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Host resistance in black pepper (*Piper nigrum* L.) against root-knot and burrowing nematode

Santhosh J. Eapen[1]*, K.V. Ramana[2] and K.V. Saji[3]

Division of Crop Protection, Indian Institute of Spices Research
Calicut - 673 012, Kerala

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Abstract

Crop resistance is a low-cost option for nematode control in subsistence agriculture and is most useful for low value crops which cannot support the cost of expensive pest management inputs. In this study an attempt has been made to screen the black pepper germplasm to locate resistance/tolerance to burrowing and root-knot nematodes. The germplasm materials collected from the field gene bank of Indian Institute of Spices Research were multiplied in a greenhouse under disease-free conditions for using in the screening studies. In the preliminary screening, 525 black pepper germplasm accessions including 100 wild, 213 each cultivated and 212 hybrids were screened against burrowing nematode. Out of these, 24 accessions showed resistance to *R. similis* which was confirmed by repeated inoculations. Similarly 431 black pepper accessions were inoculated with *M. incognita*. Sustainable resistance was observed in only 27 black pepper accessions. Out of these nine black pepper accessions were evaluated under field conditions for five years. All the *R. similis*-resistant lines viz. Hp 39, Acc. 820 (IC No. 316481), Acc. 1047 (IC No. 316602) and Acc. 1204 (IC No. 316690) were free from nematode infestation initially for two to three years. However, *R. similis* infection was noticed in all these lines subsequently, which has to be investigated further. However, among the short-listed root-knot nematode resistant lines only two accessions, Acc.1047 (IC No. 316602) and Acc. 1090 (IC No. 316635) were healthy and free from root-knot nematode infestation even after seven years of field planting. In the field evaluation trial, the highest yield was noticed for Acc. 820 and Hp 39.

Keywords: Black pepper, burrowing nematode, *Meloidogyne incognita*, *Radopholus similis*, resistance, root-knot nematode

Introduction

Burrowing nematode (*Radopholus similis*) and root knot nematode (*Meloidogyne incognita*) are the most important nematode pests of black pepper. Due to the cost and restrictions on the use of effective nematicides arising from health and environmental concerns, identifying and incorporating naturally occurring host resistance genes into crops is an increasingly favored alternative for nematode control. Crop resistance is a low-cost option for nematode control in subsistence agriculture. Therefore, in tropical agriculture, tolerance or resistance can play a key role in the reduction of crop yield losses caused by nematodes.

Nematode resistance is defined as the ability of a plant to inhibit the reproduction of a nematode species relative to reproduction on a plant lacking such resistance (Cook and Evans, 1987). A completely resistant plant allows no nematode reproduction, non-resistant or susceptible plant allows nematodes to multiply freely, and partially resistant plants support intermediate levels of reproduction. Developing markers linked to resistance genes will accelerate the introduction of novel resistance traits into cultivated plants. However, the range of single traits available for breeding programmes currently limits the number of nematode-resistant crops available. In addition, pathotypes, or species that overcome resistance, often challenge the utility of resistance.

In tropical countries like India, a much greater diversity of nematode genera and species exists. Nematodes also generally have shorter life cycles and more generations per crop season at higher temperatures (Starr *et al*., 2002). India, being one of the centres of

*[1]*Corresponding author (sjeapen@spices.res.in),* [2]*Present address: Door No. 86-2-21/2, Sangeetam Venkatareddy Street, J.N. Road, Rajahmundry-533103. Andhra Pradesh.* [3]*Div. of Crop Improvement & Biotechnology*

origins for *Piper* species, possesses a high diversity in black pepper cultivars and related species. Much of the available germplasm resources remain to be characterized for resistance to nematodes and several remains to be collected from unexplored areas. Identifying new sources of nematode resistance in the black pepper germplasm assembled in the repository of Indian Institute of Spices Research (IISR), Calicut, Kerala is the first step towards development of resistant genotypes. Efforts in this direction and the results obtained in a series of screening experiments are summarized in this paper.

Materials and Methods

Black pepper germplasm accessions

The initial materials for screening against nematodes were collected from the Germplasm Repository of Indian Institute of Spices Research (IISR). For screening purpose, black pepper rooted cuttings were raised in a greenhouse under nematode and disease-free conditions by either bamboo or serpentine methods.

Nematode inocula

Burrowing nematode (*R. similis*) was reared on carrot discs (O'Bannon and Taylor, 1968). Fresh and active nematodes collected from the carrot discs are used as inoculum for screening the germplasm. Root knot nematodes collected from infected black pepper plants were cultured on tomato or *Coleus* plants (susceptible local varieties) raised in sterile potting mixture. Infected roots from these plants showing prominent galls were collected after 30-40 days for extracting root-knot nematode eggs and juveniles to be used as inocula in screening experiments.

Screening of germplasm

Black pepper rooted cuttings raised under nematode-free conditions were screened in different batches against both *R. similis* and *M. incognita* in a screen house. The methodology developed by Ramana and Mohandas (1989) was adopted with minor modifications. Each rooted black pepper cutting was inoculated with 10 ml of an aqueous suspension of *R.similis* (~250 nematodes) harvested from carrot cultures. The nematodes were uniformly distributed around the root zone in 3-4 holes about 3 cm deep. A susceptible black pepper variety (Karimunda / Panniyur 1) was included in all batches to compare the results. The plants were uprooted after 2-3 months and washed thoroughly in water. The root system was indexed for lesions and rotting in a scale of 0-5 (0: no lesions or rotting; 1: trace infections with a few small lesions; 2: < 25 % lesions; 3: 25-50 % roots with lesions and rotting; 4: 50 -75 % roots with lesions and rotting and 5: > 75 % roots with lesions and rotting). Accessions showing < 2 root lesion index (RLI) were further tested for confirming their resistance. In the second round, besides recording the RLI, the fresh weight of roots from each inoculated plant was recorded after four months and the final nematode population from roots was also estimated by maceration-sieving method (Coolen and D'Herde, 1972). If the RLI < 2 and reproduction factor (R) < 1, those plants were rated as resistant. All other cases were considered as susceptible.

For screening against root knot nematode, the nematodes cultured on tomato or *Coleus* plants were collected through maceration and sieving. The rooted cuttings were inoculated with 1000 J2 of *M. incognita* in 3-4 holes about 3 cm deep near the vicinity of roots. Simultaneously a susceptible check (Karimunda / Panniyur 1) was also maintained in all batches. The inoculated plants were uprooted after 2-3 months and the root system was visually indexed for root galling (RKI) in a 0-5 scale (0 – no galling and 5 – maximum galling). The plants were short listed if they had < 2 RKI. They were subjected to a second round inoculation (as described above) for confirming their host status.

Field evaluation of short-listed lines

The short-listed lines in the preliminary screening were evaluated under field conditions to confirm the resistance and also to understand the effect of nematode population on growth and yield of the plant. An *in situ* screening experiment was laid out at IISR Peruvannamuzhi Farm in July 2003 to confirm the resistance of nine black pepper germplasm accessions (C.812, C.820, C.1047, C.1090, C.1204, C.4103, HP39, HP60 and HP290) short-listed in the preliminary screening. Panniyur 1, a susceptible check was also included. Each treatment was replicated five times and the design was RBD. The yellowing of the vines was visually indexed every year during January – February using a 1-5 scale (1 = no yellowing, 2 = up to 25 % yellowing, 3 = 26 – 50 % yellowing, 4 = 51-75 % yellowing and 5 = >75 % yellowing). The nematode incidence was assessed by drawing root samples (5 g) every year during the post monsoon period and processing through standard procedures. The yield was recorded by harvesting the mature berries from individual plants from 2005 (second year of planting) onwards.

Results and Discussion

Screening against burrowing nematode

In the preliminary screening, 213 cultivated accessions were screened against *R. similis* out of which

only two were resistant to the nematode even after repeated inoculations (Table 1). The resistant lines observed are IC 316481 (Perumkodi) and IC 316690, a local collection from Sagar in Karnataka. Among the 100 wild accessions screened, 18 accessions were resistant to *R. similis* indicating the high degree of resistance available in wild and related species (Table 2). These included *P. argyrophyllum* (2 accessions out of 6 - IC. 318143 and IC 318453), *P. attenuatum* (4 accessions out of 8 - IC 318446, IC 318452, IC 318455 and IC 318464), *P. colubrinum* (one accession - IC 318161), *P. hapnium* (one accession - IC 318399), *P. hymenophyllum* (2 accessions out of 6 - IC 318096 and IC 318364), *P. longum* (2 accessions out of 8 - IC 318402 and IC 318450), wild *P. nigrum* (3 accessions out of 27 - IC 318316, IC 318366 and IC 318440) and *P. sugandhi* (3 accessions out of 4 - IC 318362, IC 318390 and IC 318392). In addition, four out of 212 hybrids were also found to be resistant to *R. similis* (Table 3). The resistant hybrid lines are HP 39 (Kalluvally x Karimunda), HP 47 (Cholamundi x Panniyur 1), HP 125 (Valiyakaniakadan x Karimunda) and HP 532 (Cheppakulamunda x Karimunda). Altogether 525 black pepper germplasm accessions including 100 wild, 213 each cultivated and hybrids were screened against burrowing nematodes during this period. Several workers have made efforts to locate resistance in black pepper germplasm against *R. similis* (Ramana *et al*., 1987;

Table 1. Screening of black pepper germplasm cultivars to *Radopholus similis* and *Meloidogyne incognita*

Cultivar	Against *R. similis* (Rs)		Against *M. incognita* (Mi)		Resistant lines
	Screened	Susceptible	Screened	Susceptible	
Aimpiriyan	4	4	1	1	
Arakulammunda	8	8	5	5	
Balankotta	5	5	1	1	
Bilimalligesara	2	2	2	2	
Chengannurkodi	4	4	3	3	
Cheriyakaniakadan	2	2	1	1	
Doddigai	2	2	2	2	
Jeerakamundi	6	6	4	3	IC 317146 against *Mi*
Kalluvally	5	5	3	3	
Kaniakadan	4	4	0	0	
Karimunda	15	15	4	4	
Kottan	2	2	0	0	
Kottanadan	2	2	1	1	
Kudhirugunda	1	1	2	1	IC 316706 against *Mi*
Kurielmundi	2	2	1	1	
Kuthiravally	5	5	4	4	
Kuttianikodi	3	3	0	0	
Malamundi	3	3	2	2	
Narayakodi	7	7	2	2	
Neelamundi	8	8	5	5	
Perambramunda	2	2	0	0	
Perumkodi	4	3	1	1	IC 316481 against *Rs*
Poonjaranmunda	2	2	0	0	
Uddagara	3	3	3	3	
Uthirankotta	4	4	1	1	
Vadakkan	2	2	2	2	
Valiyakaniyakadan	2	2	1	1	
Vattamundi	6	6	1	1	
Vellamundi	5	5	3	3	
Vellanamban	4	4	1	1	
Veluthakaniakadan	2	2	1	1	
Local misc. cultivars	31	31	23	22	Chettan (IC 317062) against Mi
Unknown cultivars	56	55	28	24	IC 316475, IC 316635, IC 316695 & IC 316700 against Mi IC 316690 against Rs
Total	**213**	**211**	**108**	**101**	Against *M. incognita* – 7 Against *R. similis* - 2

Mi – *Meloidogyne incognita*; Rs – *Radopholus similis*

Table 2. Screening of black pepper germplasm wild accessions and related *Piper* species to *Radopholus similis* and *Meloidogyne incognita*

Piper species / wild accession	Against *R. similis* (Rs)		Against *M. incognita* (Mi)		Resistant lines
	Screened	Susceptible	Screened	Susceptible	
P. argyrophyllum	6	4	8	7	IC. 318143 against both Rs & Mi IC 318453 against Rs
P. attenuatum	8	4	13	13	IC 318446, IC 318452, IC 318455 & IC 318464 against Rs
P. barberi	0	0	2	2	-
P. betle	2	2	0	0	-
P. colubrinum	1	0	1	0	IC. 318161 against both Rs & Mi
P. galeatum	5	5	7	7	-
P. hapnium	1	0	2	1	IC 318399 against both Rs & Mi
P. hymenophyllum	6	4	5	5	IC 318096 & IC 318364 against Rs
P. longum	8	6	8	7	IC 318402 against RsIC 318450 against both Rs & Mi
P. nigrum (wild)	27	24	26	25	IC 318316, IC 318366 & IC 318440 against Rs IC 318503 against Mi
P. sugandhi	4	1	5	5	IC 318362, IC 318390 & IC 318392 against Rs
P. trichostachyon	1	1	3	2	IC 318469 against Mi
Unknown	31	31	67	63	Acc. 7, Acc. 37, Acc. 161 & Acc. 415 against Mi
Total	**100**	**82**	**147**	**137**	**Against *R. similis* – 18; Against *M. incognita* - 10**

Mi – *Meloidogyne incognita*; Rs – *Radopholus similis*

Table 3. Screening of black pepper hybrids to *Radopholus similis* and *Meloidogyne incognita*

Cross	Against *R. similis* (Rs)		Against *M. incognita* (Mi)		Resistant lines
	Screened	Susceptible	Screened	Susceptible	
Cheppakulamunda x Karimunda	5	4	5	5	HP 532 against Rs
Cholamundi x Karimunda	8	8	6	5	HP 7 against Mi
Cholamundi x Panniyur 1	10	9	9	9	HP 47 against Rs
Kalluvally x Karimunda	3	2	2	2	HP 39 against Rs
Karimunda x Valiakaniakadan	3	3	3	2	HP 60 against Mi
Narayakodi x Karimunda	7	7	7	6	HP 345 against Mi
Panniyur 1 x Karimunda	26	26	23	20	HP 01, HP 26 & HP 29 against Mi
Valiyakaniakadan x Aimpiriyan	5	5	4	3	HP 198 against Mi
Valiyakaniakadan x Karimunda	9	8	7	7	HP 125 against Rs
Vellamunda x Cholamundi	5	5	6	5	HP 290 against Mi
Total screened	**212**	**208**	**176**	**168**	**Against *R. similis* – 4 Against *M. incognita* - 7**

Mi – *Meloidogyne incognita*; Rs – *Radopholus similis*

Ramana, 1992). In Sri Lanka, a black pepper variety, PW 14, was immune to R. similis (Gnanapragasam, 1989). In a similar study, 370 germplasm accessions, 178 Karimunda selections and 54,000 open pollinated seedlings were screened against *R. similis* and only *P. colubrinum* was found resistant to the nematode (Ramana, 1992). However, in this study in addition to *P. colubrinum*, two cultivated lines, a number of wild and related species and four hybrids were proved to be resistant to *R. similis*.

Screening against root knot nematode

Altogether 431 black pepper accessions (108 cultivated, 147 wild and 176 hybrids) were screened against root knot nematode, *M. incognita*. Sustainable resistance was observed in only 27 black pepper accessions. Compared to burrowing nematode, more cultivated lines (7 out of 108 accessions) were resistant to root knot nematode (Table 1). One accession each of Jeerakamundi (IC 317146), Kudhirugunda (IC 316706), Chettan (IC 317062) and four unknown cultivars were resistant to *M. incognita*. Ten wild accessions out of 147 accessions were also resistant to *M. incognita* (Table 2). The resistant lines included *P. argyrophyllum* (IC. 318143), P. *colubrinum* (IC. 318161), *P. hapnium* (IC 318399), *P. longum* (IC 318450), wild *P. nigrum* (IC 318503), *P. trichostachyon* (IC 318469) and three unknown wild accessions. Seven hybrids out of 176 also did not take up root knot infection (Table 3). The resistant hybrids were HP 7 (Cholamundi x Karimunda), HP 60 (Cholamundi x Panniyur 1), Hp 198 (Valiyakaniakadan x Aimpiriyan), HP 290 (Vellamunda x Cholamundi), HP 345 (Narayakodi x Karimunda) and three hybrids (HP 1, HP 26 and HP 29) of the Panniyur 1 x Karimunda cross. Earlier too there were attempts to screen different cultivars of black pepper against *M. incognita* (Koshy and Sundararaju, 1979; Ramana and Mohandas, 1986; Paulus *et al*., 1993). Ramana (1992) too screened a number of germplasm accessions, OP seedlings etc. and obtained one cultivated type, Acc. 812 and *P. colubrinum* as resistant to *M. incognita*.

Combined resistance against both nematodes was observed only in four wild accessions *viz*. *P. argyrophyllum* (IC. 318143), *P. colubrinum* (IC. 318161), *P. hapnium* (IC 318399) and *P. longum* (IC 318450). Progenies of the same cross also varied in their reaction to nematodes indicating segregation of the resistance character.

Field evaluation of short-listed lines

Out of the nine accessions under field evaluation, all the *R. similis*-resistant lines viz. Hp 39, Acc. 820 (IC No. 316481), Acc. 1047 (IC No. 316602) and Acc. 1204 (IC No. 316690) were free from nematode infestation 2-3 years after planting. However, none of the lines were absolutely free from *R. similis* infestation at the fag-end of the experiment. The highest nematode incidence was noticed in Panniyur 1 followed by Hp 290 (Table 4). Very high incidence of the nematode was observed exceptionally in a few vines of the treatment, which can be due to predisposal by other pests/pathogens or break down of nematode resistance at flowering stage or due to high temperature prevailing at Peruvannamuzhi conditions.

root knot nematode in the present study, probably to some other species of root knot nematodes.

Among the above lot, Acc. 820 and HP 39 have recorded a mean yield (pooled for five years) of 1090 and 777.4 g (green), respectively. Except for the bulk density of the former, both these lines have excellent yield and quality attributes. However, they are susceptible to *M. incognita* and *Phytophthora capsici*. C1090, though resistant to root knot nematodes and *Phytophthtora*, was not a high yielder (the pooled mean was only 235.5 g). The biochemical profiling of promising lines showed very high caryophyllene content in Acc. 1090 and HP 39. The role of caryophyllene in imparting nematode resistance has to be further investigated. More extensive field trials using rooted cuttings of these lines are envisaged for assessing their performance at different locations.

The exact nature and mechanism of resistance in these lines have to be investigated further so that the resistance traits can be transferred into suitable commercial cultivars. The reasons for nematode resistance to be not durable in black pepper have also to

Table 4. Field performance of promising nematode resistant black pepper germplasm accessions seven years after planting (mean of four replications)

Acc. No.	Yellowing index	No. of nematodes / g root				Mean annual yield – fresh (g/plant)
		Meloidogyne sp.		*R. similis*		
		Back transformed mean	No. of pants infected	Back transformed mean	No. of plants infected	
C.812	0.8 ab	2.01 a	1	0.63 a	1	98.00 b
C.820	0.7 b	13.86 a	3	1.71 a	4	1090.00 a
C.1047	1.8 a	0.00 a	0	0.58 a	1	45.50 b
C.1090	0.2 b	0.00 a	0	0.64 a	3	235.46 b
C.1204	0.5 b	5.49 a	2	1.37 a	2	0.00 b
C.4103	0.8 ab	12.71 a	2	1.47 a	2	16.66 b
HP 39	0.1 b	0.00 a	0	0.86 a	3	877.41 a
HP 60	0.2 b	2.76 a	1	1.38 a	2	99.04 b
HP 290	1.0 ab	5.62 a	2	2.79 a	3	0.00 b
Pan. 1	1.2 ab	4.75 a	1	3.90 a	4	189.50 b

The data are pooled across different observations made during 2003-10. Means in a column followed by the same alphabet are not statistically different. Nematode counts were $\log_{10}$ transformed before statistical analysis.

The same trend was observed in the case of root-knot nematode resistant lines also. However, among the five short-listed root-knot nematode resistant black pepper lines only two accessions, Acc.1047 (IC No. 316602) and Acc. 1090 (IC No. 316635) were healthy and free from root-knot nematode infestation even after seven years of field planting. The highest root knot nematode population was recorded in the roots of C 820 and C 4103 (Table 4). Interestingly, the *M. incognita* resistant line C. 812, which was released as 'Pournami' (Ravindran *et al.*, 1996), was showing susceptibility to be critically examined. However, the partial resistance in some of these short-listed black pepper lines can be appropriately deployed to achieve satisfactory nematode control in black pepper nurseries and field.

References

Cook, R. and Evans, K. 1987. Resistance and tolerance. In: *Principles and Practice of Nematode Control in Crops*. (Eds.) Brown, R.H. and Kerry, B.R. Academic Press.

Coolen, W.A. and D'Herde, C.J. 1972. *A Method for the Quantitative Extraction of Nematodes from Plant Tissue*. 77 pp. Ghent State Agriculture Research Centre, Merelbeke, Belgium.

Gnanapragasam, N.C. 1989. Varietal response of pepper to infestation by the burrowing nematode, *Radopholus similis*. *Sri Lanka J. Tea Sci.* **58**: 5-8.

Koshy, P.K. and Sundararaju, P. 1979. Response of seven black pepper cultivars to *Meloidogyne incognita*. *Nematol. Medit.* **7**: 123-125.

O'Bannon, J.H. and Taylor, A.L. 1968. Migratory endoparasitic nematodes reared on carrot discs. *Phytopathol.* **58**: 385.

Paulus, A.D., Eng, L., Teo, C.H. and Sim, S.L. 1993. Screening of black pepper genotypes and *Piper* spp. for resistance to root-knot nematode. pp. 132-139. In: *The Black Pepper Industry – Problems and Prospects*. University Pertanian Malaysia Bintulu Campus, Sarawak, Malaysia.

Ramana, K.V. 1992. *Role of Nematodes in the Incidence of Slow Decline (Slow Wilt Disease) of Black Pepper and Screening Pepper Germplasm Against Nematodes – Final Report*. National Research Centre for Spices, Calicut, India.

Ramana, K.V. and Mohandas, C. 1986. Reaction of black pepper germplasm to root-knot nematode, *Meloidogyne incognita*. *Indian J. Nematol.* **16**: 138-139.

Ramana, K.V., Mohandas, C. and Ravindran, P.N. 1987. Reaction of black pepper germplasm to the burrowing nematode (*Radopholus similis*). *J. Plantn. Crops* **15**: 65-66.

Ramana, K.V. and Mohandas, C. 1989. Techniques for screening black pepper germplasm to *Radopholus similis* and *Meloidogyne incognita*. *Indian J. Nematol.* **19**: 144-149.

Ravindran, P.N., Ramana, K.V., Nair, M.K., Nirmal Babu, K. and Mohandas, C. 1996. Pournami - A high yielding black pepper selection tolerant to root-knot nematode (*Meloidogyne incognita*). *J Spices Aromatic Crops* **1** (2): 136-141.

Starr, J.L., Cook, R. and Bridge, J. (Eds.) 2002. *Plant Resistance to Parasitic Nematodes*. CABI Publishing, Wallingford, Oxon, UK.