

Effect of Macronutrient Contents of Resistant and Susceptible Blackgram Cultivars as Influenced by Root-Knot Nematode, *Meloidogyne incognita*

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Received 13 July 2017; Accepted 16 August 2017; Published online 2 September 2017

Abstract The present study aimed at determining the change in leaf nutrient contents of different resistant and susceptible cultivars of blackgram against root-knot nematode, *Meloidogyne incognita*. For this study four resistant cultivars, i.e., KUG-715, NUL-205, VBG 11-031, VBG-11-016 and two susceptible cultivars PU 09-36 and MU-44 were accessed for change in leaf nitrogen, phosphorus and potassium contents

of both infected and healthy plants of above six cultivars. Under pot culture condition in green house. Leaf N_2 and P_2O_5 contents were determined by Kjeldahl extraction method and K_2O content were determined by atomic absorption spectrophotometer. Maximum N_2 content of 1.32% was noted in variety MU-44 while minimum as 23.13% in variety PU 09-36. Similarly P_2O_5 maximum 6.66% noted in varieties PU 09-36 and MU-44 and minimum 17.64% in varieties VBG 11-016. The crude protein content was maximum 64.13% in the varieties KUG-715.

Keywords Macronutrient contents (N, P, K and crude protein), Biochemical changes, *Meloidogyne incognita*.

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 and qualitatively. 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

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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.

Fazal et al. [1] showed that the inoculation of *Meloidigyne incognita* race-1 caused adverse effects on growth of the blackgram, nodule development, nitrogen content of root and shoot, leg-hemoglobin, bacterioids and nitrogenase activity in all the treatments at all population level. The nematode parasitism adversely affect the nutrition uptake ability of the plants. Root-knot nematode infection also leads to the reduced photosynthetic activity of the plants by causing reduction in the leaf chlorophyll content.

Materials and Methods

A pot culture experiment was conducted in the department of nematology under greenhouse condition. Pots containing sterilized soils were arranged on greenhouse benches. Seeds collected from different sources were sown and sprinkled with water for germination purpose. To established the basis of nematode resistance four varieties namely KUG-715, NUL-205, VBG 11-031 and VBG-11-016 (resistant check),

UP 09-36 and MU-44 (susceptible check) were grown in earthen pots in green house, filled with sterilized soil and sand mixture (1:1) @ 1 kg/pot along with NPK @ 20:40:40 kg/ha. These plants were uprooted and washed 45 days after nematode inoculation and processed for chemical analysis.

Estimation of nitrogen, phosphorus, potassium and crude protein content of shoot nitrogen and crude protein estimation of leaf

Crude protein and nitrogen content of shoot were estimated by following the procedure of Mahadevan and Sridhar. 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 thiosulfate 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 indi-

Table 1. Percentage increase/decrease in Nitrogen content in healthy and root-knot infected plant.

Sl. No.	Variety	Nitrogen content % on dry weight basis			
		Infected Leaf	Healthy Leaf	Mean Leaf	% increase (+)/decrease (-) over control
1	PU 09-36	1.96	2.55	2.25	-23.13
2	MU-44	2.24	2.57	2.25	-1.32
3	VBG 11-031	3.57	3.81	3.69	-6.29
4	VBG 11-016	3.04	3.57	3.30	-14.84
5	KUG-715	2.66	3.01	2.83	-11.62
6	NUL-205	2.94	3.74	3.34	-21.39
	SEm ($\pm$)	0.37	1.40		
	CD (0.05)	1.14	4.41		

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

Sl. No.	Variety	Protein content % on dry weight basis			
		Infected Leaf	Healthy Leaf	Mean Leaf	% increase (+)/decrease (-) over control
1	PU 09-36	0.103	0.069	0.086	49.27
2	MU-44	0.231	0.106	0.168	117.9
3	VBG 11-031	0.351	0.330	0.240	6.36
4	VBG 11-016	0.239	0.178	0.208	34.26
5	KUG-715	0.151	0.092	0.121	64.13
6	NUL-205	0.202	0.141	0.171	43.26
	CD (0.05)	0.009	0.010		

cator. After completion of distillation, distillate was titrated against 0.05 NH_2SO_4 .

Calculation

$$\text{Percent N}_2 \text{ in sample} = \frac{(\text{Sample titer} - \text{blank titer}) \times \text{N}_2 \text{ of H}_2\text{SO}_4 \times 14 \times 100 \times 2.5}{\text{Sample weight (g)} \times 1000}$$

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 flask were kept as such overnight. Then the flasks containing samples were heated on a hot plate till brown fumes evolved. Five ml of diacid mixture ($\text{HNO}_2 : \text{HIO}_4$ (70%) = 3:2) was added to each flask. 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 6 N 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

Table 3. Percentage increase/decrease in phosphorus content in healthy and root-knot infected plant.

Sl. No.	Variety	Phosphorus content % on dry weight basis			
		Infected Leaf	Healthy Leaf	Mean Leaf	increase (+)/decrease (-) over control
1	PU 09-36	0.112	0.120	0.172	-6.666
2	MU-44	0.038	0.042	0.059	-9.523
3	VBG 11-031	0.042	0.045	0.064	-6.666
4	VBG 11-016	0.020	0.017	0.028	17.640
5	KUG-715	0.035	0.042	0.056	-16.666
6	NUL-205	0.022	0.042	0.043	-11.904
	CD (0.05)	0.012	0.005		

molybdate vanadate 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 Discussion

Nitrogen content in shoots influenced by the nematode

The total nitrogen content decrease in shoot system of resistant varieties of blackgram due to the infection of *Meloidogyne incognita* to the extent of 23.13% in PU 09-36 and increase by 1.32% in resistant vari-

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

Sl. No.	Variety	Phosphorus content % on dry weight basis			increase (+)/ decrease (-) over control
		Infected Leaf	Healthy Leaf	Mean Leaf	
1	PU 09-36	0.302	0.422	0.36	-23.43
2	MU-44	0.313	0.473	0.39	-33.82
3	VBG 11-031	0.441	0.661	0.55	-33.28
4	VBG 11-016	0.572	0.462	0.51	23.80
5	KUG-715	0.390	0.580	0.48	-32.75
6	NUL-205	0.515	0.565	0.53	-8.84
	CD (0.05)	0.005	0.007		

ety MU-44 over control (Table 1). The nitrogen content (%) in shoot were significantly higher in the resistant varieties as compared to that of susceptible variety. The percentage increase in N_2 -content in the shoots of resistant varieties may be due to higher absorption capacity or higher requirement for the resistance mechanisms. This may be due to lower translocation rate. Mahalik [2] showed that *Meloidogyne incognita* reduced the nitrogen content in shoot of blackgram mutant lines of Sarala, Pant U-30 and PDU-1 and the reduction was to tune of 11.4% over control.

Effect of nematode infection on crude protein content in shoot

Due to infection of root-knot nematode, the crude protein content percentage was low 6.36% in case of VBG 11-031, 34.26% in VBG 11-016, 43.26% in NUL-205, 49.27% in PU 09-36 than that of MU-44 (117.9%) and KUG-715 (64.13%) (Table 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 photolytic 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 vari-

eties. The increased crude protein content in nematode infected plants were reported earlier various workers [3, 4].

Phosphorus content in shoots influenced by the nematode

The phosphorus content (Table 3) of the shoot in the varieties, PU 09-36, MU-44, VBG 11-031, VBG 11-031, KUG-715 and NUL-205 were 6.66, 9.52, 6.66, 17.64, 16.66 and 11.904% on dry weight basis respectively. The variety VBG 11-016 contained highest percentage of phosphorus, but it was decreased to half in case of infection by the nematode and it was highest in case of resistant variety.

The result of present investigation revealed that the infected plants had decreased percent of phosphorus content in shoots of susceptible and resistant varieties. Similar trend was also observed Pandey et al. [5] in root-knot nematode infected plants. The amino acid, protein, sugar and absorbed N_2 , P_2O_5 and K_2O 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. This may be the possible reasons for the accumulation of phosphorus in nematode infected plants.

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 28.43, 33.82, 33.28 and 32.75% in PU 09-36, MU-44, VBG 11-031 and KUG-715 respectively while an increase in K_2O value to the extent of 23.80 and 8.84% in VBG 11-016 and NUL-205 respectively (Table 4).

The present study revealed there was significant increase of potassium content of both susceptible and resistant infeced plants which increase was more pronounced in shoots of resistant varieties as compared to the susceptible variety. Pandey et al. [5] have also recorded decrease in potassium content due to infection of *Meloidogyne incognita* in greengram. Potassium plays a significant role for maintaining tur-

gor pressure of tissues of plants. In the nematode infected plant tissues various compounds relating to ion-exchange may be decreased which is reflected by the reduction of K content in nematode infected plant samples.

Conclusion

The nitrogen content in shoot was significantly higher in the resistant varieties as compared to that of susceptible variety. The percentage increase in nitrogen content in the shoots of resistant varieties may be due to higher absorption capacity or higher requirement for the resistance mechanisms but there was increased percentage of phosphorus content in shoots of resistant and susceptible varieties. This may be possible reasons for the accumulation of phosphorus in nematode infected plants. In the present study there was significant decrease of potassium content of both infected susceptible and resistant infected plants by *Meloidogyne incognita* than healthy varieties and it was more pronounced in shoots of infected plants as compared to the healthy varieties. Potassium plays a significant role for maintaining turgor pressure of tissues of crop plants. The nutrient accumulation in infected roots may be due to impaired translocation to the aerial parts or the mobilization of nutrition from shoot to root.

The total protein content of infected leaves of blackgram was maximum in healthy plants than the infected. The total proteins are reduced in both shoots 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.

References

1. Fazal MY, Munawar, Hissamuddin (2009) The effect of *Meloidogyne incognita* race-1 on the functioning of rhizobial nodules on blackgram, *Vigna mungo*. J Nematol 39 : 59—64.
2. Mahalik JK (2005) Biochemical changes in blackgram mutant lines due to root-knot nematode (*Meloidogyne incognita*) infection. MSc (Ag) thesis. OUAT, Bhubaneswar.
3. Vaitheeswaran M, Mohamed Ibrahim S, Lakshmi Narasimhan K (2004) Organic amendments for the control of root-knot nematode on *Phaseolus mungo*. Ind J Nematol 35 : 112—119.
4. Nayak DK (2006) Biochemical evaluation of various metabolites as influenced by root-knot nematode, *M. incognita* in susceptible and resistant brinjal cultivars. PhD thesis. Orissa Univ Agric and Technol, Bhubaneswar.
5. Pandey RK, Nayak DK, Kar RK (2017) Effect of macronutrient contents of resistant and susceptible greengram cultivars as influenced by root-knot nematode, *Meloidogyne incognita*. Int J Curr Res 9 : 49166—49170.