots but it was reversed in the shoots. The total protein content of infected leaves of green-gram was maximum in healthy plants than the infected. The total proteins are reduced in both shoots and roots of infected plants. The enzymatic degradation of plant proteins and reduced photosynthesis caused accumulation of soluble amino acid in the infected plants. Protein increased in shoots in the infected plants due to the excessive hypertrophy and hyperplasia following nematode establishment and translocation of nucleic acid to the foliage. The nematode induced galls reported to content higher DNA, RNA. & RNA increases due to the inhibition of protein synthesis and nitrogen metabolism.

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