



Identification of the race of root knot nematode by differential host test method

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ARTICLE INFO	ABSTRACT
<p>Received : 11 November 2020 Revised : 16 February 2022 Accepted : 21 February 2022</p> <p>Available online: 29 May 2022</p> <p>Key Words: Root knot nematode <i>Meloidogyne</i> spp., Differential host test Race</p>	<p>Root knot nematode (<i>Meloidogyne incognita</i>) cause major damage to the fruit crops, vegetable crops and field crops. Infected plants showed declined symptoms and poor fruit yield also displayed stunting and yellowing symptoms. In order to choose appropriate management control techniques, nematode diagnosis and specimen identification must be accurate, quick and precise. The population of the nematode obtained from the experimental field of Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri were assessed for their host-race status by the differential host test which relies on the combination of resistant and susceptible reactions of six differential hosts to the nematode population. Result revealed that the nematode population infected tobacco (NC 95), pepper (California Wonder), watermelon (Charleston grey), tomato (Rutgers) and unable to reproduce on cotton (Deltapine- 16) and peanut (Florunner) which indicated presence of race 2 of <i>Meloidogyne incognita</i>.</p>

Introduction

The root-knot nematode is one of the world's most dangerous plant-parasitic nematodes. Estimating crop losses has already been conducted based on data published by All India Co-ordinated Research Projects on Nematodes in Agriculture over the years, phytonematodes cause 21.3 % crop losses totalling ₹ 102,039.79 million per year; losses in 19 horticultural crops were estimated to be ₹ 50,224.98 million, while losses in 11 field crops were estimated to be ₹ 51,814.81 million Kumar *et al.* (2020). With such a high potential for crop damage, it is critical to monitor and manage root knot nematode population in a field. Because all species of root knot nematode are host dependent, a farmer may be able to rotate a non-host crop if the nematode is recognised at species level. Cotton growers, for example, are frequently infested by *M. incognita*. Cotton to groundnut crop rotation is a frequent crop rotation to reduce root knot nematode population density. *M. arenaria*, on the other hand,

thrives in groundnut and can be found in the same field as *M. incognita* Davis and Timper (2000). *Meloidogyne* spp. and its races have long been difficult to identify because of physical similarities, life phases in different habitats, varied host ranges, poorly defined species borders, intraspecific variability, probable hybrid origin and polyploidy Blok and Powers (2009). In general, accurate nematode identification is very essential, especially in the initiation of the research programmes and in the development of control strategies. Major control tactics such as crop rotation and use of resistant cultivars are generally race specific. Therefore, in the process of developing resistant varieties for root knot nematode the exact identity (*i.e.*, species and race) of the nematode population being tested must be known. The differential-host test is one of the most commonly used procedures for identifying root knot nematode species. The differential-host test is one of the most commonly used procedures

for identifying root knot nematode species. Sasser and Triantaphyllou in 1977, introduced this test to distinguish the species and race of four commonly encountered plant-parasitic root-knot nematodes.

Material and Methods

The investigation on the identification of the race was conducted at glasshouse, Post Graduate Institute., Mahatma Phule Krishi Vidyapeeth, Rahuri. during December, 2020 to February, 2021. In the event of a race identification, throughout the experiment, earthen pots with a diameter of 20 cm were used. The earthen pots were cleaned by using water and then disinfected with 4% formaldehyde. Before using these pots for cultivating differential hosts plants, formaldehyde was allowed to evaporate. Medium black to black soil was obtained from experimental field at Instructional Farm of Department Agricultural of Entomology and then mixed in proportion 3:1 with FYM before being steam sterilised for 4 hours in the soil steriliser with boiler at 6.75 kg/cm² pressure. Whenever, soil population of nematodes was required, soil sample taken from the feeder root zone of guava from the field and processed by using Cobb's sieving and decanting procedure. The differential host test was used to characterise the host range. The study used six standard crop plant cultivars: tobacco cv. NC 95 (*Nicotiana tabacum* L.); cotton cv. Deltapine- 16

(*Gossypium hirsutum* L.); pepper cv. California Wonder (*Capsicum frutescens* L.); watermelon cv. Charleston Grey (*Citrullus vulgaris* Schrad.); groundnut cv. Florunner (*Arachis hypogaea* L.) and tomato cv. Rutgers (*Solanum lycopersicum* L.). Five to six leaf stage transplanted tomato, tobacco and cotton seedlings were raised from the seeds in pots having 250 g of steam sterilised soil consortium (2 soil: 1 sand) and inoculated with 100 freshly hatched J₂. Each plant cultivar was represented by four replicates. Infected plants (24) were kept at a constant temperature of 25 °C in a glasshouse with 16 hours photoperiod and fertilisers given as needed.

Plants were harvested after 60 days and the roots were cleansed under running tap water and then stained for 2 minutes with trypan blue solution (0.1 g/lit). The root system's root galls and stained egg masses were counted. The gall index was computed on a scale of 1 to 5 based on the number of egg masses produced by each plant. The lines were classified into different categories based on the gall index, as shown in Table 1.

At the end of the experiment, each plant cultivar was categorised as susceptible (+) or resistant (-) depending on whether the average root system egg mass count was higher than 3 or lesser than 3 egg mass. The obtained information was then compared to differential host test reaction chart (Table 2.).

Table 1: Gall Index Scale (Gaur, 2001)

Nematodes gall index	Number of egg masses produced by each plant	Reactions
1	0	Highly Resistant
2	1-10	Resistant
3	11-30	Moderately Resistant
4	31-100	Susceptible
5	>101	Highly Susceptible

Table 2: Differential host test reaction chart (Sasser and Triantaphyllou, 1977)

<i>Meloidogyne</i> species and race	Deltapine 16 (cotton)	NC 95 (tobacco)	California Wonder (pepper)	Charleston grey (watermelon)	Florunner (groundnut)	Rutgers Tomato
<i>M. incognita</i>						
Race-1	-	-	+	+	-	+
Race-2	-	+	+	+	-	+
Race-3	+	-	+	+	-	+
Race-4	+	+	+	+	-	+
<i>M. javanica</i>	-	+	-	+	-	+
<i>M. arenaria</i>						
Race-1	-	+	+	+	+	+
Race-2	-	+	-	+	-	+
<i>M. hapla</i>	-	+	+	-	+	+

Results and Discussion

The population distribution of root-knot nematodes (*M. incognita*) in the experimental field which specified the reactions of root knot nematode population on host differentials is depicted in Table 3. Six host differentials viz., cotton, tobacco, pepper, watermelon, groundnut and tomato were used for race identification. The population infected host differentials like tobacco, pepper, watermelon, tomato but were unable to reproduce on cotton and groundnut. The mean gall index recorded was found to be 3, 3, 3 and 4 in tobacco, pepper, watermelon and in tomato, respectively. The average number of egg mass was 22.25, 15.25, 26.00, 31.75 in tobacco, pepper, watermelon and in tomato, respectively. After comparing the observed data with differential host test reaction chart (Table 2.), it is evident that the species and the race of nematode present in the experimental field is race 2 of *Meloidogyne incognita*. These findings are in consonance with the findings of Khan *et al.* (2017) who carried out research on *Meloidogyne incognita* infecting *Passiflora edulis* (passion fruit) on NC host differentials and found that nematode population was impotent to infect and reproduce on

cotton and groundnut. However, infected and reproduced on tomato, tobacco and pepper. As a result, race 2 was assigned to the population of *M. incognita*. Similarly, Deuri *et al.* (2016) carried out a study on *M. incognita* populations infecting vegetable crops. The population of *M. incognita* found in Jorhat, Lakhimpur, Sonitpur and Kokrajhar districts of Assam was identified as race 2 based on the response of the differential hosts to *M. incognita*. These findings are in accordance with the research finding.

Results are in line with Hussain *et al.* (2008) who conducted differential host test in the nethouse of the Department of Nematology, Assam Agricultural University, Jorhat to identify the species and races of *Meloidogyne* spp. Five differential hosts viz., cotton, tobacco, pepper, groundnut and tomato were grown separately in pots containing soils collected from different localities. At the termination of the experiment, result showed galls in tobacco, pepper and tomato roots but recorded no gall (gall index 0) in the remaining hosts *i.e.*, cotton and peanut which indicated the presence of race 2 of *Meloidogyne incognita* in the area surveyed. Thus, the results are in consonance with the findings.

Table 3: Reaction of root knot nematode in differential host test

Population No.	Cotton (Deltapine 16)		Tobacco (NC95)		Pepper (California wonder)		Watermelon (Charleston grey)		Peanut (Florunner)		Tomato (Rutgers)		Species	Race
	GI	EM	GI	EM	GI	EM	GI	EM	GI	EM	GI	EM		
1	0.0	0.0	3	29	3	16	3	28	0.0	0.0	4	31	<i>M. incognita</i>	2
2	0.0	0.0	3	22	3	16	3	24	0.0	0.0	4	31	<i>M. incognita</i>	2
3	0.0	0.0	3	20	3	15	3	25	0.0	0.0	4	33	<i>M. incognita</i>	2
4	0.0	0.0	3	18	3	14	3	27	0.0	0.0	4	32	<i>M. incognita</i>	2
Mean	0.00	0.00	3	22.25	3	15.25	3	26	0.00	0.00	4	31.75		

GI = Gall index, EM = No. of egg masses/plant

Conclusion

Major control tactics such as crop rotation and resistant cultivars are generally race specific. Therefore, in the development of resistant varieties for root knot nematode resistant cultivars the exact identity (*i.e.*, species and race) of the nematode population being tasted must be known. In view of

the above facts and data, the current investigation on the identification of the race was conducted in the glasshouse, Post Graduate Institute., Mahatma Phule Krishi Vidyapeeth, Rahuri. Differential host test was performed for host range characterization. Six standard crop plant cultivars: tobacco cv. NC

95 (*Nicotiana tabacum* L.); cotton cv. Deltapine- 16 (*Gossypium hirsutum* L.); pepper cv. California Wonder (*Capsicum frutescens* L.); watermelon cv. Charleston Grey (*Citrullus vulgaris* Schrad.); groundnut cv. Florunner (*Arachis hypogaea* L.) and tomato cv. Rutgers (*Solanum lycopersicum* L.) were used for the investigation and inoculated with 100 freshly hatched J₂ of each population. Result revealed that the nematode population infected tobacco (NC 95), pepper (California Wonder), watermelon (Charleston grey), tomato (Rutgers) and unable to reproduce on cotton (Deltapine- 16)

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and peanut (Florunner) which indicated presence of race 2 of *Meloidogyne incognita*.

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Conflict of interest

The authors declare that they have no conflict of interest.

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