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Research Article
Biochemical Changes Induced by *Meloidogyne graminicola* in Resistant and Susceptible Pearl Millet (*Pennisetum glaucum* L.) Hybrids

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Abstract

Background and Objective: *Meloidogyne graminicola* is a potential threat to the successful cultivation of the pearl millet, significantly reducing its yield and produce quality. Moreover, biochemical and physiological changes induced by *Meloidogyne graminicola* susceptible and resistant hybrids of pearl millet is not known in detail. The study was focused on the biochemical changes induced by the root-knot nematode, *Meloidogyne graminicola* on pearl millet hybrids to determine the role of different biochemical on nature of resistance of the plants. **Materials and Methods:** Resistant (HHB 146) and susceptible (HHB 272) pearl millet hybrids were grown in steam-sterilized soil with two different sets, i.e., inoculated and uninoculated. Data was recorded on various biochemical parameters from roots and shoot portion on 15, 30 and 45 Days After Inoculation (DAI). **Results:** Total protein and phenols were increased in the roots and shoot portion of the infected plants. Total sugar was reduced in *M. graminicola* infected plants and this reduction was more pronounced in case of susceptible inoculated plants. Phenol content increases as a result of nematode infection. On the other side, due to nematode infection sugar content in roots of susceptible hybrid is comparatively lesser as compared to resistant hybrid. **Conclusion:** It is concluded that root-knot nematodes, *M. graminicola* bring about significant biochemical changes in infected plants, which appear to employ physiological and biochemical strategies either to avoid or to tolerate the nematode infection.

Key words: *Meloidogyne graminicola*, biochemical changes, pearl millet hybrid, phenol, sugars, protein

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Pearl millet (*Pennisetum glaucum* L.) is the fifth important cereal crop in the world and the fourth important food crop in India after rice, wheat and sorghum. It is a short day plant that belongs to Gramineae family and usually grown as a dry land dual-purpose grain and fodder crop although it is sometimes irrigated in India. It is a coarse grain crop and is considered as poor man's staple food. The crop is grown on the poorest soil and under harsh climatic conditions where no other crop can grow[1]. Due to its efficient root system as well as high ability to produce tillers, pearl millet proves to be drought and heat tolerant crop. It certainly originated in tropical western Africa, where the greatest number of both wild and cultivated forms occurs. In India, pearl millet is grown on 7.81 mha with grain production of 9.21 MT and productivity[2] of 1231 kg ha^{-1}. Lead pearl millet producing states in India are Rajasthan, Maharashtra, Gujarat, Uttar Pradesh and Haryana which account for more than 90% of pearl millet acreage in the country. Pearl millet has emerged as a substitute for cereal crops to combat with food shortage for the increasing population. It is not only rich in fiber, proteins and vitamins, but also carries phosphorus, manganese in abundance. Apart from this, it also promotes heart health, reduces weight, improves blood sugars and possesses anti-carcinogenic activity. Several plant-parasitic nematodes including ecto, endo and semi endoparasites have been reported to be associated with pearl millet. Among different genera of nematodes, burrowing nematode (*Radopholous similis*), cyst nematode (*Heterodera gambiensis*) root-knot nematode (*Meloidogyne* spp.), lesion nematode (*Pratylenchus* spp.) and stunt nematode (*Tylenchorhynchus* spp.) are considered most important. Initially, *Meloidogyne graminicola* was encountered in West Bengal, Odisha, Assam and Kerala only, but now has also spread to newer areas of northern and southern India[3]. *Meloidogyne graminicola* has a wide host range[4] that comprises many of the common weeds of rice fields and agricultural crops that are grown in rotation with rice. It has also been found infecting crops of Gramineae, including pearl millet[5]. Pearl millet is found to be the second most preferred and susceptible host for *Meloidogyne graminicola*[6]. Reports on damage caused by this nematode are available in rice, but no work on yield losses has been carried out in pearl millet. Jain *et al.*[7] reported 10.54% reduction in rice yield due to various nematodes together with *Meloidogyne graminicola*. Eggs of this nematode are laid inside the cortex of the root, contrasting with other *Meloidogyne* spp. that allows the juveniles to endure in the maternal gall and re-infect the same root[8]. The second-stage juveniles (J_2) of *Meloidogyne graminicola* make their point of entry at the zone of elongation just behind the root cap. These J_2 penetrate into the roots and lead to formation of multinucleate giant cells. Root-knot nematode, *Meloidogyne graminicola* feeds on the vascular tissues that hamper water and nutrient uptake and also create hindrance in their translocation. Peculiar small gall (hook shaped) is an indication of *Meloidogyne graminicola* infection. Further, excessive branching of affected roots is also observed due to infection. Root-knot nematode affected plants show reduced vigour, poor growth, chlorotic and curled leaves. Wide ranges of phenolic compounds are synthesized in plant tissues during normal growth and development. These compounds are building blocks for cell wall structure and plant pigment production. They serve as protection from ultraviolet light and as a defense against pathogens[1]. They act as hydrogen donors/acceptors in oxidation reduction reaction. During plant nematode interaction, plant shows variety of chemical changes to curb nematode menace[9]. Like other nematodes, *Meloidogyne graminicola* is also quite effective in manipulation of the hosts metabolism. Whereas primary metabolic pathways are induced, secondary metabolism is inhibited in the galled tissue. Chlorophyll a, b and carotenoids, accumulate inside the galled tissue. During nematode infection plants show biochemical changes leading to wilting and death due to blockage of water conduction, but resistant plants produce inhibitions/phytoalexin including histological changes. Nematode damage causes nutrient shortage, which triggers depletion of chlorophyll in the healthy tissues, which in turn reduces carbon assimilation and successive metabolic processes. Resistance offers one of the most economical and effective method to check infestation of plant parasitic nematodes. Several kinds of chemicals are present in the plant system that affects the metabolism of the nematode feeding upon them. In rice, resistance to *Meloidogyne graminicola* is found to be associated with high aspartic acid and alanine contents and low valine, tryptophan and methionine contents in the roots. Amino acids normally increase in resistant rice plants due to nematization[10]. Phenolic compounds are secondary metabolites that play a major role in the defence mechanisms of plants against pathogens and tend to increase in the resistant cultivars. Identifying new sources of resistance will help in breeding new nematode-resistant cultivars. Finding out sources of resistance and generate information on the mechanism of resistance can be utilized for breeding purpose and cultivation. Some studies have been done on screening, life cycle, pathogenicity and biochemical aspects (phenol, sugars, amino acids and gallic acid) on rice, but no work has been done on effect of *Meloidogyne graminicola* on

pearl millet. The importance of the subject, the present study was planned to gather information on biochemical changes induced by *Meloidogyne graminicola* in pearl millet hybrids.

MATERIALS AND METHODS

Plant material: In order to understand the basis of nematode resistance biochemical analysis of two pearl millet hybrids, i.e., resistant HHB 146 and susceptible HHB 272 were done. These hybrids were grown in steam-sterilized soil having 1 kg capacity earthen pots. The inoculum of *Meloidogyne graminicola* required for experimentation was collected from the infected roots of rice plant from nearby farmer field. Clean galled portions of roots were taken in the Petri plate containing small amount of water and were tested under the stereo-microscope with needle and forceps, for the collection of the eggs and J_2. Galled roots and residue of the root were removed.

Study area: This study was conducted in the screen house located at the research farm of the Department of Nematology, CCS Haryana Agricultural University, Hisar, India, during Kharif season August-October, 2018.

Inoculation treatment: Two sets of pots were maintained; in one set inoculation was done and another set was left uninoculated. After germination, one plant per pot was retained and inoculated with 2000 eggs and J_2 per pot in inoculation treatment. At fortnightly intervals, i.e., 15, 30 and 45 days after inoculation, plants were uprooted and washed properly. In this way, total four treatments with two sub-treatments were replicated five times.

Biochemical traits and data analysis

Extraction and estimation of total sugar: To extract total soluble sugars, 100 mg of crushed leaves or root sample were mixed separately in 10 mL of 80% ethanol. The resultant mixtures were heated for 1 h in a water bath at 80°C and intermittently the mixture was shaken vigorously on a vortex. After cooling, the homogenate was centrifuged at 4000 rpm for 20 min. The supernatant obtained was kept in a tube. This sample was again heated with 5 mL of 80% ethanol in a water bath at 80°C for 30 min. The supernatant thus obtained was pooled into the supernatant obtained earlier. This was repeated thrice and the supernatants collected were pooled. The pooled supernatant was concentrated until dryness at 40°C under vacuum concentrator and dissolved in distilled water to make the final volume 5 mL.

For estimation of total soluble sugars, the method given by DuBois *et al.*[11] was used. In a test tube, 0.5 mL of the above solutions were taken and 2 mL of 2% phenol and 5 mL of concentrated sulphuric acid (H_2SO_4) was added. Shaking of the solution was done on a vortex in order to mix the solutions well. The temperature of the solution was high; therefore, the solution was kept at room temperature for cooling. The developed colour was read by using UV-Spectrophotometer at 490 nm against reagent blank. The standard curve prepared by using dextrose (100-1000 μg mL^{-1}) was used to determine the concentration of sugar in samples.

Extraction and estimation of phenolic content: The procedure for the phenolic extraction was similar to the procedure used for extracting total soluble sugars. For estimation of total phenolic contents, Bary and Thorpe[12] method was used where 0.5 mL of aliquot was pipetted out in a test tube from the prepared extract. To this extract, freshly prepared 0.5 mL of 1N Folin-Ciocalteu reagent was added. After 3 min, 2 mL of 20% sodium carbonate (Na_2CO_3) was added to each of the test tube containing this solution. The resulting solution was shaken for the development of blue colour which was read at 650 nm using UV-spectrophotometer against blank reagent. The concentration of total phenol in each sample was determined by using catechol (10-100 μg mL^{-1}) as standard.

Extraction and estimation of crude proteins: From the ground leaves and root, a sample of 100 mg was taken in a digestion flask of 150 mL separately. After that, concentrated H_2SO_4 was mixed in 4:1 ratio and poured at 10 mL in each flask through measure and left it as such for one day. On the next day, for digestion, the flask was kept over the hot plate in an open area, until the solution become colourless. After cooling, the digested material was diluted with a small quantity of distilled ammonia-free water, so that temperature did not increase. After that, by pouring extra distilled water the solution was made up to 100 mL. This solution was transferred to the distillation apparatus.

Analysis of the crude protein was done by using the standard Kjeldahl method[13]. For the estimation of protein, the first nitrogen was extracted from the solution prepared, over the hot plate. The Kjeldahl flask was rinsed with a successive small quantity of water. A 100 mL of conical flask containing 10 mL of 0.01 H_2SO_4 with a few drops of mixed indicator (methyl red solution) was placed with the tip of condenser dipped below the surface of the solution. Then, 40% NaOH and 10 mL digested solution (test solution) were added in the

apparatus. Afterwards, ammonia was collected in the sulphuric acid (at least 40-50 mL of distilled solution was collected) by distillation. The tip of the condenser was rinsed off and the solution was titrated against standard acid until the permanent appearance of violet colour, the endpoint. A reagent blank with an equal volume of distilled water was run and titration volume was subtracted from that of a sample of titer volume.

The data obtained in the experiments were analyzed using OPSTAT software available online at CCS HAU, Hisar, (www.hau.ernet.in).

RESULTS

The data on the effect of *Meloidogyne graminicola* infestation on total protein content on a dry weight basis in pearl millet roots depicts that overall protein content in roots increased with the advancement of plant age. The *M. graminicola* infestation led to a significant increase in protein content of root, but the levels of the rise were different in both hybrids (Table 1). Increase in protein content of root of pearl millet was higher in susceptible (HHB 272) as compared to the resistant hybrid (HHB 146). Maximum increase (27.24%) in protein content in the root of pearl millet was found in susceptible (HHB 272) at 30 DAI. Percent increase in protein content of both hybrids at 45 DAI was lower as compared to 30 DAI.

The data on the effect of *M. graminicola* infestation on total protein content in pearl millet shoot estimated on dry weight basis showed that overall protein content in shoots of pearl millet hybrid increased up to 30 DAI. Nematode infection caused a significant increase in protein content of shoot however; increase in the level in both the hybrid was different. The rise in protein content of stalk of pearl millet was higher in susceptible (HHB 272) as compared to resistant (HHB 146). Maximum percent increase (26.09%) in protein content in the shoot of pearl millet was found in susceptible on 30 DAI (Table 2). Protein content in the resistant inoculated shoots was higher as compared to resistant uninoculated. Percent increase in protein content of resistant hybrid at 45 DAI was lower as compared to 30 DAI.

It is evident from Table 3 that there was a significant increase in phenol content in roots with the advancement of the age of the plant. Data revealed that overall phenol content in roots of pearl millet hybrid increased with observation period. Inoculated plants showed a significant increase in phenolic content as compared to uninoculated plants, irrespective of both the hybrids and all the three observation periods (Table 3). The maximum increase in phenol (73.33%) was recorded in roots of resistant inoculated plants on 15 DAI followed by 45 DAI (66.04%). In comparison to resistant inoculated there was lesser production of phenol in susceptible inoculated plants. Phenol content in susceptible hybrid started increasing on 15 DAI onwards, but it was lower

Table 1: Protein content in roots of resistant and susceptible hybrids of pearl millet inoculated with *Meloidogyne graminicola* (Mean of five replications)

	Protein content (Dry weight basis (%))						
	HHB 146 (Resistant)			HHB 272 (Susceptible)			
Observation period	UI	I	Increase over (%) (UI)	UI	I	Increase over (%) (UI)	Overall mean
15 DAI	16.04	16.76	4.49	14.24	16.48	15.73	15.88
30 DAI	16.80	18.50	10.12	15.42	19.62	27.24	17.59
45 DAI	17.23	18.54	7.60	16.54	19.60	18.50	17.98
Mean	16.69	17.93		15.4	18.57		
C.D. at 5%	T = 0.37, H = 0.42, T×H = 0.73						

Initial nematode population: 2000 eggs and J_2, T: Treatment, H: Hybrid, DAI: Days after inoculation, UI: Uninoculated, I: Inoculated

Table 2: Protein content in shoots of resistant and susceptible hybrids of pearl millet inoculated with *Meloidogyne graminicola* (Mean of five replications)

	Protein content (Dry weight basis (%))						
	HHB 146 (Resistant)			HHB 272 (Susceptible)			
Observation period	UI	I	Increase over (%) (UI)	UI	I	Increase over (%) (UI)	Overall mean
15 DAI	17.22	18.96	10.10	15.96	20.07	25.75	18.05
30 DAI	18.34	20.43	11.40	16.48	20.78	26.09	19.01
45 DAI	18.75	19.51	4.51	17.49	21.71	24.13	19.37
Mean	18.10	19.63		16.64	20.85		
C.D. at 5%	T = 0.39, H = 0.45, T×H = 0.78						

Initial nematode population: 2000 eggs and J_2, T: Treatment, H: Hybrid, DAI: Days after inoculation, UI: Uninoculated, I: Inoculated

Table 3: Phenol content in roots of resistant and susceptible hybrids of pearl millet inoculated with *Meloidogyne graminicola* (Mean of five replications)

	Protein content (Dry weight basis (%))						
	HHB 146 (Resistant)			HHB 272 (Susceptible)			
Observation period	UI	I	Increase over (%) (UI)	UI	I	Increase over (%) (UI)	Overall mean
15 DAI	0.30	0.52	73.33	0.35	0.37	5.71	0.39
30 DAI	0.44	0.72	63.64	0.43	0.47	9.30	0.52
45 DAI	0.53	0.88	66.04	0.51	0.56	9.80	0.62
Mean	0.43	0.71		0.43	0.47		
C.D. at 5%	T = 0.012, H = 0.014, T×H = 0.024						

Initial nematode population: 2000 eggs and J_2, T: Treatment, H: Hybrid, DAI: Days after inoculation, UI: Uninoculated, I: Inoculated

Table 4: Phenol content in shoots of resistant and susceptible hybrids of pearl millet inoculated with *Meloidogyne graminicola* (Mean of five replications)

	Protein content (Dry weight basis (%))						
	HHB 146 (Resistant)			HHB 272 (Susceptible)			
Observation period	UI	I	Increase over (%) (UI)	UI	I	Increase over (%) (UI)	Overall mean
15 DAI	0.43	0.73	69.77	0.32	0.50	56.25	0.50
30 DAI	0.60	1.10	83.33	0.53	0.75	41.51	0.75
45 DAI	0.73	1.21	65.75	0.62	0.78	25.81	0.84
Mean	0.59	1.01		0.49	0.68		
C.D. at 5%	T = 0.013, H = 0.015, T×H = 0.026						

Initial nematode population: 2000 eggs and J_2, T: Treatment, H: Hybrid; DAI: Days after inoculation, UI: Uninoculated, I: Inoculated

Table 5: Total sugar in shoots of resistant and susceptible hybrids of pearl millet inoculated with *Meloidogyne graminicola* (Mean of five replications)

	Total sugar (Dry weight basis (%))						
	HHB 146 (Resistant)			HHB 272 (Susceptible)			
Observation period	UI	I	Decrease over (%) (UI)	UI	I	Decrease over (%) (UI)	Overall mean
15 DAI	2.20	1.98	10.00	2.29	1.85	19.21	2.08
30 DAI	2.35	2.16	8.09	2.52	2.01	20.24	2.26
45 DAI	2.49	2.30	7.63	2.61	2.25	13.79	2.41
Mean	2.35	2.14		2.47	2.04		
C.D. at 5%	T = 0.17, H = 0.06, T×H = 0.04						

Initial nematode population: 2000 eggs and J_2, T: Treatment, H: Hybrid, DAI: Days after inoculation, UI: Uninoculated, I: Inoculated

than resistant inoculated plants at each observation. In the susceptible hybrid, maximum phenol content (9.80%) was found on 45 DAI.

Total phenol content in shoots significantly increased in all the growth stages viz. 15, 30 and 45 DAI (Table 4). Data showed that overall phenol content in shoots of pearl millet hybrid increased with observation period. There was a significant increase in phenol content in the inoculated plants as compared to uninoculated plants in all the stages of plant growth. Maximum increase (83.33%) was recorded in resistant inoculated plants over uninoculated at 30 DAS followed by 15 DAS (69.77%) and 45 DAS (65.75%). In the susceptible hybrid, the maximum increase in phenol content (56.25%) was observed at 15 DAI and it rapidly declined with the age of the plant. Minimum increase (25.81%) in phenol content of pearl millet shoot was found on 45 DAI.

The overall mean of total sugar content in shoots of pearl millet estimated on dry weight basis indicates that overall total sugar content in shoots of pearl millet hybrid increased with the advancement of plant age. It is evident from the data that total sugar content in the shoot of both the hybrids decreased as compared to their respective control (Table 5). It was found that lowest (1.85) in susceptible inoculated (HHB 272) on 15 DAI and highest (2.61) in susceptible uninoculated (HHB 272) on 45 DAI. Susceptible hybrid (HHB 272) showed a maximum decrease (20.24%) in total sugar content in the shoot on 30 DAI while least percent reduction (13.79%) on 45 DAI. In the resistant hybrid, HHB 146 maximum decrease (10.00%) in total sugar was recorded on 15 DAI which kept on reducing with the advancement in age of plant and was found minimum (7.63%) at 45 DAI.

The data on the total sugar content in roots, estimated on a dry weight basis, in resistant and susceptible pearl millet hybrids are given in Table 6. Overall entire sugar content in roots of pearl millet hybrid increased with observation period.

Table 6: Total sugar in roots of resistant and susceptible hybrids of pearl millet inoculated with *Meloidogyne graminicola* (Mean of five replications)

	Total sugar (Dry weight basis (%))						
	HHB 146 (Resistant)			HHB 272 (Susceptible)			
Observation period	UI	I	Increase over (%) (UI)	UI	I	Increase over (%) (UI)	Overall mean
15 DAI	1.76	2.15	22.16	1.51	2.16	43.05	1.90
30 DAI	1.81	2.27	25.41	2.18	3.76	72.48	2.51
45 DAI	1.96	2.39	21.94	2.29	4.05	76.86	2.67
Mean	1.85	2.27		2.00	3.32		
C.D. at 5%	T = 0.01, H = 0.02, T×H = 0.03						

Initial nematode population: 2000 eggs and J_2, T: Treatment, H: Hybrid, DAI: Days after inoculation, UI: Uninoculated, I: Inoculated

The mean sugar content was the highest (4.05 mg g^{-1}) in susceptible hybrid (HHB 272) on 45 DAI (3.76) and the same hybrid on 30 DAI. Irrespective of hybrids, roots of infected plants had significantly higher sugar than healthy plants. Susceptible hybrid showed a maximum increase (76.86%) in roots after nematode infection over healthy roots on 45 DAI. In resistant pearl millet hybrid (HHB 146), maximum increase (25.41%) in roots was recorded on 30 DAI and minimum (21.94%) on 45 DAI. Irrespective of observation periods, total sugars in root was more in susceptible hybrid than the resistant in both inoculated as well as uninoculated conditions.

DISCUSSION

Root-knot nematodes form giant cells in the roots that disrupt the root vascular system, reduce uptake of water and nutrients and their transport from the roots to the shoots. The plant response to nematode parasitism thus causes morphological, physiological and biochemical changes that affect the photosynthetic process. These changes upsurge with the duration of infection. Therefore, the study was conducted to determine the changes in the pearl millet.

Study of the biochemistry of host-pathogen relationship plays a vital role in characterising the plant discases. Root-knot nematodes, *Meloidogyne graminicola* is capable of disturbing the host metabolism. In the present study, *Meloidogyne graminicola* infestation led to a significant increase in protein content of root, but the levels of the rise in protein content were different in resistant and susceptible hybrids. Increase in protein content of root of pearl millet was higher in susceptible (HHB 272) as compared to the resistant hybrid (HHB 146). The possible reason for increasing protein concentration may be due to production of new enzyme proteins in infected plants or may be the contributions from the nematode[14]. The slight decrease in the protein content at 45 DAI as compared to 30 DAI suggested that developing nematodes continuously withdraw large amounts of nutrients from the giant cells[15]. These cells are major sinks for amino acids, which are imported into the roots via the vascular system.

Meloidogyne graminicola infestation caused significant increase in protein content of shoot however; increase in the level in both the hybrid was different. Rise in protein content of shoot of pearl millet was higher in susceptible (HHB 272) as compared to resistant (HHB 146) hybrid. Similar results were also determined by Upadhyay and Banerjee[16] who recorded that increase in protein content of roots and shoots of *Meloidogyne javanica* infected chickpea plant is a function of initial nematode inoculum level. The increase was more pronounced in stem than root. Increase in proteins in nematode infected plant seems to be disease related as the synthesis of protein was stimulated by the infection of *Meloidogyne javanica*.

Phenolic compounds are associated with nematode injury, leading to the browning of plant tissues. In the present investigation, there was a significant increase in phenol content in roots with the advancement of the age of the plant. However, Ahmed *et al.*[17] recorded that at 15, 30 and 45 DAI of *Meloidogyne javanica* amount of total phenols amplified in both infected and uninfected plants, but the differences were not significant. This variation in response may be due to the difference in crop/nematode/observation period. In comparison to resistant inoculated, there was lesser production of phenol in susceptible inoculated plants. Similar observations were also determined by Senthilkumar *et al.*[18], they found that total phenol content increased in *Meloidogyne graminicola* infested resistant rice cultivars. The increase in phenolic compounds during the infection period might be attributed to the rapid breakdown of bound phenols or switching over of phenols to different pathways leading to the formation of various compounds like lignin, which plays a significant role in plant resistance. There was a significant increase in phenol content in the inoculated plants as

compared to uninoculated plants in all the stages of plant growth. Maximum increase (83.33%) was recorded in shoot of resistant inoculated plants over uninoculated at 30 DAI followed by 15 DAI (69.77%) and 45 DAI (65.75%). In case of susceptible hybrid (HHB 272), maximum increase in phenol content (56.25%) was observed at 15 DAI and rapidly declined with the age of pearl millet plant. Result of the present investigation are in agreement with those of Mishra and Mohanty[19] who found that percent increase in total phenol was lower in susceptible *Meloidogyne graminicola* infected rice cultivar as compared to resistant one.

Sugar is the major source of metabolic energy for all living organisms. Total sugar content in the shoot of both the hybrids decreased as compared to their respective uninoculated controls. It was found lowest in susceptible inoculated (HHB 272) on 15 DAI and highest in susceptible uninoculated (HHB 272) on 30 DAI. The possible reason for the decrease of sugar content as suggested by Upadhyay and Banerjee[16] is the secretion of some hydrolyzing enzymes by nematodes or induction of production of hydrolyzing enzymes by nematode in the host, which causes the conversion of the stored form of sugars into its utilizable form.

The data on the total sugar content in roots, estimated on a dry weight basis, in both pearl millet hybrids depict that mean sugar content increased in the infected pearl millet plant as compared to healthy ones. The highest increase (4.05) in susceptible hybrid (HHB 272) on 45 DAI (3.76) and the same hybrid on 30 DAI. These findings corroborated earlier workers result[19-22], who found that sugar production decrease in shoot due to the reduction in chlorophyll. Increase in total sugar of the roots is due to metabolically active giant cells that demand higher energy which plant supply by converting the starch in the shoots into sugar and transport it to roots. Nayak and Mohanty[23] also suggested that increase in the sugar content of roots is due to movement of various metabolites towards the infection site from other parts of plants. Alternatively, more of these metabolites are produced by cell at the infection site as a result more of carbohydrates are required for respiration and metabolism that leads to the lower concentration of total sugars in the shoot portion[10].

It is inferred that *M. graminicola* induces biochemical alterations in the pearl millet. Plant resistance features one of the more inexpensive and effective processes to control the infestation of plant-parasitic nematodes. Quite a few substances exist during the plant procedure that affects the rate of metabolism of nematode feeding. Phenolic compounds are secondary metabolites that participate in the defence mechanisms of crops against pathogens and have a tendency to enhance the plant resistance. Here, also phenols were determined to be involved with resistance in pearl millet. Pinpointing new resources of resistance might help in breeding new nematode-resistant pearl millet cultivars.

CONCLUSION

In the present study experiment, percent increase in protein and phenol of root and shoot of pearl millet was recorded in both the tested hybrids. Protein content were increased more in roots of susceptible hybrids as compared to the resistant hybrid. The higher increase in the protein content of susceptible pearl millet roots and shoots up to 45 DAI lead to the conclusion that a portion of plants is still metabolically active according to the need of nematode requirement. Contrary to it, phenol content in root and shoots was increased more in resistant hybrid as compared to the susceptible hybrid. It indicates that phenol has a significant role in providing resistance against *Meloidogyne graminicola*. Total sugars in shoot portion of decreased in both the hybrids while increased in the infected roots. Increase in the sugar percent in susceptible hybrid was much higher than the resistant hybrid.

SIGNIFICANCE STATEMENT

It is inferred from the present study that *M. graminicola* induces biochemical changes in the pearl millet hybrid. Resistance offers one of the most economical and effective method to check infestation of plant-parasitic nematodes. Several kinds of chemicals are present in the plant system that affects the metabolism of the nematode feeding upon them. Phenolic compounds are secondary metabolites that play a significant role in the defence mechanisms of plants against pathogens and tend to increase in the resistant cultivars. In the present study phenol content also seem to be associated with resistance in pearl millet. Identifying new sources of resistance will help in breeding new nematode-resistant cultivars. Finding out sources of resistance and generate information on mechanism of resistance can be utilized for breeding purpose and cultivation.

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