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RESEARCH ARTICLES

Genetic analysis of root-knot nematode (*Meloidogyne incognita*) resistance in carrot (*Daucus carota* L.)

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Abstract

The genetics analysis for a specific characteristic is critical to monitor plant health along with developing and testing novel, cost-effective, and long-term management. Root knot nematode by formation of galls causes a significant yield lossess in tropical carrots. Severe infestations result in forking or fanging of the roots, reducing their marketability. In carrot production, genetic resistance considerably reduce the requirement for broad-spectrum soil fumigants. The genetics and nature of root-knot nematode resistance in carrot were identified using root gall index in the plastic bag settings using a pure culture of root-knot nematode. In order to carry out this research, we used one immune parent (Acc-88) and one highly susceptible parent (Acc-113B) to produce F_1, F_2, BC_1P_1 and BC_1P_2 progenies. In cross between Acc-113B x Acc-88, chi-square analysis indicated that root-knot nematode (*Meloidogyne incognita*) resistance is controlled by a recessive gene. Further, the non-significant scaling test in the cross confirmed the absence of epistatic interaction in this study. By evaluating three parameters [m, d, and h] through generation mean analysis, the results suggested that cross had a predominance of additive types. All of the above results showed that it might be possible to improve resistance to root-knot nematodes by backcross breeding.

Keywords Additive · Carrot · Chi-square · Non-significance · Single recessive gene

Introduction

Carrot (*Daucus carota* L.) 2n = 18 belongs to Apiaceae family, an important root vegetable. Edible roots of carrot are famous around the world for both their fresh and processed form. Carrot contains dietary nutrients (carotenoids, anthocyanins, and flavonoids) that protect human health and lower the risk of cardiovascular disease (Selvakumar et al. 2019). Carrot roots are good source of vitamin A, accounting for 14 to 17% of total vitamin A (Block, 1914). Carrot is the most widely grown root vegetable in India, occupying 112 ha and producing 2042MT with a yield of 18.23 t/ha (National Horticulture Board 2020). Carrot production in the world has increased fourfold in last 45 years, from 5.8 million MT in 1961 to 23.6 million MT in 2014 (FAO, 2015). Among fruits and vegetables, carrot has been placed 7th in nutrition contribution (Alasalvar et al. 2001; Simon 2000). India's agricultural sector suffers from various challenges that limit its productivity, including inadequate farming practices, a shortage of crop varieties that are suitable for different agro-ecologies, and the use of low-quality planting seeds. These factors collectively contribute to the country's low agricultural productivity, which poses significant obstacles for farmers in terms of yields and profitability. These issues can be addressed through improved practices and use of disease resistance varieties.

Carrot is vulnerable to biotic stresses caused by pathogens such as bacteria, fungi, viruses, and soil-borne pathogens. Root knot nematode is among the soil-borne pathogen that are damaging carrot production and marketability. There have been 98 reported species of root knot nematodes (Subbotin et al. 2021), but only four species (*Meloidogyne incognita*, *M. javanica*, *M. arenaria*, and *M. hapla*) account for approximately 99 percent of the population (Mai and Abawi 1987). *M. incognita* and *M. javanica* are the two serious

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threats in tropical carrot while *M. hapla* is common in temperate carrots (Seenivasan 2017). Infective juveniles of RKN enter the roots and cause galling of tap and lateral roots besides causing characteristic forking of the roots (Taylor et al. 1982). It harms the tap root by causing galls and deformation of tap root due to forking, making the roots unpalatable to consumers. In southern regions of India, *Meloidogyne* spp. emerging as a serious menace to carrot growers result in 45–50 per cent of yield loss (Nisha et al., 2012). Through All India Coordinated Research Projects (Nematodes), percent yield loss in carrot due to *Meloidogyne* spp. was assessed as 34%, highest among other vegetable crops, and the monetary loss was estimated about Rs. 745.12 million (Kumar et al., 2020). Control of RKN is difficult and requires comprehensive management. The most commonly used strategies are chemical based non-biological and genetic techniques (Dias Arieral et al. 2013). Because there is no effective management method, the researchers are working on developing resistant varieities which are effective and environment friendly (Huet 2014; Namisy et al. 2019). Genetics analysis is an important element to consider before beginning any breeding effort, as it aids in determining the best breeding approach for developing resistant varieties or hybrids. Genetic research is needed because genotype and environment contribute to the inheritance of RKN resistance (Barik et al. 2021). Studies have already been made to identify carrot varieties resistant to root-knot nematode (*M. hapla*). Wang and Goldman 1996 reported two different homozygous recessive genes conditioned for root-knot nematode resistance(*M. hapla*). There is no report conferring root knot nematode resistance (*M. incognita*) in tropical carrots. As a result, a study was recently undertaken at ICAR-IIHR (Indian Institute of Horticultural Research) in Bengaluru, and potential immune (Acc-88) and highly susceptible (Acc-113B) lines to root knot nematode were identified using the root gall index (Taylor and Sasser, 1998) in field and in plastic bags. Keeping this in mind, the current study aimed to learn more about the genetics of resistance in order to better understand the gene activity and assist breeders in developing an appropriate breeding strategy.

Material and methods

Plant material and population development

In this study, one immune line Acc-88 was used, which was identified through the number of galls and RGI (root gall index). To generate six generations of populations, highly susceptible line Acc-113B was crossed to the root-knot immune line Acc-88 to produce P_1, P_2, F_1, F_2, BC_1P_1 (F_1 crossed with susceptible parent) and BC_1P_2 (F_1 crossed with the resistant parent). Four progenies (F_1, F_2, BC_1P_1 and BC_1P_2) along with parental lines involving Acc-88 and Acc-113B were assessed for nematode infection during November 2021–April 2022 under plastic bag (Fig. 1). The bags were filled with sterilized growing media (mixture of sand, soil and farm yard manure at the ratio of 1:1:1). Completely randomized design (CRD) was adopted in this experiment with three replications. The inoculation method regularized by Division of Crop Protection was followed to hatch and release J_2 stage of root-knot nematode to the crop for evaluation against root-knot nematode. Data were recorded for number of galls and root gall index at harvesting time from 90 days after sowing as described by Taylor and Sasser, 1978 on 0–5 scale (Table 1).

Root knot nematode identification, screening and chi square analysis

RKN culture was obtained from Division of Crop Protection, ICAR-IIHR, Bengaluru and the species was confirmed as *M. incognita* by observing females' perineal cuticular pattern (Sasser and Carter 1982). Further, maintenance of nematode culture was done in susceptible tomato plants (*cv.* PKM-1) for future use in experiments. For genotype screening, fully developed egg masses were collected from the infected roots of these plants. After uprooting the plants, roots were washed gently and then, matured egg masses were collected using forceps. These egg masses were placed in Petri dishes containing distilled water and kept at 28 ± 2 °C for hatching. After 72 h, freshly hatched J_2 were collected, concentrated in a beaker and counted under stereo zoom microscope (Motic, Hongkong). The number of active J_2 ml^{-1} water was determined and this nematode inoculum was used for further screening experiments. The seeds were sown in one kg of plastic bag. The bags were filled with sterilized growing media (mixture of sand, soil and farm yard manure at the ratio of 1:1:1). Freshly hatched juveniles were inoculated into each plastic bag at 2000 J_2 (approx.) near the root zone at 25 days after sowing (DAS). Juveniles were released into soil by making three holes near root zone around the plant and the holes were closed with soil after J_2 release (Coyne and Ross 2014). Thereafter, scoring for number of galls per root and root gall index (RGI) was done as described (Table 1) on 0–5 scale for examining the roots individually and independently.

Plants from F_2 and backcross progeny were recorded for number of galls and categorized as immune (0 galls), highly resistant (1–2 galls), resistant (3–10 galls), moderately resistant (11–30 galls), susceptible (31–100 galls) and highly susceptible (> 100). When plants from different filial and backcross populations showed reaction to RKN, the segregation ratio between resistant (R) and susceptible (S) nematode reactions were subjected to chi-square tests to conclude the goodness of fit with various classical mendelian ratios with assumed

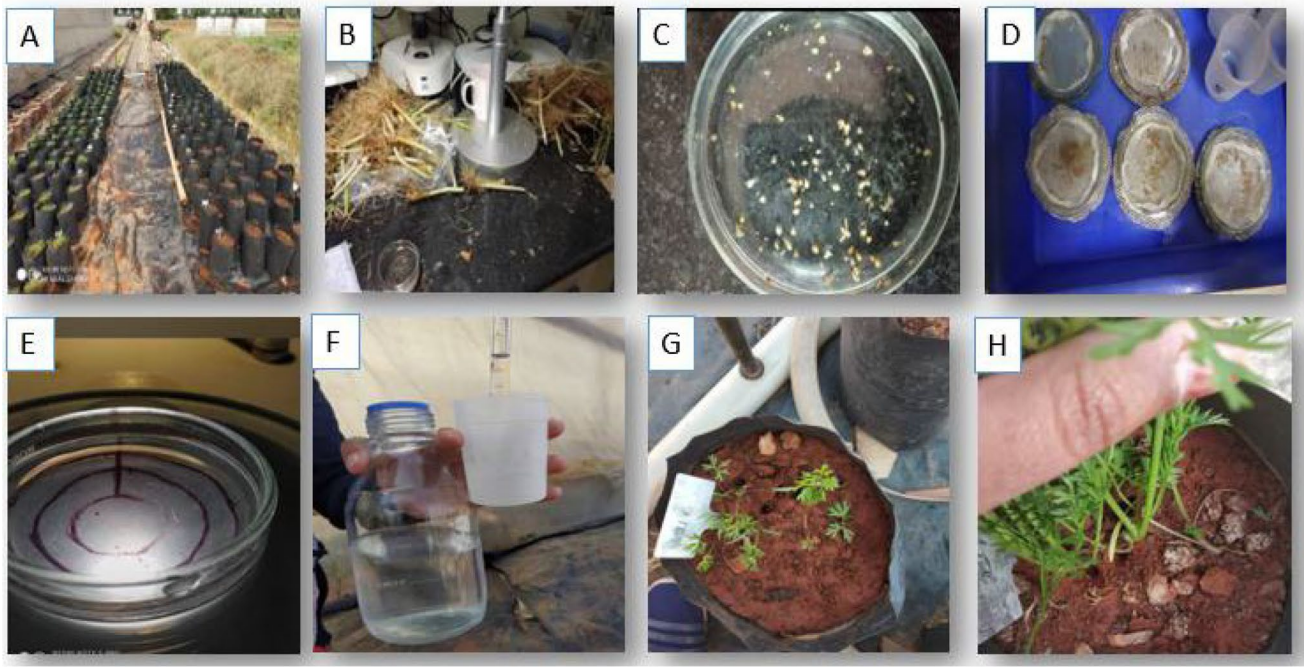

Fig. 1 Procedure for screening accession for nematode resistance in artificial epiphytotic conditions(**A**. Seeds were sown in 1 kg plastic bag. **B**&**C** Egg masses were collected from infected nematode susceptible plants. **D** Kept for hatching for 24 h. **E** Counted under stereo microscope. **F** 2000 J_2 population was used. **G**&**H** Inoculated near root zone by making hole)

Table 1 Scoring of root gall index as per Taylor and Sasser (1978)

Root Gall index	Number of galls root^{-1}	Reaction
0	0	I
1	1–2	HR
2	3–10	R
3	11–30	MR
4	31–100	S
5	> 100	HS

Immune

HR Highly Resistant; *R* Resistant; *MR* Moderately Resistant; *S* Susceptible; *HS* Highly Susceptible

phenotypic ratios of F_2 and backcross progeny as proposed by Panse and Sukhatme (1985). Based on ratio (hypothetical) presumption, expected values corresponding to observed values were calculated. On the basis of chi-square test, deviations from these values were calculated as follows

$$\chi^2 = \sum (\text{Observed number} - \text{Expected number})^2 / \text{Expected numbers}$$

Estimation of gene effects for root-knot nematode resistance

Based on three replications combined, average score value for nematode infection was calculated for the parents (P_1 and P_2), F_1s ($P_1 \times P_2$), and F_2s (F_1s selfed) in the cross, as well as their first generation backcrosses ($BC_1 = F_1 \times P_1$ and $BC_2 = F_1 \times P_2$). It served as the basis for calculating various statistics. Prior estimating the parameters, Hayman and Mather (1955) and Mather (1949) scaling tests were performed to detect non-allelic interactions. Based on the formula proposed by Jinks and Jones (1958), three parameters were evaluated for non-interacting cross. WINDOSTAT version 8.6 (statistical software developed by Indostat Services, Hyderabad) was used to conduct all statistical analyses at ICAR-IIHR, Hesaraghatta, Bengaluru.

Phenotyping of root knot nematode after J_2 stage nematode inoculation under artificial epiphytotic condition

The manifestation of RKN upon J_2 nematode inoculation has been characterized by formation of number of galls on root system. The galls were formed by females, a swelling of central cylinder, and highly deformed vascular elements. The stained roots demonstrate female's spherical part surrounding the parenchyma. As a result of severe *Meloidogyne*

infection, plants loose a great deal of their normal root system as well as completely disorganized vascular system, resulting in small number of severely galled roots in their root system. Roots' functions of water and nutrient uptake along with transport are severely hampered. Under growing conditions, plants wilt rapidly and are often stunted (Coyne and Ross 2014).

Validation of root knot nematode infection using stereo microscopic technique

To collect J_2, after uprooting the plants, roots were washed gently and then matured egg masses were collected using forceps. These egg masses were placed in Petri dishes containing distilled water and kept at 28 ± 2 °C for hatching (Coyne and Ross 2014). After 72 h, newly produced J_2 were collected, concentrated in a beaker and counted under stereo zoom microscope (Motic, Hongkong). The number of active J_2 individuals ml^{-1} water was determined and this nematode inoculum was used for further screening experiments. The population of J_2 nematodes in roots and number of egg masses per root were counted under a microscope (Motic SMZ-180) after staining the roots with acid fuchsin method (Byrd et al. 1983).

Results

Different generations' average performance against root knot nematode reaction

Means and variances were calculated to get an understanding of RKN response across generations of the cross (Table 2). After 90 days, when roots reached their maximum size and have been infected with nematode (90–100 percent), number of galls score in the cross was used for statistical analysis (genetic estimation) and interpretation of root-knot nematode resistance. Evaluation of different generations based on number of galls $root^{-1}$and RGI scores for the cross Acc-113B B × Acc-88 revealed that P_1(399.33), F_1(51.40), F_2(67.04) and BC_1P_1 (55.40) manifested lower resistance compared to P_2(0.33) and BC_2P_2 (2.30) (Fig. 2).

Chi-square analysis

"Acc-88" was immune and did not displayed any reaction to RKN. Different plants from the F_1, F_2, BC_1 and BC_2 populations exhibited varying responses, ranging from no reaction to RKN to gall formation on the roots, which is a distinguishing feature of the root-knot nematode (Fig. 3). 220 F_2 plants resulting from the cross of Acc-113B × Acc-88 was assessed for nematode response where, 59 plants were resistant and 161 plants were susceptible. The plants from back-crosses with susceptible parent were susceptible to nematode. When F_2 population data was estimated to chi-square analysis; only monogenic segregation ratio was satisfied and showed segregation pattern of 3:1 (susceptible: resistant) with chi-square value of 0.67 with percent probability of 53.00. The back cross population with immune parent (Acc-88) was segregated into 16 highly resistant and 14 susceptible plants ($\chi 2 = 8.35$; $p = 0.003$). Due to Mendelian segregation pattern (1:1) being recognized clearly in the backcross population (BC_2), the results were conclusive. On the other hand, genetic analysis of F_2 population indicates the involvement of a major recessive gene causing root-knot nematode resistance (*M. incognita*) in the cross Acc-113B × Acc-88 in carrots (Table 3).

Estimation of gene effects for root knot nematode resistance

Results of a statistical analysis (scaling test) were used to identify epistasis in the population, and are presented in Table 4. In number of galls, standard scaling test was not significant, indicating the simplicity of additive-dominance model as a sufficient mechanism to explain resistance against nematodes and absence of non-allelic interactions for the cross Acc-113Bx Acc-88. Table 4 illustrates the estimates of different genetic components, viz., mean, additive, and dominance effects. The additive(d) effect in cross, for number of galls, contributes to resistance.

Table 2 Different generations' average performance against root-knot nematode reaction in carrot

Cross		P_1	P_2	F_1	F_2	BC_1	BC_2
Acc-113BB × Acc-88	Mean ± SE	399.33 ± 50.33	0.33 ± 0.33	51.40 ± 15.97	67.04 ± 4.83	55.40 ± 5.90	2.30 ± 1.04
	Variance	2533.77	0.11	2551.37	5139.52	348.48	10.90

Note: Here, P_1 and P_2 represent susceptible and resistant parents respectively and F1 is the first filial generation, F_2 is the second filial generation, BC_1P_1 is backcross with the susceptible parent and BC_1P_2 backcross with the resistant parent

Fig. 2 Six-generation mean analysis population of Acc-113B × Acc-88 in carrot

Fig. 3 Evaluation of different generations based on number of galls per root

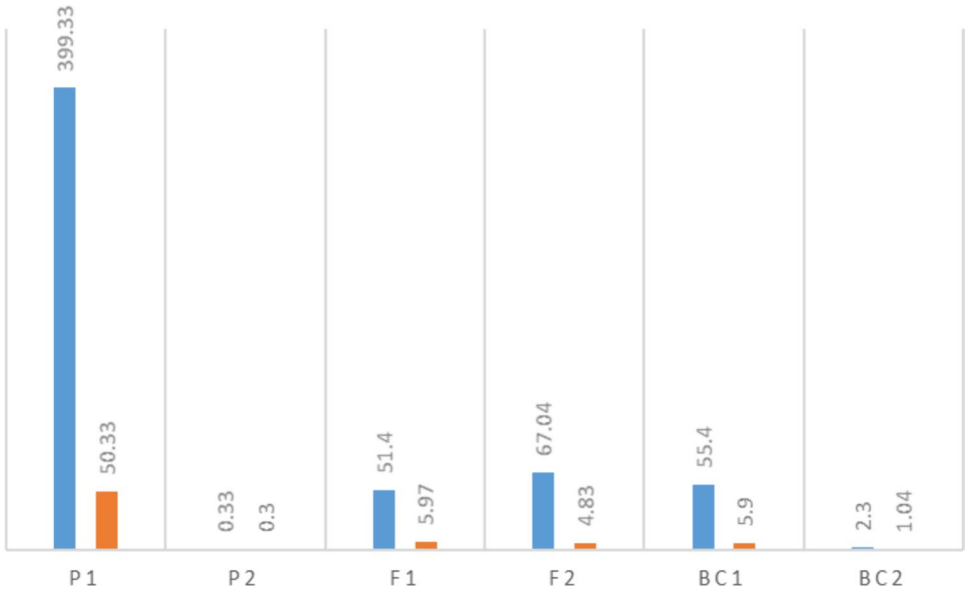

Table 3 Calculated chi-square values and their probability for nematode resistance intensity of carrot F_2 and test cross through classical Mendelian ratios

Cross	Generation	Description	Number of Plants			Genetic Ratio	χ2 value	Probability (5%)
			Susceptible	Resistant	Total			
Acc-113B × Acc-88	Acc-113B (P_1)	Acc-113B	30	0	30	–	–	–
	Acc-88 (P_2)	Acc-88	0	30	30	–	–	–
	F_1	Acc-113B × Acc-88	30	0	30	–	–	–
	F_2	Acc-113B × Acc-88	161	59	220	**3:1**	0.67	0.41
	BC_1	(Acc-113B × Acc-88) × Acc-113B	29	1	30	**1:0**	–	–
	BC_2	(Acc-113B × Acc-88) × Acc-88	14	16	30	**1:1**	8.35	0.03

The value of chi-square(χ2) value is 0.67 and probability (5%) value is 0.41

Table 4 Scaling test and three parameters genetic model for nematode resistance in carrot

Trait	A	B	C	D	[m]	[d]	[h]
Acc-113B × Acc-88	−47.13 ± 16.12 ^{ns}	−339.93 ± 54.11 ^{ns}	−270.75 ± 62.89 ^{ns}	−58.15 ± 11.67 ^{ns}	72.87 ± 5.14*	72.54 ± 5.138*	−67.43 ± 6.01 ^{ns}

m mean effect, *d* additive effect, *h* dominance effect

*significant at $p = 0.05$

**significant at $p = 0.01$, ns = not significant

Discussion

Root knot or gall caused by RKN (*M. incognita*) has become major production constraint and results in considerable yield loss in carrot, especially in tropical and sub-tropical regions of India. As a result, there is a significant need to produce nematode-resistant cultivars and hybrids. However, before producing desired variety/hybrids, a thorough understanding on resistance of nematode (*M. incognita*) to carrot accessions and its genetic nature is required to develop a breeding approach for improving this feature. In present study, plastic bag screening was adopted to screen the segregating generations through examining resistance performance of parental lines and their progeny, we have gained insights into genetic characteristics of the resistant species. The inheritance pattern of root knot nematode was studied in cross involving Acc-113B as susceptible (female) parent and Acc-88 as resistant donor (pollen) parent. F_1 plants of the cross was susceptible, indicating that resistance was inherited as a recessive trait which was inherited through a homozygous recessive trait. The segregation of F_2 populations into susceptible and resistant plants fit in 3:1 ratio, which indicated that susceptibility was dominant over resistance and root knot nematode resistance was inherited through a homozygous recessive trait at a single locus. In genetic background of *Daucus carota* genotype 'Acc-88', it was confirmed by the segregation pattern in both backcross populations where resistance to root knot nematode is conferred by a single recessive gene (Table 3). Further, scaling tests conducted on each of the two crosses were found to be non-significant which indicated the absence of epistasis. Therefore, the cross was of non-interacting types (Table 4) and in conformity with the results of chi square analysis. Wang and Goldman (1996) found that root-knot nematode (*M. hapla*) resistance is influenced by two homozygous recessive genes in $R_1 \times R_2$ cross. The single gene *Mj-2* with incomplete dominance in Asiatic genotype PI 652188 which imparts resistance against *M. javanica* was mapped by Ali et al. (2014). Using molecular markers, they could reveal that *Mj*-1 and *Mj*-2 are two different locations on chromosome 8. For resistance against *M. incognita,* Parsons et al. (2015) reported 5 non-overlapping QTLs using three diverse resistance sources HM (from Syria), SFF (from Europe) and Br1091 (South America) located on chromosomes 1, 2, 4, 8, and 9. One QTL was present in all three populations, in the same region of *Mj-1*. Acc-88 appeared to have a higher level of resistance in this study than Acc-113B, which has major recessive genes. Acc-88 can be used as a resistant line in susceptible varieties to introduce resistance to RKN (*M. incognita*). This is the first information of resistance to RKN (*M. incognita*) in carrots in India using a plastic bag inoculation of J_2-stage nematodes.

Conclusion

The results of current study indicated that presence of a single recessive gene controlled root-knot nematode (*M. incognita*) in the cross Acc-113 B × Acc-88 in carrot. The cross showed a predominance of additive types. Results indicated monogenic recessive resistance to root knot nematode in the genetic background which need to be confirmed through repeated experiments with large population size. These results are imperative for breeding root knot nematode resistant cultivars in *Daucus* species.

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Declarations

Conflict of interest Authors have no conflict of interest to express.

Data availability The current manuscript have included all related data.

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