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Assessment of root-knot nematode, *M. incognita* and identification of the source of resistance in cauliflower, *Brassica oleracea*

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Abstract

Root-knot nematodes of genus *Meloidogyne* are the most devastating plant parasitic nematodes of vegetable crops responsible for severe global economic losses. The objective of the present work was to study the diversity of *Meloidogne* species in cauliflower growing areas of Punjab and to identify the source of resistance against *M. incognita.* The root and soil samples were collected from the cauliflower growing areas and associated nematode specie(s) were identified based on perineal pattern morphology and morphometry of adult females. *M. incognita* sp. was found most prevalent in all the cauliflower growing areas surveyed. Thirty genotypes of cauliflower (*Brassica oleracea*) were screened against root-knot nematode, *M. incognita,* in pots. Four genotypes *viz.*, LSMVAR-6, PI1654, LSMVAR-2 and LS-4 were resistant to *M. incognita* with root galling index (RGI) less than 2.0 and reproduction factor (Rf) less than 1.0. Among others, fourteen genotypes showed hypersusceptible (RGI 2.0- 2.6; Rf 0.57-1.0) and eleven genotypes showed a susceptible reaction. The resistant genotypes identified in the present study may be utilized to incorporate resistance against *M. incognita* in commercial cauliflower cultivars.

Key words: *Brassica oleracea*, Root-knot nematodes, resistance

Introduction

Root-knot nematodes (*Meloidogyne* spp.) have been designated as the most devastating plant-parasitic nematodes attacking most vegetable crops (Singh and Kumar, 2015; Gowda *et al.,* 2019). Their polyphagous nature, short life cycle and high fecundity rate make it difficult to control these nematodes. Root-knot nematodes attack the tender roots of host plants and form permanent feeding sites in the roots leading to the formation of characteristic root galls or root knots (Agrios, 2005). The formation of these galls hinders the host plant's water and nutrient uptake capacity. The root-knot-infected plants appear yellow, stunted and less vigorous than healthy plants. Being soil-borne, nematodes also play an important role in disease complexes involving soil-borne bacteria and fungi (Back *et al.,* 2002; Rao *et al.,* 2017). The plants infected with root-knot nematodes become more vulnerable to soil-borne pathogens leading to huge economic loss.

Cauliflower, *Brassica oleracea* var. botrytis, is an important member of the family Cruciferae. It is one of the major winter vegetable crops grown in India, consumed as fresh or cooked vegetables and processed for freezing or pickling. It is cultivated over an area of 22.53 thousand hectares with a production of 418.36 thousand tons in Punjab (Anonymous, 2020). Crucifer crops are generally regarded as not good hosts for *Meloidogyne* spp. and are being suggested as cover crops to manage root-knot nematodes (Liébanas *et al.*, 2004). Also, cruciferous plants of *Brassica* spp. are known to produce glucosinolate compounds on incorporation into the soil and are, therefore, reported to be used as bio-fumigants against nematodes (Mohamed *et al.,* 2020). However, the susceptibility of each crucifer crop varies considerably against *Meloidogyne* spp. (Liébanas *et al.*, 2004).

Root-knot nematodes have been found prevalent in major vegetable-growing areas of Punjab affecting chili (Kaur *et al.,* 2016), cucumber (Singh, 2017) and bitter gourd (Sharma *et al.,* 2018). To know the incidence and prevalence of *Meloidogyne* spp. in cauliflower crops the present study was planned and random samples were collected from major cauliflower growing areas of Punjab. Further, the available cauliflower germplasm with the Department of Vegetable Science, Punjab Agricultural University, Ludhiana, was screened against the prevalent root-knot nematode species.

Material and methods

Incidence and severity of root-knot nematodes: Samples were collected from cauliflower growing areas in districts Ludhiana, Sangrur, Moga and Tarn Taran during the crop season in 2018-19. Soil and roots were collected from the rhizosphere of plants showing stunting, yellowing and poor growth. The soil samples were collected at 15-20cm depth with the help of an auger. These samples were packed in polythene bags, labeled, sealed properly and brought to the laboratory for nematode detection and population estimation. Soil samples were analyzed to estimate the root-knot nematode population using Cobb's sieving and decanting technique (Cobb, 1918). Root-knot nematode incidence (%) was calculated as follows

$$\text{Incidence (\%)} = \frac{\text{Number of sites infected with root-knot nematodes}}{\text{Total number of sites surveyed}} \times 100$$

Identification of root-knot nematode species associated with cauliflower: Mature females were extracted from the infected root samples collected during the survey and processed to study perineal patterns. The root-knot nematode-infested roots showing galls were stained with acid fuchsin stain (0.1%) (Eisenback *et al.,*1981). The mature females were collected in clear lactophenol by teasing out the stained roots with the help of a pair of forceps and a needle. A single female specimen was then placed on a glass slide in a drop of lactophenol, and the posterior end region of the female was cut using a surgical blade. The cut portion was cleaned by carefully removing the internal tissue using a nylon bristle and transferred to a clear slide in a drop of glycerol. A cover slip was placed gently on the cut portion, sealed with the help of glyceel, and examined under the microscope. The root-knot nematode sp. associated with the collected samples was identified based on perineal pattern descriptions given by Eisenback (1985). The perineal pattern study selected ten mature females randomly from each population. The perineal cut patterns were observed under the Leica microscope (DME EC3) at 10x and 40x magnification. Photographs were taken with the help of an in-built Leica digital camera and a Leica image analysis application system was used to record morphometric observations for the length of the vulval slit (LVS), Anus to vulval slit (AVS) and Anus to tail terminus length (ATT).

Screening of cauliflower genotypes: A set of 30 cauliflower genotypes available with the Department of Vegetable Science, Punjab Agricultural University, Ludhiana, was screened against root-knot nematode, *M. incognita* in the year 2019-20. The seedlings were raised in rectangular cemented pots (90cm x 60cm) filled with autoclaved soil. The one-month-old seedlings of the cauliflower genotype were transplanted in 20 cm diameter individual pots with one seedling per pot. Seven days after transplanting, as the plants got established properly, pots were inoculated with freshly hatched second-stage juveniles @ two juveniles per gram of soil. Pots were arranged in a randomized complete block design with five replications for each cultivar. Plants of susceptible tomato cultivar, 'Punjab Upma' were also inoculated simultaneously to check the pathogenicity of the inoculums and to determine the termination time of the experiment.

After sixty days of observations on the root galling index, the final soil nematode population and reproduction factor (Rf = Pf/Pi) were calculated. Each plant was uprooted and rated as per 0-5 rating scale (Taylor and Sasser, 1978) (where 0 = no galls or egg masses, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, and 5 = > 100 galls or egg masses per root system) and average root galling index (RGI) was worked out for each genotype. Based on RGI and Rf each genotype was categorized as resistant or susceptible as per the host suitability scheme given by Canto-Saenz, (1983). According to this scheme, genotypes with RGI= 0 and Rf = 0 were designated as immune. Genotype with RGI < 2 and Rf ≤ 1 as resistant. Genotypes with RGI ≤2 and Rf>1 were designated as tolerant, while genotypes with RGI>2 and Rf≤ 1 were categorized as hypersusceptible. Genotypes with RGI >2 and Rf> 1 were designated as susceptible.

The data were statistically analyzed using SPSS software. The analysis of variance and differences among means were compared against check by the Dunnett test ($P < 0.05$).

Results and discussion

Incidence and severity of root-knot nematodes: 46 samples were collected from cauliflower growing areas in districts; Ludhiana, Sangrur, Moga and Tarn Taran. The cauliflower samples collected from symptomatic plants from farmer's fields showed characteristic root knots on the roots (Fig 1). It was observed that root-knot nematode disease incidence in cauliflower growing areas of districts under study ranged from 10 to 45 percent (Table 1). Overall, the maximum incidence was observed in district Sangrur (35.6 %), followed by Moga (33.3%) and Ludhiana (24.3%). The minimum incidence of root-knot disease was recorded in district Tarn Taran (16.6 %). Soil nematode population was also highest in Sangrur (474.2 J_2/250g soil) district and lowest (284.9 J_2/250g soil) in Tarn Taran district. Mean Root galling index was maximum in Sangrur (4.61) district, followed by Moga (4.91) and the minimum was recorded in Tarn Taran (2.50) district. The incidence of root-knot nematode on cauliflower was comparatively higher in areas where okra, tomato, chili and cucurbit crops were in rotation with cauliflower as compared to fields where wheat or onion crop was there in rotation with cauliflower.

The perineal pattern studies of mature females extracted from

Fig. 1. Roots of cauliflower plants collected from farmer's field with characteristic galls/ knots on roots due to root-knot nematode infestation.

Table 1. Incidence of root-knot nematode in cauliflower growing areas of Punjab

Location District/village	Crop rotation	Incidence (%)	Soil nematode population/ 250g soil	RGI (0-10) scale
LUDHIANA				
Jassian	Chilli –Cauliflower-Onion	13.0	366.0	3.33
Jassian	Okra – Cauliflower- Tomato	35.0	386.6	4.56
Jodhan	Chilli- Cauliflower-Okra	25.0	350.3	2.66
Mean		24.3	367.6	3.51
SANGRUR				
Bhaini Kalan	Okra- Chilli –Cauliflower	26.0	456.0	4.33
Bhaini Kalan	Okra – Cauliflower- Tomato	36.0	530.3	5.66
Bhaini Kamboan	Cucurbits- Cauliflower- Chilli	45.0	436.3	3.85
Mean		35.6	474.2	4.61
MOGA				
Raoke Kalan	Brinjal – Cauliflower- Cucurbits	45.0	550.0	5.56
Rania	Okra –Cauliflower- Bitter gourd	35.0	456.5	4.33
Ajitwal	Tomato- Cauliflower- Onion	20.0	256.3	2.86
Mean		33.3	420.9	4.25
TARN TARAN				
Bohru	Okra - Cauliflower - Onion	15.0	333.0	2.33
Bohru	Wheat- Cauliflower -Chilli	10.0	236.3	1.85
Mandiala	Chilli - Late Cauliflower	25.0	285.6	3.33
Mean		16.6	284.9	2.50

the root samples collected during the survey showed a high dorsal arch and distinct wavy forked striae (Fig 2). This is the characteristic feature of *Meloidogyne incognita* sp. of root-knot nematode. This confirms the association of *M. incognita* as a prominent root-knot nematode species associated with cauliflower crop in areas under survey. However, the morphometric observations of the perineal patterns of females varied among the samples collected from different locations (Table 2). The LVS was maximum in the Ludhiana population (23.33 µm), followed by the Tarn Taran population (21.53 µm) and the minimum in the Moga population (19.38 µm). AVS was recorded highest in the Sangrur population (17.31 µm), followed by the Ludhiana population (16.67 µm) and the minimum in the Moga population (15.64 µm). ATT was maximum

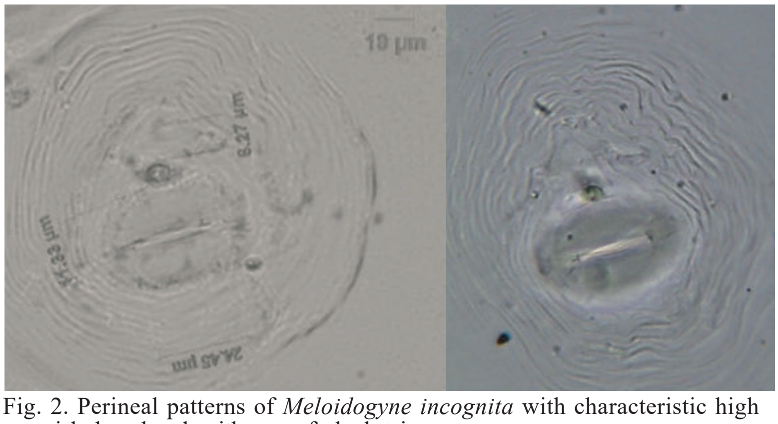

Fig. 2. Perineal patterns of *Meloidogyne incognita* with characteristic high squarish dorsal arch with wavy forked striae.

Table 2. Morphometric observations of perineal pattern of *M. incognita* females (Mean±SD, range) (µm)

Characters[a]	Ludhiana	Sangrur	Moga	Tarn Taran
LVS (µm)	23.33±2.20 (21.5-24.66)	20.69±2.84 (16.98-23.65)	19.38±2.36 (15.67-21.66)	21.53±2.12 (17.55-21.86)
AVS (µm)	16.67±3.36 (11.32-18.05)	17.31±2.24 (12.67-19.66)	15.64±2.06 (14.26-19.37)	16.36±3.26 (15.26-17.34)
ATT (µm)	15.33±2.16 (10.66-16.36)	16.38±1.83 (13.34-18.35)	17.34±1.76 (15.35-20.11)	14.67±2.11 (13.46-19.88)

[a]Characters LVS= Length of vulval slit, AVS= Anus to vulval slit, ATT= Anus to tail terminus

in the Moga population (17.34 µm), followed by the Sangrur population (16.38 µm) and minimum in the Tarn Taran population (14.67 µm).

The random survey revealed the association of root-knot nematode, *M. incognita,* in cauliflower growing areas of Punjab. The infested cauliflower plants exhibited the formation of typical knots or galls on the roots. Earlier, Kaur *et al.* (2016) documented the association of *M. incognita* and *M. javanica* root-knot nematode species in chili growing areas in the Indian Punjab, with *M. incognita* sp. as the more prominent species. *M. incognita* is the most prominent species of root-knot nematode associated with major vegetable growing areas worldwide (Anwar and McKenry, 2012; Singh and Kumar, 2015). Anwar and McKenry (2010) reported a 13.6 percent incidence of *M. incognita* on cauliflower with a root gall severity of 1.5 in Punjab, Pakistan. Singh and Khanna (2015) reported a prominent association of plant parasitic nematode*, M. incognita* and *Helicotylenchus dihystera* with cauliflower crop in Himachal Pradesh. Arun *et al.* (2019) also reported that *M. incognita* was the most prevalent root-knot nematode species with cabbage and cauliflower crop in Tamil Nadu. Knowledge about the diversity and association of root-knot nematode species with any crop is vital for designing management strategies because the interaction of different species during concurrent infections manipulates the host's defense reaction (Ogallo and Mcclure, 1995).

Screening of genotypes: Different genotypes of cauliflower showed a variable degree of resistance against *M. incognita* (Table 3). The tomato plants inoculated along with the cauliflower genotypes showed heavy galling (RGI 4.33), which shows the viability of the inoculum. Among the total thirty genotypes screened against *M. incognita,* none showed an immune reaction, while four genotypes (LSMVAR-6, PI1654, LSMVAR-2 and LS-4) showed resistant reaction with RGI ranging from 0.83-1.85 and Rf ranging from 0.51 to 0.68. As RGI and the Reproduction factor (Rf) are low, these genotypes are poor hosts to *M. incognita*. The resistant lines showed healthy roots free from root galls, while distinct root galls were observed on roots of susceptible genotypes. Fourteen genotypes were susceptible to *M. incognita,* with RGI ranging from 2.06-2.66 and Rf ranging from 0.57-1.0. These genotypes also showed comparatively less Rf, which shows that these are poor hosts to M. incognita. However, these genotypes recorded considerable galling of the root system, meaning significant damage may occur in these lines under heavy nematode infestation. Among others, eleven genotypes were found susceptible with RGI ranging from 2.89-3.55 and Rf from 1.03-1.17, showing that they are efficient hosts to the nematode and significant damage may occur.

The cauliflower genotypes showing resistant reaction against *M. incognita* were recorded with lower root gall index and reproduction factor than hyper susceptible and susceptible genotypes. The ability of nematodes to reproduce on a given host is considered as a indicator of the resistance level and is successfully used in selecting

Table 3. Reaction of different cauliflower genotypes to root-knot nematode, *M. incognita*

Genotypes	Soil population/ 250g soil	Rf (Pf/Pi)	RGI (0-5) Scale	Disease reaction
LSMVAR-6	300*±18	0.60	0.92*±0.32	Resistant
PI1654	257*±16	0.51	0.83*±0.29	Resistant
LSPSuk	387*±25	0.77	2.25*±0.27	Hyper susceptible
LSPPja	367*±15	0.73	2.10*±0.36	Hyper susceptible
LS-11	452*±14	0.90	2.56*±0.27	Hyper susceptible
LSP-1	442*±10	0.88	2.35*±0.44	Hyper susceptible
LC-1	387*±25	0.77	2.23*±0.33	Hyper susceptible
LS 3	523±20	1.05	3.29±0.37	Susceptible
MKL-1	387*±22	0.77	2.36*±0.13	Hyper susceptible
KRT-1	354*±10	0.71	2.30*±0.38	Hyper susceptible
PKS-1	342*±8	0.68	2.25*±0.22	Hyper susceptible
KRT-3	287*±12	0.57	2.06*±0.24	Hyper susceptible
LSPAs	541±26	1.08	3.25±0.09	Susceptible
LC-13	517±20	1.03	3.33±0.18	Susceptible
LSMVAR-3	359*±08	0.72	2.45*±0.43	Hyper susceptible
LSPMna	557±22	1.11	3.30±0.20	Susceptible
LC-5	529±16	1.06	3.55±0.36	Susceptible
LS-2	523±25	1.05	3.11±0.24	Susceptible
LSPSh	535±31	1.07	3.35±0.23	Susceptible
LSMVAR-7	530±22	1.06	3.11±0.24	Susceptible
LS 104	587±12	1.17	3.50±0.31	Susceptible
LS 4	341*±8	0.68	1.85*±0.34	Resistant
LSMVAR-1	439*±8	0.88	2.66*±0.25	Hyper susceptible
LSMVAR-2	289*±10	0.58	0.83*±0.20	Resistant
LSTar	528±08	1.06	2.89±0.27	Susceptible
LSCrs	466*±20	0.93	2.62*±0.24	Hyper susceptible
Sel -10	500*±20	1.00	2.51±0.45	Hyper susceptible
LSYmd	357*±18	0.71	2.15*±0.26	Hyper susceptible
PUstar	383±12	0.67	2.28±0.22	Hyper susceptible
PKS-6 Susceptible variety	560±10	1.12	3.55±0.24	Susceptible
Tomato cv Punjab Upma	686±10	1.37	4.33±0.11	Susceptible

Rf: Reproduction factor; Pf: Final nematode population; Pi: Initial nematode population; RGI: Root gall index; Initial population: 500juveniles/250g of soil. Values having * are significantly different from control (Susceptible variety:

resistance against root-knot nematodes in different crops (Gomes *et al.,*2015; Kaur and Dhatt, 2017; Sharma *et al.,* 2019). Khan and Khan (1991) reported that Pusa Snowball and 74-6C cauliflower cultivars were resistant to *M. incognita* race 1 whereas cultivar Superial Maghi was susceptible to all races of *M. incognita*. The resistant cultivars, Pusa Snowball and 74-6C0, were recorded with Rf of less than one. McSorley and Frederick (1995), also reported that 'Early Snowball' cultivar of cauliflower was susceptible to *M. javanica* and all races of *M. incognita.* Developing and utilizing resistant crop cultivars is the most adapted strategy for managing crop pathogens (Fawke *et al.,* 2015; Mundt, 2002).

In the present scenario, when most of the chemical nematicides have been banned due to their harmful effects on humankind and the environment, some economically viable approach is required to manage these nematodes to minimize crop losses. The information about the prevailing root-knot nematode species associated with the cauliflower crop will help select the appropriate management strategy. The *M. incognita* resistant genotypes identified during the current investigation will help strengthen resistance breeding against this nematode. They may also be further investigated for the release of relative glucosinolate concentration and their potential in biofumigation against these nematodes.

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