Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(4): 1191-1193

Received: 04-05-2019
Accepted: 06-06-2019

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Protein estimation through biochemical analysis in resistant and susceptible cultivars of cowpea against infection by root-knot nematode, *Meloidogyne incognita*

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Abstract
Biochemical alterations brought about in resistant and susceptible cultivars of cowpeaas a result of root-knot infection was estimated in roots uprooted after 45 days of *Meloidogyne incognita* inoculation. Quantitative analysis of protein metabolites in resistant varieties such as Arka variety, IT-35956-1 and TVX-944 cultivars showed that protein content increased by 42.81 per cent, 89.93 per cent and 57.67 per cent respectively, in diseased roots. Similarly, in leaves of inoculated susceptible varieties like KM-5 and Gomati, protein content was highly reduced by 63.10 per cent and 52.27 per cent, respectively.

Keywords: Cowpea, protein, nematode, *meloidogyne incognita*, resistant, susceptible

Introduction
Meloidogyne incognita is a serious pest of cowpea, *Vigna unguiculata* (L.) Walp. In most cowpea growing areas of the world [1]. *Meloidogyne incognita* was first reported in Nigeria on cowpea in 1958 and documented in 1960 this nematode constitutes a major constraint to cowpea production. Root-knot nematodes like many other obligate parasites are capable of disturbing the host metabolism. The changes in the physiological and biochemical processes of infected host as consequences of disturbed metabolism decide whether the host becomes susceptible or resistant to nematode attack. Root knot nematode infection is the formation of typical root galls on the roots of susceptible host plants. Nutrient and water uptake are substantially reduced because of the damaged root system, resulting in weak and low-yielding plants [2]. Root-knot nematodes cause measurable changes in the morphology and physiology of the host. Root damage from the nematode results in stunted and chlorotic plants. In cowpea leaves, root-knot infection reduces the photosynthetic rates [3]. [4]Protein content decreased and most amino acids and amides increased after inoculation in susceptible as well as resistant cultivars. Phenolic compounds have also been associated with nematode injury, leading to the browning of plant tissues. In addition, a marked increase in pre-existing phenols as well as the synthesis of small amounts of phenols in infected roots has been found to result from nematode infection. The study examined the physiological and biochemical changes caused by the root-knot nematode *Meloidogynejavanica*in mung bean (*Vigna radiate* [L.] Wilczek) at various post inoculation times.

The changes in the physiological, growth and yield parameters and biochemical processes of infested host decide whether the host becomes susceptible or resistant to nematode attack. In this context an intimate knowledge of nematode physiology and biochemistry along with its host is absolutely essential for developing plant resistance against the nematodes. In recent past some progress has also been made in this direction. the penetration of J_2 into the root tips and later migration into the root tissue causing cortical hypertrophy, giant cell formation and increase in size of epidermal, cortical and stellar cells. The infected roots shows increased amount of insoluble polysaccharides, proteins and nucleic acid compared to healthy roots. In this context an intimate knowledge of nematode physiology and biochemistry along with its host is absolutely essential for developing plant resistance against the nematodes. In total sugar content, starch contents, chlorophyll contents, protein contents, phenol contents, amino acids and proline contents in relation to resistant and susceptible cowpea inoculated with root-knot nematode, *Meloidogyne incognita.*

India is the world's largest producer and consumer of a wide Variety of pulses which is dominated by tropical and sub-tropical crops. Presently, pulse accounts for 294.65 Lakh ha of the total area in India and contributes about 22.95 million tonnes of the total production with the productivity of 779 kg/ha (Anon, 2017-18). The largest producer states in India are U.P, Bihar, Jharkhand, West Bengal and Odisha. The estimated area under cowpea cultivation in India is around 23012 ha. The production quantity is about 133587 tonnes and productivity accounting to 5.8 t/ha in India. The total area under pulse in Odisha is about 52.97 thousand hectares, with a production of 39.54 thousand tonnes (Odisha Agricultural Statistics, 2014-2015). The productivity is approximately 746 kg/ha.

The production of pulse crops unfortunately suffers from several constraints of which pest and disease are the most important once. Among pests, phytoparasitic nematodes have been recognized as one of the major constraint in pulse production. The extent of losses due to nematodes especially in pulse crops is yet to be estimated properly but in cowpea production is estimated to cause annual yield losses of nearly 15% worldwide. In India average loss caused by root-knot nematode on pulses may be 14.6% which could go as high as 50-80% in some crops. In another study estimated 28.60 per cent losses due to root-knot nematode, *M. incognita* in cowpea.

Materials and Method

Estimation of protein by Lowry's method

1g of fresh or 0.2g dry sample is to be macerated by pestle and mortar in 10ml of TCA solution. The sample so obtained is to be transferred to centrifugal tube and centrifuged at 5000rpm for 10mins. The supernatant from centrifugal tube is to be discarded. 10ml of 1N NaOH solution is to be added and mixed well with the help of glass rod. The prepared sample is again centrifuged at 10,000 rpm for 10 mins. The true protein is estimated using the collected supernatant.

Estimation

Prepared the standards by taking 0, 0.2, 0.4, 0. 6, 0.8 and 1 ml of the working solution of standard and added to series of test tubes. 0.2ml of 1N NaOH solution added into each test tube. 0.1ml & 0.2ml of the sample extract transferred into 2 other test tubes. The volume made to 1 ml in all the tubes including the sample tubes by adding distilled water. A tube with 1 ml of water served as blank. 5 ml of Reagent C added to each tube including blank. Mixes well and incubated at room temp for 10 min. Then 0.5 ml of Reagent D was added, mixes well immediately and incubated at room temperature in the dark for 30 min. Blue colour were developed. Measure the sample at OD 660nm. Drawn a standard graph and calculated amount of protein in sample and expressed the results as mg/g or mg/100g sample/ percentage.

Result and Discussion

Protein content in leaves

The present study (table 1) was carried out for five varieties *viz.*, Arka variety, IT-35956-1, TVX-944 variety, KM-5 and Gomati. Among these Arka variety, IT-35956-1 and TVX-944 variety were found to be resistance to *M. incognita.* Protein content in leaves of these inoculated resistance varieties was found to be reduced from 7.83 mg/g to 6.59 mg/g, 8.96 mg/g to 8.03 mg/g, and 9.33 mg/g to 8.40 mg/g respectively whereas KM-5 and Gomati showed susceptibility with values of 23.16 mg/g to 8.55 mg/g and 18.36 mg/g to 8.76 mg/g, respectively.

Among the susceptible varieties KM-5 showed difference between inoculated and uninoculated cowpea varieties which was found to be noticed highest reduction per cent of Protein content (63.10%) in KM-5 followed by (52.27%) in Gomati. Among the resistant varieties there is decreased in Protein content of (15.82%) in Arka variety, (9.92%) in TVX-944 variety and (10.41%) in IT-35956-1 respectively in comparison to uninoculated crop.

Table 1: Change in protein content in cowpea shoots influenced by *M. incognita*

Sl. No.	Varieties	Protein analysis in cowpea			
		Leaf			% Increase(+)/ decrease(-)
		Inoculated	Uninoculated	Mean	Over Uninoculated
1	Ark seeds(R)	6.59	7.83	7.21	-15.82
2	IT-3596-1(R)	8.03	8.96	8.49	-10.41
3	TVX-944(R)	8.40	9.33	8.87	-9.92
4	KM-5(S)	8.55	23.16	15.85	-63.10
5	Gomati(S)	8.76	18.36	13.56	-52.27
	SE(M)±	0.76	1.33		
	CD(0.05)	1.67	2.90		

Resistant(R), Susceptible(S)

Protein content in Root

The present study was carried out for five varieties *viz.*, Arka variety, IT-35956-1, TVX-944 variety, KM-5 and Gomati. Among these Arka variety, IT-35956-1 and TVX-944 variety were found to be resistant to *M. incognita*. Protein content in Root of these inoculated resistance varieties was found to be increased from 25.51 mg/g to 36.48 mg/g, 16.36 mg/g to 31.68 mg/g, and 13.33 mg/g to 21.02 mg/g respectively whereas KM-5 and Gomati showed susceptibility with values of 18.38 mg/g to 18.94 mg/g and 14.34 mg/g to 15.36 mg/g, respectively.

Among the resistant varieties IT-35956-1 showed difference between inoculated and uninoculated cowpea varieties which was found to be noticed highest increased per cent of protein content 89.93% in IT-35956-1 followed by 57.69% in TVX-944 variety, 42.81% in Arka variety. Among the susceptible varieties there is increased in protein content of 7.11% in Gomati and 3.05% in KM-5 variety in comparison to uninoculated crop.

Table 2: Change in protein content in cowpea roots influenced by *M. incognita*

Sl. No.	Varieties	Protein analysis in cowpea			
		Root			% Increase (+)/ decrease(-) over
		Inoculated	Uninoculated	Mean	Uninoculated
1	Ark seeds(R)	36.43	25.51	30.97	+42.81
2	IT-3596-1(R)	31.68	16.68	24.18	+89.93
3	TVX-944(R)	21.02	13.33	17.18	+57.69
4	KM-5(S)	18.94	18.38	18.66	+3.05
5	Gomati(S)	15.36	14.34	14.85	+7.11
	SE(M)±	0.71	0.50		
	CD(0.05)	1.56	1.09		

Resistant(R), Susceptible(S)

Present study showed in the table 1 and 2 discussed following reasons *M. incognita* infestation resulted in difference of Total protein(63.10%) content of leaves were highest reduction in KM-5 variety whereas, in root Total protein content (89.93%)were more increased in IT-3596-1 as compared to Uninoculated resistant and susceptible cowpea. These variation take place in plant due to chemical component produces during infection of nematode. It can explain that protein and nucleic acid are varied with the severity of the nematode infection caused. Protein content has reduced in the nematode infected plant because of its giant cells utilizes the amino acid pool which is required for protein synthesis. The quantitative increase of various amino acids during post infection period may be due to proteolysis of existing tissue proteins or synthesis of new compounds through various metabolic pathways during plant-nematode interaction. Under stress condition protein was reduced by proteolysis of proteins. It leads the low concentration of nucleic acids in infected plants due to enhanced ribonuclease activity. The activity of this enzyme is possibly increased in susceptible plants due to the growth and multiplication of the nematodes in the roots. Results are in accordance with the earlier findings [5] [6].

Conclusion

Studies on physiological and biochemical processes of tested cowpea plant regarding This may be useful in understanding the advanced mechanism of plant resistance which can be exploited through advanced biotechnological research for planning appropriate management strategies against *Meloidogyne incognita* and other plant parasitic nematode.

Acknowledgement

The authors are thankful to Department of Agril. Biotechnology, College of Agriculture, OUAT, Bhubaneswar and Department of Nematology, College of Agriculture, OUAT, Bhubaneswar for providing space for research work.

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