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Evaluation of a resistant line of tropical carrot to root-knot nematode *Meloidogyne incognita* using conventional method and molecular markers

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Abstract Carrots are highly susceptible to root-knot nematodes (RKN) which reduce their marketability due to deformation of roots. Hence, there is a need to identify resistant sources against RKN to accelerate resistance breeding programmes in carrot. In the current study, forty-eight carrot accessions were screened by artificial inoculation of nematodes to locate the lines resistant to *Meloidogyne incognita*. Among all, Acc- 88 was found to be immune to *M. incognita* followed by Acc-72, Acc-145 and KSP-123 which showed a moderately resistant reaction. Acc -113B was highly susceptible while the other 44 lines showed a susceptible reaction towards *M. incognita*. The accessions were validated for the *Mj* gene using linked molecular markers conferring resistance to RKN. The immune line, Acc-88 amplified at 266 bp proving the presence of resistance allele and Acc-113B generated the product size of the susceptible allele revealing a polymorphic difference with the ESSR0110 marker. The *Mj* resistance locus carrying Acc-88 has the potential to introgress RKN resistance in susceptible varieties of carrot. The validated ESSR0110 co-dominant molecular marker is promising because it could effectively distinguish resistant and susceptible individuals, thus helps the breeder to develop stable source resistance to root knot nematode. This finding is the first report on identification of immune linse and linked molecular marker in tropical carrot against *M. incognita.*

Keywords Carrot · Root knot nematode · *Meloidogyne incognita* · Screening · Resistance · Marker · *Mj* gene

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Introduction

Carrot (*Daucus carota* L. 2n = 18) is an important root vegetable crop grown worldwide for its nutritional importance whilst contributing flavor and texture as a dietary component. It is consumed both in fresh and processed form, and in confectionery preparation and pickles. Carotene is the principal precursor of pro vitamin A, which is an important nutrient. Deficiency of this vitamin causes xerophthalmia, an eye ailment

prevalent among pregnant women and children aged below 5 years, especially in rural areas of India (Riaz et al., 2021). Carrot is a highly remunerative crop and in India, it is being cultivated in 112 thousand ha producing 2024 thousand tonnes per year (Anonymous, 2016). However, carrot productivity is hindered by several diseases and pests, including root-knot nematodes (RKN). Among several species of RKN, *M. incognita* and *M. javanica* are the two most serious threats in the tropical carrot while *M. hapla* is common in temperate carrots. The infective juveniles of RKN enter the roots and cause galling of the tap and lateral roots besides causing characteristic forking of the roots (Ali et al., 2014). The RKN damage causes severe losses to marketable quality (Gugino et al., 2006). *M. hapla* alone causes yield losses up to 19.1% and market losses up to 59.1% in the Nilgiris and Kodaikanal hills of Tamil Nadu (Seenivasan, 2017). In southern regions of India, *Meloidogyne* spp. is emerging as a serious menace to carrot growers resulting in 45–50 per cent of yield loss (Nisha et al., 2012). Through an All India Coordinated Research Projects (Nematodes), the percent yield loss in carrot due to *Meloidogyne* spp. was assessed as 34%, the highest compared with the other vegetable crops, and causing a monetary loss of Rs. 745.12 million (Kumar et al., 2020). Due to climate change the severity of biotic and abiotic diseases has increased and resistance breeding has the potential to mitigate the severity of diseases in various crops including carrot. Chemical nematicide based control measures have proved their efficacy in reducing nematode damage (Gugino et al., 2006). However, owing to their toxic effects on humans and biotic soil life, many chemical pesticides are either banned or in the verge of being withdrawn from the market for commercial use. Resistance breeding provides an effective, economical and ecofriendly solution for reducing the nematode population. Hence, there is always a need to identify resistance sources and incorporate them in breeding programmes.

Simon et al., 2000 reported the inbred line 'Brasilia' to be immune to *M. javanica*. The single dominant locus '*Mj-1*' governs resistance to *M. javanica* in the 'Brasilia' derived line 'Br1252' (Boiteux et al., 2004; Simon et al., 2000). Co-dominant sequence tagged sites (STS) flanking markers linked to the RKN resistance locus have been developed by Boiteux et al., (2004). The single gene *Mj-2* with incomplete dominance in an Asiatic genotype PI 652188 which produces resistance against *M. javanica* was mapped by Ali et al. (2014). Using molecular markers, they revealed that the *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 as *Mj-1*. The total phenol content (mg/100 g) and carotenoid content (mg/100 g) was estimated to investigate any relationship between these biochemical parameters and nematode resistance. Considering these complexities in trait handling, the current study was designed to identify stable sources of resistance to RKN, *M. incognita* in tropical carrot accessions through conventional assays followed by validation through available molecular markers.

Material and methods

Location of the experiment and the source of planting material

The experiment was conducted at ICAR-Indian Institute of Horticultural Research (IIHR), Hesaraghatta, Bengaluru, Karnataka, India (13.58° N; 78° E and 890 m MSL). The experiment was carried out for two seasons during 2018 and 2019. Seeds of all carrot accessions were procured from the germplasm collection of Division of Vegetable Crops, ICAR- IIHR, Bengaluru.

Maintenance of nematode culture

The RKN culture was sourced from the Division of Crop Protection, ICAR-IIHR, Bengaluru and the species was confirmed as *M. incognita* by observing the females' perineal cuticular pattern (Sasser & Carter, 1982) (Fig. 1). Further, maintenance of the 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, the roots were washed gently and mature egg masses were collected using forceps. These egg masses were placed in Petri dishes containing distilled water at 28 ± 2^0C for hatching. After 72 h, freshly hatched second stage juveniles (J_2) were collected, concentrated in

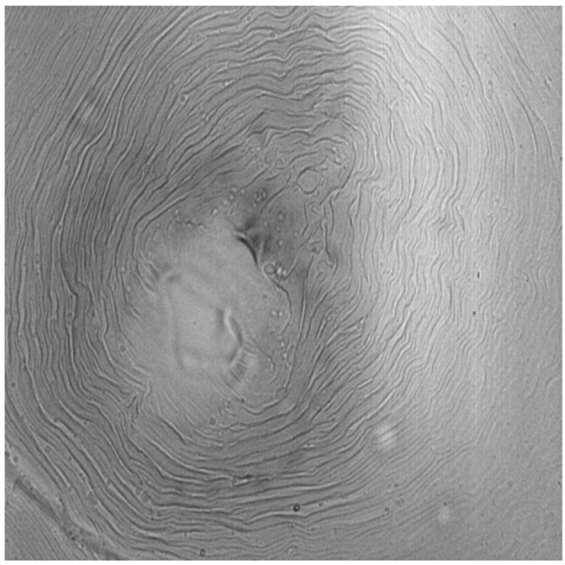

Fig. 1 Perineal cuticular pattern of *M. incognita*

a beaker and counted under a 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 the number of egg masses per root were counted under a microscope (Motic SMZ-180) after staining the roots with the acid fuchsin method (Byrd et al., 1983).

Screening of carrot accessions for M. incognita resistance

For evaluating carrot accession resistance against RKN, seeds were sown in black polythene bags (1 kg capacity). Sterilized potting media containing equal proportion of sand, red soil and Farm Yard Manure was placed in the bags. Forty-eight lines had been evaluated for nematode resistance including Arka Suraj as susceptible check. Freshly hatched juveniles were inoculated into each pot containing a single seedling at 2000 J_2 (approx.) near the root zone at 15 days after sowing (DAS). Juveniles were released into the soil by making three holes near the root zone around the plant and the holes were closed with soil after J_2 release. A completely randomized design (CRD) was adopted in this experiment with five replicates per accession and ten plants for each replicate. 90 days after nematode inoculation, plants were uprooted and the roots were gently washed with water to get rid of the adhering soil. The number of galls per root, egg masses per root and root gall index (RGI) were scored on 0–5 scale as described by Taylor and Sasser (1978). This experiment was conducted twice from Dec to April, 2018 and April to July, 2019. The number of egg masses in root and number of J_2 population per 100 cc soil were also recorded (Cobb, 1918).

Biochemical Analysis after nematode inoculation

The total carotenoid content (mg/100 g), total phenol content (mg/100 g) and TSS (°Brix) was assesssed in immune, moderately resistant and highly susceptible accessions. The total carotenoid content (mg/100 g) was extracted using 100% acetone. (AOAC, 2000). Total phenol content was estimated by the spectrophotometric method using Folin Ciocalteu's Reagence (FCR) (Singleton et al., 1999).

DNA extraction and Genotyping

The extraction of genomic DNA was done from healthy leaves by the Cetyl Trimethyl Ammonium Bromide (CTAB) method as describe by Doyle and Doyle (1990). Molecular markers (Boiteux et al., 2004; Cavagnaro et al., 2011; Ali et al., 2014 and Parson et al., 2015) were screened to determine polymorphy of markers between the lines. The primer list and sequence information are given in Tables 1 and 2. Initial denaturation at 95 °C for 4 min was followed by 35 cycles of denaturation at 94 °C for 30 s, annealing as directed by the primers for 30 s, extension at 72 °C for 4 min, and a final extension at 72 °C for 5 min in a Technie TC-500. On a 4 percent agarose gel electrophoresis, the PCR products were visualized using a UV–tech gel documentation system. The amplicon size was determined using a 100-bp DNA ladder. This was done three times to make sure the results were correct. To determine polymorphisms among immune and highly susceptible accessions, fifteen molecular markers were used. Genotyping was performed on ten samples from each accession and repeated three times.

Root Gall Index (RGI) scoring for nematode screening

Following inoculation, the roots were rated on a 0–5 scale for the number of galls per root, egg masses per root, and root gall index (RGI) as described by Taylor and Sasser (1978). Plants with zero galls and

Table 1 Molecular markers linked to *Mj* gene against RKN in carrot

S.No	Primer	Forward	Reverse	Reference
1	GSSR-35	AATTCACAATCACCGACTC	TCCACGTCAAAGCTCCTGTTCATTT	Parson et al., 2015
2	GSSR-96	AGCGTCGTTTTCGCGAGTC	GCGGTTAAAGCAAAGCTAAT	Parson et al., 2015
3	BSSR-15	TGTAATCGACTTATTATCGTCTTTTG	AAGGATGATGTAGAGGTGAATGAT	Parson et al., 2015
4	ESSR0009	ATCTGGGGAACTTGCTGTTG	AGCATCAGCAGCAGCTACAA	Parson et al., 2015
5	ESSR0041	AATTCACGAAGCCACGATTC	ATCGTCCCACAGATCCAATC	Parson et al., 2015
6	ESSR0029	GGGCGAAGTGAAAGTAG	CAGGTTAACCGGCTCAATCGAAA	Parson et al., 2015
7	ESSR0110	TTTCTCCATCCCTCCCTTCTTG	GAAGAAGCCCAAGGACTA	Parson et al., 2015
8	SQ1	GGAcgATGGCCAGGGAAAGCAACC	AAGTCACGCCAACAGTAATT	Boiteux et al., 2004
9	SQ6	GAGcgCCTTGATTGATGCTGTGT TGCC	GAGcgCCTTGGCAGCATCGATTA GTAG	Boiteux et al., 2004
10	SCAR21	TCCATACCGGCTTACAGTC	CTTGGTCTAGCATGCTCCAAG	Ali et al., 2014
11	BSSR132	GAAAGGAGGTGGCTGAAGAGTCA	GCACAATACTAATAGGGAAATGCAA	Cavagnaro et al., 2011
12	GSSR44	AACTTCACCCCAGCTCACCCA	AAGCAAGTAAAGAGACAGCG	Ali et al., 2014
13	GSSR044	AACTTCACCCCAGCTCACCCA	AAGCAAGTAAAGAGACAGCG	Parson et al., 2015
14	GSSR046	GTCCAACTATCCACTTCTTGGCG	CTATGTAAAATCTCCCACTACCTCT	Parson et al., 2015
15	BSSR026	GTTGTTCCTATTCAGAGGACTTG	TGGTAGTCTTGGAGGAGTTGAAGT	Parson et al., 2015

a zero root gall index showed an immune response, but plants with 1–2 galls and a one root gall index showed a high resistance response. The number of galls (3–10) and root gall index of two indicated nematode resistance response. The plants with moderate resistance had 11–30 galls and a root gall index of 3. The root gall index (4) and the number of galls (31–100) were susceptible to nematode. The highly susceptible plants have over 100 galls and a root gall index of 5.

Statistical analysis

The experimental data from both seasons were statistically evaluated by analysis of variance (ANOVA). Mean values were compared and the critical differences were determined using Duncan's Multiple Range Test (Panse & Sukhatme, 1985).

Results

Screening trials –Season 1

Among the 48 accessions screened in season 1, there were no galls observed in Acc -88 (RGI score-0) showing an immune reaction to *M. incognita*. Acc-88 also had no egg masses in their roots nor a J2 population in the soil. The highest number of galls was recorded in Acc-113 B (RGI- 5) in season 1 indicating a highly susceptible reaction to *M.*

Table 2 Reaction of carrot accessions against root knot nematode (*Meloidogyne incognita*) Inoculation (season –I)

Acc. No	Number of galls	Root gall index	Number of egg masses per root	Number of J_2 population per 100 cc soil	Reaction
Acc-88	$0(0.00)^{a}$	$0(0.00)^{a}$	$0.00(0.00)^{a}$	$0(0.00)^{a}$	I
Acc-72	$22.36(1.37)^{b}$	$3(0.60)^{b}$	$10.20(0.97)^{b}$	$17.2(1.26)^{b}$	MR
Acc-145	$28.20(1.47)^{c}$	$3(0.60)^{b}$	$20.40(1.29)^{c}$	$22.2(1.36)^{c}$	MR
KSP123	$23(1.36)^{b}$	$3(0.60)^{b}$	$25.20(1.39)^{d}$	$26.6(1.51)^{d}$	MR
Acc-113B	$310.20(2.51)^{j}$	$5(0.78)^{e}$	$92.20(1.89)^{v}$	$109.6(2.19)^{j}$	HS
Arka Suraj	$51.80(1.70)^{no}$	$4(0.70)^{c}$	$32.60(1.50)^{fg}$	$44(1.65)^{ijk}$	S
SE(d)	0.104	0.0075	0.0251	0.017	
CD(0.05)	0.02	0.015	0.049	0.033	

Fig. 2 Reaction of carrot accessions to *M. incognita* after 90 days of inoculation: A –Immune line (Acc-88), B – Highly susceptible line (Acc-113B), C- Susceptible check—(Arka Suraj)

incognita. ACC-113B also showed the highest number of egg masses and the highest J_2 population in roots in season 1. (Table 2; Fig. 2).

Three accessions viz., Acc-72, KSP-123 and Acc-145 displayed a moderately resistant reaction in season trial 1 with 22.36, 23 and 28.20 galls, respectively, with RGI of 3. The number of egg masses in their roots was 10.20, 25.20 and 20.40, respectively, and the J_2 population in soil was 17.2, 26.6 and 22.2, respectively (Table 2).

All the remaining accessions were found susceptible to *M. incognita* with RGI as 4. The number of galls ranged from 31.20 to 99.50, the number of egg masses from 26.40 to 102 and the number of J_2 (per 100 cc soil) from 28.4 to 97.7 in all these accessions (Supplementary file 1; Fig. 2). ANOVA results on screening of carrot accessions against RKN in the season I trial and the significant mean differences among the treatments are shown in Table 3.

Screening trials –Season II

In season 2, similar results were found and Acc- 88 no galls and the root gall index was also zero, showing an immune reaction against RKN. The number of egg masses per root and the number of J_2 in the population was zero. Contrary, Acc-113B had the highest number of galls RGI of 5 showing a highly susceptible reaction to *M. incognita*. ACC-113B also showed the highest number of egg masses and the highest J_2 population in the soil.

Acc-72, KSP-123 and Acc-145 again showed a moderately resistance reaction to RKN with the number of galls ranging from 22.20 to 26.40 and RGI of 3. All the remaining accessions were susceptible to *M. incognita* with RGI of 4 (Table 4). In all these accessions, the number of galls ranged from 31.60 to 97.60, the number of egg masses from 22.60 to 83.8, and the number of J_2

Table 3 Analysis of variance for the reaction of carrot accessions to root knot nematode (*M. incognita*) inoculation—season 1

Source	Degree of freedom	Sum of squares			
		Number of galls	Root gall index	Number of egg masses per root	Number of J_2 population per 100 cc soil
Treatment	47	24.63	2.57	19.61	20.82
Error	196	0.05	0.02	0.30	0.14
Total	243	24.68	2.60	19.92	20.97
F value		1911.70	381.13	259.93	600.14
P value		0.001*	0.001*	0.001*	0.001*

*Significant at a level of 1% probability

Table 4 Reaction of carrot accessions against root knot nematode (*Meloidogyne incognita*) Inoculation (Season -II)

Acc. No	Number of galls	Root gall index	Number of egg masses per root	Number of J_2 population per 100 cc soil	Reaction
Acc-88	0.00(0.00)^{a}	0(0.00)^{a}	0(0.00)^{a}	0(0.00)^{a}	I
Acc-72	22.20(1.36)^{b}	3(0.60)^{b}	13.2(1.15)^{b}	15.2(1.28)b	MR
Acc-145	28.20(1.47)^{d}	3(0.60)$_{b}$	18(1.27)^{c}	21.4(1.35)^{d}	MR
KSP123	26.40(1.44)^{c}	3(0.60)^{b}	19(1.40)^{c}	26.4(1.44)^{e}	MR
Acc-113B	321.80(2.51)^{j}	5(0.78)^{e}	112(2.05)^{y}	154.8(2.19)^{z}	HS
Arka Suraj	54.00(1.74)^{rst}	4(0.70)^{c}	51(1.72)^{no}	53.6(1.74)^{ij}	S
SE(d)	0.010	0.003	0.019	1.443	
CD(0.05)	0.022	0.006	0.038	2.846	

*Numericals followed by different alphabets in each column indicate significant differences according to Duncan's Multiple Range Test at $P \leq 0.05$

**Figures in parentheses are log transformed values

Table 5 Analysis of variance for the reaction of carrot accessions to root knot nematode (*M. incognita*) inoculation—season 2

Source	Degree of freedom	Sum of squares			
		Number of galls	Root gall index	Number of egg masses per root	Number of J_2 population per 100 cc soil
Treatment	47	23.72	2.53	21.12	148819.60
Error	196	0.05	0.005	0.18	1020.48
Total	243	23.78	2.53	21.30	149840.08
F value		1651.59	2063.41	461.68	595.48
P value		0.001*	0.001*	0.001*	0.001*

(per 100 cc soil) from 18.2 to 97.2 (Supplementary file 1). Table 5 shows the ANOVA results of screening trials of season -II showing significant differences among the accessions for their reaction to nematodes.

The carotenoid content, total phenol content and TSS of immune, moderately resistant and highly susceptible accessions was recorded and presented in Table 6. Acc-88 showed a higher total phenol content and TSS as compared to the highly susceptible accession Acc-113B (Fig. 3).

Marker validation

In the present study, fifteen molecular markers (SSR, SCAR and STS) as shown in Table 1 were used to detect the presence or absence of polymorphism in the test accessions. Among these, ESSR0110 was able to differentiate between immune and highly susceptible accessions by producing different PCR amplicon sizes. The immune line, Acc-88 gave an amplicon size of 266 bp and the highly susceptible line, Acc 113B generated 259 bp band size (Fig. 4). Thus, the results revealed the presence of the *Mj* locus in the carrot line immune to *M. incognita.*

Discussion

In the present study, carrot accession Acc-88 showed an immune reaction recording no galls and egg masses in season 1 & 2. A similar observation was done by Khan et al. (2018). He observed a low root gall index (1.2) and a low number of egg masses (6.0) in the resistant line Golden Rosy. Among the traits that differentiate nematode

Table 6 Biochemical reaction of carrot accessions after root knot nematode (*M. incognita*) inoculation

Accessions	Carotenoid content (mg/100 g)	Total phenol content (mg/100 g)	TSS (°Brix)
Acc-88	13.44	66.42	13.90
Acc-72	12.82	56.40	14.17
Acc-145	11.45	55.00	14.13
KSP123	9.61	-	11.30
Acc-113B	9.01	49.50	9.63
CD (0.05)	2.72	0.065	2.65

*Significant at a level of 1% probability

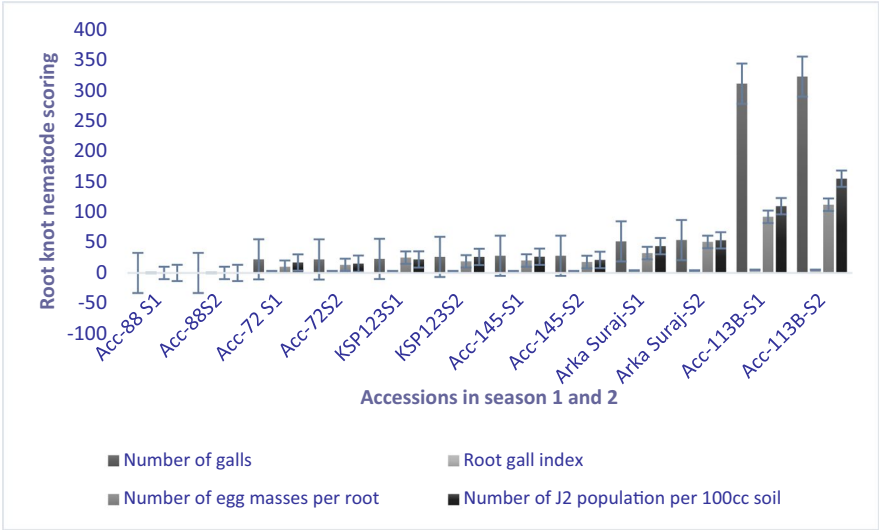

Fig. 3 Basis of screening and Effect of Root knot nematode on different accessions

resistant plants from susceptible plants is the inability of the nematode to create functional feeding sites in the host after invasion, as well as hypersensitivity reaction towards the nematodes (Williamson & Kumar, 2006). Also in the current study, the juvenile (j_2) population was 0 per 100 cc soil in Acc 88. This may be due to an incompatibility reaction and death due to starvation or due to antagonistic effects of root exudates of Acc 88. The exact mechanism of the resistance needs further investigations.

Both resistant and susceptible roots are attractive to root-knot nematodes. Nematodes migrate intercellularly following host penetration, usually near the tip of the root (Eisenback & Triantaphyllou, 2020). Galls form in infected roots, which are the primary symptom of root-knot nematode infection. Root-knot nematode eggs develop poorly on resistant cultivars compared to susceptible cultivars, according to Cousins and Walker (1998). The above events do not occur when a genotype is resistant to nematode attacks, but larvae may enter the roots in small numbers and develop into adults. When larvae enter the roots, but the cells immediately surrounding them die, resulting in localized necrosis of cells

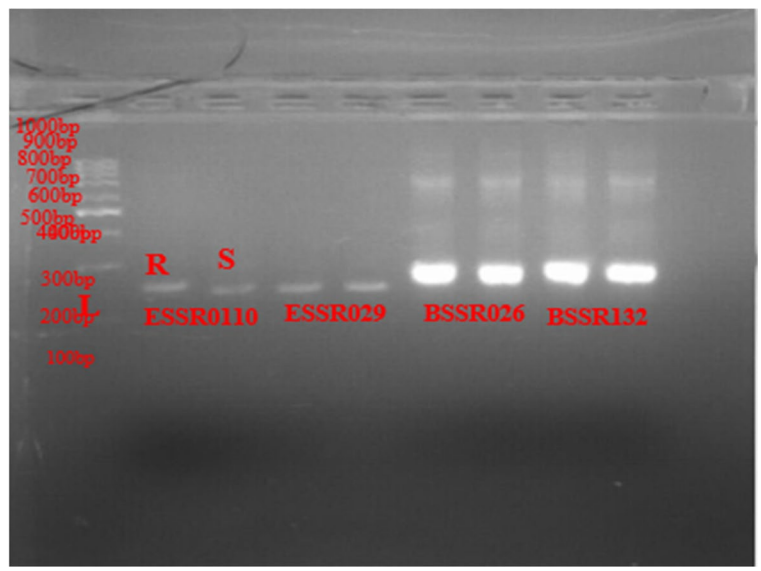

Fig. 4 Validation of ESSR0110 marker in highly susceptible and immune accessions of carrot to *M. incognita.* Molecular Ladder: 100 base pair, R: immune accession with 266 bp and S: Highly susceptible accession with 259 bp

around the nematode's anterior end, an important resistance reaction occurs (Dropkin et al., 1969).

Different levels of susceptibility to *M. incognita* in carrot accessions resulting from genetic differences are demonstrated by the number of galls and egg masses in each accession. In the current study, we found that the highly susceptible cultivar (Acc-113B) produced the highest number of galls, eggs and J_2 individuals per 100 cc soil. The moderately resistant accessions Acc-72, Acc-145, and KSP-123, on the other hand, allowed a small number of *M. incognita* juveniles to penetrate the roots, resulting in numerous galls and egg masses. Nematode populations varied significantly across all cultivars tested, as shown by disease severity and root gall counts. The highly susceptible cultivar was penetrated by a large number of nematodes, which resulted in the formation of giant cells, allowing the population density and number of root galls to increase. According to Vovlas et al. (2005), Nelson et al. (1990), and Khan et al. (2018), the number of root galls is approximately equal to the nematode population density, with the susceptible host's juvenile population reaching full development while the resistant cultivar had less development.

The current study also revealed that there is a significant difference in reaction of the accessions to *M. incognita*, suggesting that the variation in levels of resistance in these alleles is due to differences in the genotypes. One line, Acc-88 was immune to *M. incognita* in both seasons, which indicated that this genotype might possess the *Mj* gene as reported by Simon et al. (2000), Ali et al. (2014) and Kalia et al. (2019). This is the first report of a trait linked marker specific to *M. incognita* in carrot. The *Mj* gene does provide resistance to nematode in carrot, however, the marker validated by us in this study may or may not be linked to the *Mj* gene or linked to some other gene. Nematode response can be reduced by secondary metabolites produced by plants. The total phenol content of the accessions was estimated and found that it was slightly higher in immune line as compared to highly susceptible line.

The *Mj-1* resistance locus, which is carried by the European carrot lines 6526 B Sun2000 and 8542B Vilmorin, which have been differentiated with the STS-SQ1 marker, has the potential to introduce RKN resistance in susceptible carrot varieties (Kalia et al., 2019). Resistance to *M. javanica* was controlled by the monogenic dominant locus *Mj*-1 in the carrot line Br1252, which was derived from the variety 'Brasilia' (Simon et al., 2000). For *M. incognita* resistance, five non-overlapping QTLs were found in carrot F2 populations of Br1091 x HM1 and three in SFF x HM2, one each on chromosomes 1, 2, 4, 8, and 9 (Parsons et al., 2015). Co-dominant sequence tagged sites STS flanking markers linked to the RKN resistance locus were developed by Boiteux et al. (2004). Ali et al. (2014) identified the single *Mj*-2 gene, which confers resistance to *M. javanica*, in an Asiatic carrot genotype PI 652188 with incomplete dominance. Using molecular markers, they were able to determine that *Mj*-1 and *Mj*-2 are two distinct locations on chromosome 8. On sequencing, the primer ESSR0110 was found to exhibit 5 nucleotides difference between the resistant and susceptible parents. In the susceptible parent, the deletion of 5 nucleotides was observed in comparison to the resistant parent. Sequence analysis of ESSR0110 showed a 94.56 per cent homology with the endoglucanase 6 like protein in resistant parents contributing to nematode resistance.

Heterozygotes and homozygotes can thus be distinguished using codominant markers, allowing genotypes and allele frequencies to be determined at loci which is important for handling segregating material during resistance breeding for RKN. As a result, by crossing for nematode resistance and pyramiding the *Mj* gene with other important genes, Acc-88 could be used to introduce the resistance gene into cultivated carrot backgrounds. The above study also shows that using *Mj* gene specific markers is reliable for screening carrot germplasm against *M. incognita* and identifying long-term sources in carrot breeding programmes.

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Data Availability All data supporting the findings of this study are available within the paper and its Supplementary Information.

Declarations

Ethical approval This paper does not contain on human or animal participants.

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

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