Biochemical studies of *M. incognita* and *M. javanica* towards their identification

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Received: 10 July 2013 / Accepted: 11 May 2014 / Published online: 3 June 2014
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Abstract Biochemical analysis is a reliable, quick, yet effective way for profiling any organism. The same tools when employed in taxonomic queries, they aid substantially and support the process. Taxonomic queries have been since beginning presenting numerable challenges towards the studies of different plant parasitic nematodes. In the present study various biochemical tests have been employed to assess the differentiation among the two dominant sps. of *Meloidogyne* i.e., *M. incognita* and *M. javanica*. Some of the biochemical parameters considered were the specific concentrations of carbohydrates and cholesterol and were monitored in the juvenile and the female of the respective species. Results have been discussed in details along with its employability towards the taxonomical studies especially with reference to plant parasitic nematodes.

Keywords *Meloidogyne* · Biochemical analysis · *M. incognita* · *M. javanica* · Taxonomic queries

Introduction

Root-knot nematodes (*Meloidogyne* species), are the most prevalent and destructive of all plant-parasitic nematodes. These nematodes are known to parasitize important food crops such as Okra, Brinjal, Tomato, Chili, Sweet Potato,

Potato, Onion, Garlic, Banana, Citrus, Ginger, Rice, Sugarcane, Tobacco And other vegetables. There are 95 nominal species of root-knot nematodes described so far (Skantar et al. 2008) and over 5500 plant hosts (Trudgill and Blok 2001). Four species (*M. incognita*, *M. javanica*, *M. arenaia*, and *M. hapla*) account for more than 95 % of the root-knot nematodes in agricultural soils, and collectively attack nearly every crop that is grown. But two species *M. javanica* and *M. incognita* were found to be the most prevalent in the area of study i.e., in the northern parts India.

Root-knot nematode species identification requires specialized skills but is of fundamental importance to a wide range of scientific studies on virulence and nematode management. Identification of *Meloidogyne* species is becoming increasingly important for the design of effective nematode management practices such as crop rotation and plant resistance which require precise species identification and for quarantine purposes (Hussey 1990; Zijlstra et al. 2000; Zijlstra and Van Hoof 2006). Recently the whole genome of one of the most common species, *Meloidogyne incognita* has been sequenced to provide insights into the adaptations required by metazoans to successfully parasitize and counter defenses of immunocompetent plants (Abad et al. 2008).

The accuracy of species identification increases with taxonomic characters such as cytological, biochemical and physiological data (Hirschman 1985). Biochemical, cytological and reproductive characters have also been useful in studies focusing on host response and parasitism mechanisms (Williamson and Hussey 1996; Abad et al. 2003).

The use of biochemical analysis has considerable potential for identification of plant parasitic nematodes. Various biochemical tests were performed to find out the differences in between the chemical composition of the two

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dominant species of root knot nematodes in the region, on the basis of specific concentrations of carbohydrate, cholesterol, free amino acid, lipid and protein, present in these nematodes.

Considering the importance of the genus *Meloidogyne* for agriculture, this study was undertaken with the objective of easy identification of *Meloidogyne* populations based on biochemical characteristics.

Materials and methods

Stock cultures of all the RKN *M. incognita* and *M. javanica* obtained from single egg masses were cultured multiplied and maintained on tomato, potato, lady finger and cucurbits and raised in 40 cm pots containing sterilized soil. Procedures for surface sterilization of seed and other general methods for raising seedlings were the same as given by Dasgupta and Ganguly (1975). As and when, required the growing plants were supplemented with sterilized FYM. The nematode populations were sub cultured periodically at 45–60 days intervals either by transplanting fresh tomato, egg plant in infested soil or by inoculating new transplants with second stage juvenile suspensions after removal of old plants to ensure constant and large supply of J₂, females and egg-masses. For the collection of second stage juveniles J₂, of *Meloidogyne* spp. (*M. incognita* or *M. javanica*), the fully developed egg masses containing embryonated eggs were isolated from infested fresh roots. These were repeatedly washed with distilled water to make them free of sand, followed by 3–4 washings in sterile water blanks and poured on the sieve and isolated.

Fig. 1 Total carbohydrate concentration in (μg/g) in female and juvenile in *M. incognita* and *M. javanica*

Further analysis were made by following biochemical methods

1. Total Carbohydrate content of parasites was determined by Anthrone method given by Yemm and Willis (1954).
2. Total cholesterol percentage was estimated using the one step method of Warnick et al. (1985).
3. Free amino acids so extracted in solvent were utilized for subsequent estimation of free amino acid by Ninhydrin Method after Plummer (1990).
4. Total lipid content of the parasite was estimated using the method as given by Zollner and Krish (1962).
5. Total protein content of the parasite was estimated following the method of (Lowry et al. 1951).

Observations

Total Carbohydrates: The carbohydrate concentration in female *M. incognita* was estimated to be 0.564 μg/g. Almost similar concentrations of carbohydrate were estimated in its juvenile, while a little higher values were observed in case of *M. javanica*. Similar to *M. incognita* juveniles of *M. javanica* also exhibited a little difference in their carbohydrate content with respect to its female (Fig. 1).

Total Cholesterol: A very low value of cholesterol 0.0162 % as given in Fig. 2 was estimated in female *M. incognita*. Similarly, the female of *M. javanica* also exhibit low concentration of cholesterol. However, a significantly high concentration of cholesterol was observed in both the

Fig. 2 Total cholesterol concentration % in female and juvenile in *M. incognita* and *M. Javanica*

Fig. 3 Free Amino acid concentration ($\mu\text{g}/\text{mg}$) in female and juvenile in *M. incognita* and *M. Javanica*

juveniles, although there was little difference in concentration of cholesterol contents in the two juvenile (Fig. 2).

Free Amino Acid: The concentration of total free amino acid of female *M. incognita* was estimated to be 129.196 $\mu\text{g}/\text{g}$. However, free amino acid concentration of its juvenile was significantly lower. There was a significant

higher value estimated in female *M. javanica*. Significant difference in the value of free amino acid in the both the juveniles were also observed (Fig. 3).

Total Lipid: The concentration of lipid in *M. javanica* was estimated at 674 $\mu\text{g}/\text{mg}$ in female. The lipid content was significantly lower in its juvenile. The lipid content of

Fig. 4 Total Lipid concentration ($\mu\text{g}/\text{mg}$) in female and juvenile in *M. incognita* and *M. javanica*

female *M. incognita* does not show any significant difference from female *M. javanica*. Similar to the *M. javanica* juvenile, the juvenile of *M. incognita* also exhibits a significantly lower value of lipids than its female. The concentration of lipid however does not show significant difference between females and juveniles of the corresponding species (Fig. 4).

Total Protein: The concentration of protein was estimated to be $157 \mu\text{g}/\text{g}$ for *M. incognita* female. The concentration was slightly higher in the juvenile as compared to the female. While, in the other dominant species i.e., *M. javanica* the concentration of protein in female was significantly higher than *M. incognita*. The observations of *M. javanica* juveniles also exhibited significantly higher concentrations of protein as compared to its female (Fig. 5).

Results and discussion

Till date limited attempts have been made to use biochemical parameters to identify intra specific populations of nematodes differing in their ability to infect and reproduce on different hosts. Therefore, this study was undertaken to differentiate between dominant *Meloidogyne* spp. i.e., *M. incognita* and *M. javanica*. On the basis of various biochemical parameters the following interesting and

noteworthy features were recorded during the course of present study.

Total Carbohydrates: Sugars and proteins are utilized to a greater extent during the adult stage in the life history of the root knot nematodes, because survival and infestation of J_2 is known to be dependent on the food stored in the giant cell (Van Gundy et al. 1967). Thus the requirement of the storage products by the juvenile is responsible for higher concentration of carbohydrate in female. The results indicate that the content of carbohydrate in both, juvenile and adult female are almost similar, this may be due to the presence of a number of eggs in the female body prior to oviposition (Sharma 1992) also reported that root knot nematodes exhibited a higher level of insoluble polysaccharides maximum being in ovipositing female. Similar reports state that egg and egg matrix produced by female in plants are rich insoluble polysaccharides (Shah and Raju 1977 and Sharma 1992).

Total Cholesterol: The cholesterol percentage does not show much significant difference in both the species in adult females, but the juvenile of both the species have higher cholesterol concentration than female, while cholesterol concentration in both the juveniles is also similar. The difference in content and composition of sterol in various stages of life cycles has been recorded by various workers Chitwood et al. (1987) stated that sterol composition of *Meloidogyne* spp. differed from other genera of plant parasitic nematodes.

Fig. 5 Total Protein concentration ($\mu\text{g}/\text{mg}$) in female and juvenile in *M. incognita* and *M. javanica*

Free amino acids: Free amino acids are the translational of blue prints DNA. There are a variety of amino acids, which represent advance metabolic activities of the root knot nematode. The free amino acids are the functional units of proteins. Not only, do the regular amino acids form the integral part of the nematodes but also play an important role in growth stimulation for the proliferation of the host tissue. An increase, in the percentage, of the basic amino acids, i.e., arginine and lysine, on the pre-feeding site, is reported by (Veech et al. 1981), in *M. incognita*. Our results exhibit more concentration of total amino acids, in female *M. incognita* than in female *M. javanica*. However, their juveniles exhibit less amino acid content. A number of studies have reported on higher rate of use up of the amino acids to form the building blocks of juvenile (J_2) body, in the root knot nematode and our study also corroborates with the same. Myuge (1956) in his study on amino acids in the nematodes stated that the increase in amount of amino acids at the feeding sites is due to faster break down of protein and slower uptake of the same by the nematodes.

Total Lipid: Lipids form an integral part of the life cycle of *Meloidogyne* sp. It forms a large percentage of the eggshell and egg reserve. It also forms, food reserves of juveniles, as well as, the body wall of juveniles and adult forms (J_1 , J_2 , and J_3 , male and female). Yoo et al. (2000) reported that lipids are important entomopathogenic nematode nutritional components as they are energy reserves and serve as indicators of nematode quality. The results of the present study indicate that adult females of both the species have higher concentration of lipids than their juveniles, but the concentration of lipids in the two different species of *Meloidogyne* i.e., *M.*

javanica and *M. incognita* do not show considerable differences. In a study made by Krusberg et al. (1973) comparing the lipid and fatty acid composition of adult females and eggs of *M. incognita* and *M. arenaria* no qualitative differences were detected in fatty acids, although relative quantities of certain fatty acids might differ between the two species. Lipids are more concentrated in nematode body and eggs. These amounts increase with the development of giant cell, (Trivedi and Tiagi 1980 and Sharma and Widhi 1992) and the nematode females stain dark on account of high lipid content. This accumulation of lipids is supposed to be the storage material for nematode (Kannan 1986c). Gelatinous matrix and eggs were found to be rich in lipids as reported by Bird and Rogers (1965), Bird (1967), Bird (1968), Arya (1980), Tiagi (1982) and Trivedi and Tiagi (1986) revealed that the egg shell consisted of an outer chitinous layer and an inner lipid layer and during hatching of *M. javanica*, egg material of shell increased due to the break down of lipid layer.

Total Protein: There may be a number of enzymatic and non-enzymatic proteins in a nematode. The non-enzymatic proteins may be similar or dissimilar according to the different body forms, while the enzymatic proteins may differ or may show similarity according to differences in various functional requirements and so may be their quantity. All stages of nematodes stain positively for proteins (Bird 1974; Trivedi and Tiagi 1986 and Sharma and Widhi 1992). The observations reveal that the protein concentration was less in adult females than in juveniles of the species, *M. javanica* as well as *M. incognita*. However, the protein content of *M. javanica*, female was found to be greater than that of *M. incognita*. However, protein content of galled tissue induced by the root knot nematode

increases many fold (80 %). There is an increase in protein content due to the mobilization of the metabolite from the shoot tips to the galled roots.

As the results indicate the concentration of protein varies with different developmental stages of the phytonematodes, probably the juveniles require more enzymes like pectate lyase (Huang et al. 2005), cellulase (Rosso et al. 1999), Polyglycol dehydrogenase (Jaubart et al. 2002) and other similar cell wall degrading enzymes, as they have to survive and move through/within plant roots by migrating between plant cells along the middle lamella, that contain the most concentrated pectin.

Other enzymes used in the metabolic activity are viz, proteases, β -esterases, glutamates, oxalo-acetates, transaminases, glucose-6-phosphatases, dehydrogenases, catalases, malate dehydrogenases, superoxide dismutases, glycol-3-phosphate-dehydrogenases etc. (Ganguly and Dasgupta 1979, 1980). The proteins are utilized to a greater extent during the adult stage (Van Gundy 1965).

The lesser amount of protein in the adult than the juvenile is probably due to their less functional requirements and therefore, less activity of the adult. However, the mature adult female at the time of oviposition may have the highest amount of protein.

Conclusion

The biochemical analysis can be concluded with the fact that there are chances that the different biological forms of the *Meloidogyne* are there in different physiological state and age. Therefore there are equal chances for their different biochemical composition depending upon their biological requirements. The biological requirement of the organism is determined by a number of factors such as surrounding environments, host status and host response.

But under specific type of environmental conditions, differences in biochemical parameters can be considered important for easy, cost effective and less time taking identification various sps, so similar kind of work can be done on many more species of *Meloidogyne*.

Acknowledgements The authors are highly grateful to their respective institutions for all supports and authorities of CSIR (India) for financial assistance as JRF/SRF to first author.

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