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RESEARCH ARTICLE

Screening of carrot cultivars against root-knot nematode *Meloidogyne incognita*

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Abstract

To evaluate the 13 carrot cultivars against *Meloidogyne incognita* under green house conditions; different levels of susceptibility were observed in all the examined cultivars. Cultivar Golden Rosy was resistant to nematode attack with a low root gall index (1.2) while cultivar Kamini was found highly susceptible showing the highest root gall index (5.0). Similarly, the cultivars Rose Red, Noorie, Lali, Sindhuri, and Selection 80 were susceptible; the cultivars Pearl Red, Super Red, Surbhi, and Kamboj were moderately susceptible and the cultivars Desi Red and Red King were found moderately resistant against the nematode damage. A negative and significant correlation was found between root-knot nematode infestation and plant growth parameters.

Keywords Root-knot nematode · Resistance · Susceptibility · Screening

Introduction

The genus carrot (*Daucus carota* L.) is a member of the family—Apiaceae, one of the important vegetables commercially grown worldwide. Its edible part is fresh taproot which can be eaten as raw or cooked. In USA, the yield loss to carrot production due to root-knot nematodes was 45% (Widmer et al. 1999), whereas in Nilgiris of India it was approximately 36% (Anita and Selvaraj 2011).

Plant-parasitic nematodes are among the most destructive soil parasites which causes severe economic yield losses to agriculture crops, estimated to $US 118 billion per year in the world (Atkinson et al. 2012). These tiny parasites consist of ectoparasites as well as endoparasites which feed on the cytoplasm of living plant cells. Functionally, nematodes create metabolic sinks in infected plants by utilizing photo assimilates prepared to roots through metabolic activity of gall tissues. Among the plant parasitic nematodes, most of the vegetable crops are dangerously attacked by *Meloidogyne* species due to their polyphagy. In India, *Meloidogyne* spp. infestation is a limiting factor in the production of carrot and it is necessary to find sustainable control measures which engage the attention of many growers and researchers.

The nematode population in infested fields can be controlled by the use of several approaches as nematicides treatments, application of biocontrol agents (Vagelas and Gowen 2012), soil amendments (Asif et al. 2016, 2017), cultural practices in terms of crop rotation and the use of antagonistic plants (Hussain et al. 2011; Kayani et al. 2012).The application of chemical nematicides has been found to be a potent and effective means to control root-knot nematodes but they are losing their popularity due to high costs and detrimental effects on Man and environment (Taba et al. 2008).Among all the management tactics, utilization of resistant cultivars is cited as one of the primary, economically feasible and environmentally benign method to combat nematode menace (Mukhtar et al. 2013; Kinlock and Hinson 1972; Ansari et al. 2018).

The main objective of this work was to explore the magnitude of disease resistance in 13 carrot cultivars for root-knot nematode, *M. incognita* in greenhouse conditions which can be further used in nematode management programme.

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Materials and methods

Collection of carrot cultivar and nematode inoculum

Seeds of 13 carrot cultivars viz. Lali, Super Red, Rose Red, Red King, Desi Red, Golden Rosy, Noorie, Kamini, Sindhuri, Pearl Red, Kamboj, Surbhi, and Selection 80 were procured from agro company Chola Beej Bhandar, Aligarh (UP) India. The pure population of *M. incognita* was reared and maintained on eggplant in the greenhouse of Department of Botany, Aligarh Muslim University, Aligarh (India). The population was originally established from root-knot nematode infected roots collected from tomato and eggplant fields and identified by perineal patterns (Eisenback 1985). The Second stage juveniles (J2s) were obtained from hatched eggs by incubating handpicked egg masses in sterile distilled water at 27 ± 2 °C. The hatched juveniles were collected after every 24 h and distilled water was added. The concentration of freshly hatched second stage juveniles was standardized.

Screening carrot cultivar

Screening of carrot cultivars for resistance to *M. incognita* was performed in clay pots (30 cm diameter). Three sterilized seeds (1.0% NaOCl for 15 min) of each cultivar were sown in clay pots containing 2 kg autoclaved sandy loam soil and manure in the ratio 3:1. 2 weeks after germination, one healthy seedling of each cultivar was maintained in each pot including the control.

Inoculation technique

Each pot was inoculated with 3000 freshly hatched second stage juveniles (J2s) of *M. incognita* by making three holes around the roots at the same distance so that roots were not damaged. Then the nematode suspension having 3000 second stage juveniles (J2s) was poured into the holes. The experiment was carried out according to a completely randomized design (CRD) with eight replications for each cultivar. The experiment was performed two times in the year 2016 and 2017 with the same protocol. Uninoculated plants were used as control. The plants were watered when needed and handled with proper care to reduce the chances of error in sampling.

Data collection and observations

After 3 months from inoculation, roots of carrot cultivars were gently uprooted from the pots and washed in a basket filled with water to avoid egg mass losses during the entire process and observations were recorded. The assessment was carried out on the basis of following parameters such as shoot and root length; fresh weights, root gall index (RGI), egg masses/root, eggs/egg mass and population of nematodes/250 g of soil. The per cent reduction or increase in growth parameters were calculated using the following mathematical formula (Irshad et al. 2012; Mukhtar et al. 2014)

$$\%\ \text{Reduction or increase} = \frac{\text{Value in uninoculated} - \text{Value in inoculated}}{\text{Value in uninoculated}} \times 100.$$

Extraction of nematode population

To know the final *M. incognita* population in soil at the time of termination of the trials, nematodes were extracted as per Cobb's sieving and decanting technique (Cobb 1918) followed by modified Baermann's funnel technique (Southey 1986).

Categorization of cultivars for resistance

The carrot cultivars were categorized for the resistance/susceptibility by the degree of root-knot nematode infection i.e., root gall index which was recorded according to rating as proposed by Taylor and Sasser (1978).

Statistical analysis

The experimental data were analyzed statistically by one-way analysis of variance (ANOVA) using SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA). Mean values were statistically compared by Duncan's Multiple Range Test at $P \leq 0.05$.

Results and discussion

The foremost emphasis of our investigation is to produce healthy and good quality carrot as well as to control the root-knot nematode, *M. incognita* using eco-friendly tactics. The results revealed that all the tested cultivars of carrot behaved significantly different to nematode infestation i.e., root gall index, egg masses/root, eggs/egg mass, and nematode population (Table 1). The carrot cultivars categorized on the basis of the root gall index. Among the tested cultivars, Golden Rosy was found resistant against *M. incognita* whereas Kamini showed highly susceptible behavior. The cultivar Kamboj, Surbhi, Super Red and Pearl Red were found moderately susceptible (MS); Rose Red, Noorie, Lali, Sindhuri, and Selection 80 were found susceptible (S) to root-knot nematode. Two cultivars Red King and Desi Red with root gall indices (1.8 and 2.0) displayed moderately resistant (MR) reaction against the nematode (Table 1).

Table 1 Effect of root-knot nematode, *Meloidogyne incognita* on different cultivars of carrot in relation to nematode infestation parameters

Cultivars	Treatment	Egg masses/root	Eggs/egg mass	Nematode population/250 g soil	Root gall index	Reaction
Lali	Control	–	–	–	–	
	Inoculated	78^{d}	104^{cd}	1853^{cd}	4.0^{abc}	Susceptible
Super Red	Control	–	–	–	–	
	Inoculated	43^{gh}	79^{ef}	1226^{hi}	2.8^{cde}	Moderately susceptible
Rose Red	Control	–	–	–	–	
	Inoculated	67^{e}	85^{de}	1631^{f}	3.4^{bc}	Susceptible
Red King	Control	–	–	–	–	
	Inoculated	16^{i}	38^{i}	514^{l}	1.8^{ef}	Moderately resistant
Desi Red	Control	–	–	–	–	
	Inoculated	24^{h}	44^{h}	1043^{jk}	2.0^{def}	Moderately resistant
Golden Rosy	Control	–	–	–	–	
	Inoculated	6^{j}	37^{i}	371^{m}	1.2^{fg}	Resistant
Noorie	Control	–	–	–	–	
	Inoculated	73^{de}	93^{d}	1673^{de}	3.8^{abc}	Susceptible
Kamini	Control	–	–	–	–	
	Inoculated	131^{a}	137^{a}	2029^{a}	5.0^{a}	Highly susceptible
Sindhuri	Control	–	–	–	–	
	Inoculated	93^{c}	108^{c}	1898^{bc}	4.1^{ab}	Susceptible
Pearl Red	Control	–	–	–	–	
	Inoculated	45^{gh}	69^{gh}	1106^{j}	2.8^{cde}	Moderately susceptible
Kamboj	Control	–	–	–	–	
	Inoculated	56^{f}	74^{fg}	1380^{g}	3.2^{bcd}	Moderately susceptible
Surbhi	Control	–	–	–	–	
	Inoculated	48^{fg}	88^{de}	1309^{gh}	3.2^{bcd}	Moderately susceptible
Selection 80	Control	–	–	–	–	
	Inoculated	102^{b}	119^{b}	1935^{b}	4.2^{ab}	Susceptible

Each value is the mean of eight replicates. Means in each column with different letters denote significant differences according to Duncan's Multiple Range Test at $P \leq 0.05$. Data with same letters do not differ significantly

In addition to galls, the nematode also induced a significant increase in the incidence of other undesirable characteristics on the infected carrots. The most prominent was sudden and localized constrictions with twisting and distortion in carrot. The forking, twisting and cracking cause complete damage and distortion of roots in terms of their shape, length, weight, and external appearance (Figs. 1, 2). Besides the above symptoms, the formation of root hairs also affected the root length of cultivars.

From the roots of highly susceptible cultivar (Kamini), the highest number of egg masses was recorded (Table 1) which revealed that the maximum number of juveniles penetrated the roots and completed their life cycles in a successful manner. On the other hand, cultivar Golden Rosy allowed only a limited number of juveniles of *M. incognita* to penetrate the roots, leading to maturity as it is confirmed by the number of egg masses (Table 1). Comparatively larger number of egg masses was obtained from the susceptible carrot cultivars such as Noorie, Lali, Sindhuri, and Selection 80 (Table 1). Similarly, the maximum eggs were recorded on highly susceptible cultivar roots compared with the susceptible, moderately susceptible, moderately resistant and/or resistant cultivars (Table 1). Formation of galls over the roots of the susceptible cultivar is the primary symptom of root-knot nematode infection. According to Cousins and Walker (2000), root-knot nematode eggs developed poorly on resistant cultivars compared to susceptible ones.

It was concluded from this study that, the highly susceptible cultivar (Kamini) recorded the highest nematode population/250 g soil (2029) which was significantly different from all other tested cultivars (Table 1). All the tested cultivars showed variability in nematode population that can be attributed to the disease severity and number of root galls. The increased population density and number of the root galls might be due to penetration of a large number of nematodes favored by the highly susceptible cultivar and finally stabilized them to form the giant cells. Vovlas et al. (2005) and Nelson et al. (1990) stated

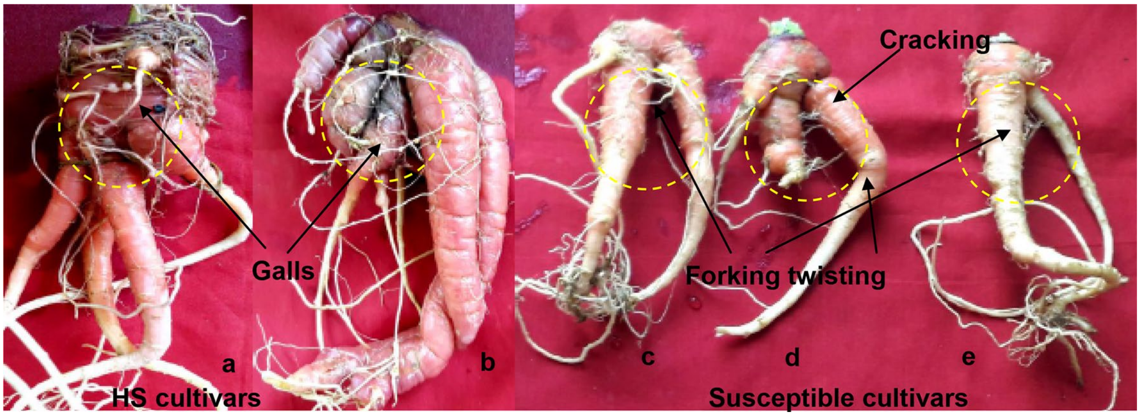

Fig. 1 Forking, galling and twisting in highly susceptible (HS) cultivar (**a**) and susceptible cultivars (**b**–**e**)

Fig. 2 Comparative effect of nematode infestation on the root lengths, root weights, and root shapes of 13 carrot cultivars named as, **a** Golden Rosy, **b** Red King, **c** Desi red, **d** Pearl Red, **e** Super Red, **f** Surbhi, **g** Kamboj, **h** Rose Red, **i** Noorie, **j** Lali, **k** Sindhuri, **l** Selection 80 and **m** Kamini

in their findings that number of root galls was directly proportional to the nematode population density where the juvenile population in the susceptible host attains their full development while lesser development was observed in resistant cultivar.

All the tested cultivars showed significant differences in reductions in shoot length, root length, shoot weight and root weight based on their resistance/susceptible behavior to the root-knot nematode as compared to their controls. The minimum reductions of 11.68% and 13.81% were observed in the shoot and root lengths of cultivar Golden Rosy followed by Red King (16.64% and 18.32%), whereas the maximum reductions of 47.60% and 50.26% were recorded in cultivar Kamini. A similar remark was made for the reductions in shoot and root fresh weights, where the minimum reductions of 16.57% and 13.34% were found in cultivar Golden Rosy followed by Red King (20.78% and 19.29%), whereas the maximum reductions of 44.70% and 49.16% were recorded in cultivar Kamini (Table 2). The reductions in growth parameters are the result of injured roots caused by the penetration or feeding of nematodes that ultimately reduced the efficiency of absorbing water by the root system. Our results are supported by the findings of Di Vito et al. (2004); Caveness and Ogunforowa (1985), they reported that *Meloidogyne* spp. cause infection in roots and induce the formation of nurse cells that favors the larger displacement of photosynthates towards infected roots while the above ground parts appears deficient.

Positive and significant relationships were occurred between root gall indices and the percent reductions in shoot length ($R^2=0.91$), root length ($R^2=0.89$), shoot fresh weight ($R^2=0.92$) and root fresh weight ($R^2=0.78$) (Fig. 3). In most of the studies researcher mostly focused on the nematode infestation parameters versus plant growth parameters. So, among the root-knot infestation parameters, the more emphasis was given to root gall index. According to Pearson's correlation coefficients, very strong and negative correlations were found between nematode infestation characters and almost all the plant growth characters of tested carrot cultivars (Table 3). Table 3 also revealed that a strong, negative and highly significant correlation was observed between root gall index and root length ($r=-0.97$ at $P=0.01$) followed by root fresh weight ($r=-0.84$ at $P=0.01$), shoot length ($r=-0.74$ at $P=0.01$) whereas the weak, negative and non-significant correlation was displayed by shoot fresh weight ($r=-0.20$).

Table 2 Effect of root-knot nematode, *Meloidogyne incognita* on different cultivars of carrot in relation to plant growth parameters

Cultivar	Treatment	Plant length (cm)				Fresh weight (g)			
		Shoot	% Reduction over control	Root	% Reduction over control	Shoot	% Reduction over control	Root	% Reduction over control
Lali	Control	57.2^{bcde}	31.68^{b}	17.5^{abc}	32.57^{bc}	80.5^{bcd}	31.60^{cd}	79.4^{ab}	29.21^{cd}
	Inoculated	39.1^{kl}		11.8^{ef}		55.1^{ghijk}		56.2^{hi}	
Super red	Control	62.4^{abcd}	21.90^{def}	18.6^{a}	22.58^{d}	84.6^{bc}	26.14^{def}	73.7^{abcde}	20.62^{e}
	Inoculated	$48.7^{efghijk}$		14.4^{cde}		62.5^{efghi}		58.5^{ghi}	
Rose red	Control	64.8^{abc}	27.29^{bcd}	18.4^{ab}	29.34^{ef}	97.4^{a}	30.09^{cde}	74.7^{abcd}	23.82^{de}
	Inoculated	47.1^{ghijk}		13.0^{de}		68.1^{def}		56.9^{hi}	
Red king	Control	57.9^{bcdef}	16.64^{fg}	19.1^{a}	18.32^{def}	70.8^{defg}	20.78^{fg}	76.7^{abc}	19.29^{ef}
	Inoculated	48.3^{fghijk}		15.6^{abcd}		56.1^{ghijk}		61.9^{efghi}	
Desi red	Control	$53.8^{cdefghi}$	18.25^{ef}	18.5^{ab}	19.45^{def}	70.8^{defg}	23.47^{ef}	81.5^{ab}	19.38^{ef}
	Inoculated	44.0^{ijkl}		14.9^{bcde}		54.2^{hijk}		65.7^{cdefgh}	
Golden Rosy	Control	58.9^{bcde}	11.68^{g}	18.1^{ab}	13.81^{f}	67.5^{efgh}	16.57^{g}	80.2^{ab}	13.34^{f}
	Inoculated	$52.0^{defghij}$		15.6^{abcd}		56.3^{hijk}		69.5^{bcdefg}	
Noorie	Control	55.4^{cdefg}	28.68^{bc}	17.8^{abc}	30.89^{c}	81.2^{bcd}	30.21^{cde}	84.3^{a}	25.74^{cde}
	Inoculated	39.5^{kl}		12.3^{def}		56.7^{fghijk}		62.6^{defghi}	
Kamini	Control	68.2^{ab}	47.60^{a}	18.9^{a}	50.26^{a}	87.7^{ab}	44.70^{a}	71.8^{bcdef}	49.16^{a}
	Inoculated	35.7^{l}		9.4^{f}		48.5^{k}		36.5^{k}	
Sindhuri	Control	63.6^{abc}	32.29^{b}	17.9^{abc}	34.07^{bc}	70.8^{defg}	34.94^{bc}	76.7^{abc}	31.55^{c}
	Inoculated	43.1^{jkl}		11.8^{ef}		46.0^{k}		52.5^{ij}	
Pearl red	Control	58.6^{bcdefg}	21.54^{def}	17.8^{abc}	21.91^{de}	72.5^{def}	25.04^{def}	75.8^{abc}	20.58^{e}
	Inoculated	44.4^{hijkl}		13.9^{de}		54.4^{hijk}		60.2^{fghi}	
Kamboj	Control	59.9^{bcd}	24.66^{cde}	18.0^{ab}	28.88^{c}	85.5^{b}	29.64^{cde}	74.7^{abcd}	22.48^{e}
	Inoculated	45.1^{hijkl}		12.8^{de}		60.2^{efghij}		57.9^{ghi}	
Surbhi	Control	71.7^{a}	22.61^{cdef}	17.9^{abc}	23.12^{d}	73.6^{cde}	28.66^{cde}	69.2^{bcdefg}	22.25^{e}
	Inoculated	$55.5^{cdefghi}$		13.7^{de}		52.5^{hijk}		53.8^{hij}	
Selection 80	Control	54.5^{cdefgh}	33.98^{b}	18.5^{ab}	37.29^{b}	79.3^{bcd}	39.50^{ab}	73.1^{abcde}	38.71^{b}
	Inoculated	35.9^{l}		11.6^{ef}		48.0^{jk}		44.8^{jk}	

Each value is the mean of eight replicates. Means in each column with different letters denote significant differences among inoculated and control according to Duncan's Multiple Range Test at $P \leq 0.05$. Data with same letters do not differ significantly

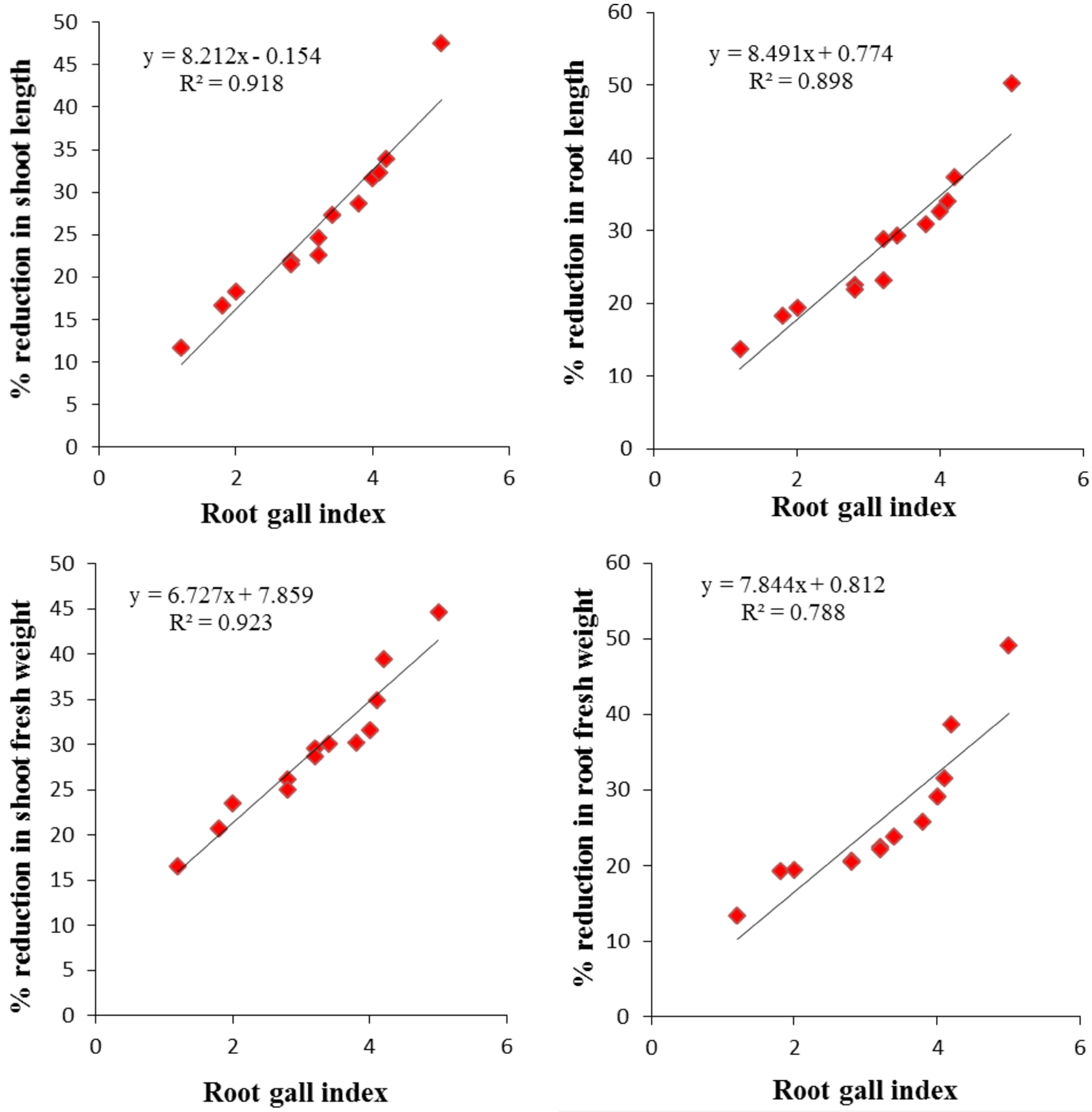

Fig. 3 Relationship between root gall index and % reduction in different growth parameters of carrot cultivars

Conclusion

The current investigation reported that the significant differences were observed among all tested cultivars against *M. incognita* in terms of growth factors and nematode infestation level. The least development and multiplication of juveniles were observed on resistant cultivar. The magnitude of plant infestation was comparatively less in moderately resistant cultivars. The moderately resistant cultivars also faced comparatively lesser nematode destruction and could be used in breeding programs to develop new nematode resistant cultivars.

The major symptoms such as forking, twisting and cracking completely damage the roots which cause a huge reduction in the healthy taproots and are a threat to the carrot industry. Due to excessive damage, the reduction in yield and market value of carrot greatly affects the economy of the country and this might be the subject of worry in the future with increasing population. So, keeping this view in mind, it can be concluded that an effective and environment-friendly approach for root-knot nematode management is required i.e., crop resistance, a cost-effective strategy for nematode management in subsistence agriculture. The exploration of resistance in the cultivars may be the most pronounced and environment-friendly strategy for reducing the nematode infestation and encouragement of disease-free carrot production.

Table 3 A matrix of Pearson's correlation coefficients between measured variables of carrot cultivars

Variables	SL	RL	SFW	RFW	RKI	EGMS	EGGS	NMP
SL	1							
RL	0.798**	1						
SFW	0.299	0.255	1					
RFW	0.606*	0.850**	0.335	1				
RKI	−0.744**	−0.970**	−0.208^{ns}	−0.849**	1			
EGMS	−0.798**	−0.983**	−0.275^{ns}	−0.891**	0.978**	1		
EGGS	−0.698**	−0.953**	−0.260^{ns}	−0.885**	0.979**	0.973**	1	
NMP	−0.747**	−0.926**	−0.138^{ns}	−0.742**	0.969**	0.937**	0.939**	1

SL shoot length, *RL* root length, *SFW* shoot fresh weight, *RFW* root fresh weight, *RGI* root gall index, *NMP* nematode population

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

^{ns}Correlation is not significant

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