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# Differential responses of certain field pea and cowpea cultivars to root-knot nematode, *Meloidogyne incognita* for commercial release

Mahmoud M. A. Youssef<sup>\*</sup>  and Wafaa M. A. El-Nagdi

## Abstract

**Background:** Legumes are considered staple foods for many countries in different areas of the world. Cultivation of nematode-resistant newly introduced field pea and cowpea cultivars is an important, cheap, and economical way to reduce damaging the nematode population that comes from improper use of nematicides and pesticides.

**Results:** The cultivars of two examined crops were planted in pots inoculated with second-stage juveniles of *M. incognita* under screen house conditions. The results proved significant differences ( $P \leq 0.05$ ) among the tested cultivars regarding their responses to *M. incognita*. Host resistance/susceptibility was rated on the basis of potential reproduction index (PRI). Host reaction was based on average percent reduction potentials of plant growth and yield. The combination between host resistance/susceptibility and host reaction rates was used to give clear and accurate determination to these cultivars. According to the later scale, field pea cultivars were grouped into three categories of highly susceptible (Consessa), moderately susceptible (Diacole), and intolerant cultivars (Cerdon, Samantha and 337), while cowpea cultivars were grouped into two categories of highly susceptible (Kafr El-Sheikh) and susceptible cultivars (Dokki and 331).

**Conclusions:** None of the tested cowpea and field pea cultivars was found to be resistant or immune to root-knot nematode, *M. incognita*. Subsequently, plant growth and yield of the tested cultivars seemed to be severely damaged by root-knot nematode that might be attributed to less nutrient uptake by field pea and cowpea roots due to nematode infection. Planting of highly susceptible or susceptible cultivar in progression with resistant cultivars may help in limiting or reducing these defects. This is keeping management processes and production more economical. Further studies are needed to evaluate more cultivars/accessions against root-knot nematode.

**Keywords:** Response, Field pea, Cowpea, Root-knot nematode, *Meloidogyne incognita*

## Background

Vegetables are important components of the daily diet of humans and are considered high-value cash crops for many growers in the world. They have been grown in Egypt for exporting and local consumption and have been reported to be infested by root-knot nematode, *Meloidogyne* spp. (Netscher and Sikora 1990; Montasser et al. 1992; Youssef 1993; Youssef and Abd-Elgawad 1993; Youssef et al. 1998; El-Nagdi Wafaa and Youssef

2004; Ibrahim 2006; Abd-Elgawad et al. 2007; Amponsah et al. 2008; Youssef and Korayem 2008; El-Nagdi Wafaa et al. 2015). Legumes are considered staple foods for many countries in different areas of the world. Cultivation of nematode-resistant cowpea cultivars is an important and economical way to reduce damaging nematode population that come from improper use of nematicides and pesticides, due to hazards caused by improper use of nematicides and pesticides (Swanson and Van Gundy 1984; Witcher and Ogle 1987; Patel et al. 1990; Heffes et al. 1992; Olowe 2007). Also, some studies focused on screening of pea cultivars for reaction against

\* Correspondence: [myoussef\\_2003@yahoo.com](mailto:myoussef_2003@yahoo.com)

Plant Pathology Department, Nematology Lab., National Research Centre, Dokki, Post Code, Cairo 12622, Egypt

root-knot nematode infection (Hadisoeganda and Sasser 1982; Santo and Ponti 1985; Montasser et al. 2017).

Therefore, this study was conducted to evaluate newly introduced cultivars of two legume crops, cowpea as summer crop and field pea as winter crop for their response to root-knot nematode, *Meloidogyne incognita*.

## Methods

In the first study, five field pea, *Pisum sativum* cultivars (Cerdon, Concessa, Diacole, Samantha and 337) newly introduced in Egypt were evaluated for host suitability to *M. incognita* under screen house conditions. Pots of 25-cm diameter were filled with 4 kg solarized sandy loam soil. Three seeds of each pea cultivar were sown in each pot. After germination, seedlings were thinned to 2 seedlings per pot. One week later, in January 20, 2017, each plant was inoculated with 600 of second-stage juveniles of root-knot nematode, *M. incognita*, obtained from pure stock culture. There were five replicates for each cultivar. Another set of pots for each cultivar served as control (non-inoculated check). All pots were arranged in a completely randomized block design on a bench in screen house at  $20 \pm ^\circ\text{C}$ . Plants were irrigated with water as needed. All pots were inoculated with Al-Okadean (containing nitrogen-fixing bacterium namely *Rhizobium legumisonarum*). Three months after inoculation, in April 13, 2017, plants were uprooted to record nematode parameters including number of eggmasses and galls and calculate them on the whole root system of pea. Number of nematode juveniles in soil was extracted by sieving and decanting methods (Barker 1985). Data on vegetative criteria of pea plants including shoot length, shoot fresh and dry weights, and fresh weight of roots were measured. Also, yield criteria including number and weight of pods were recorded. Number of nodules was recorded on roots of cowpea. Potential reproductive index (PRI) according to Montasser et al. (1992) was calculated by dividing final nematode population of a given cultivar on final population of the highest one multiplied

by 100. Where 21–40.9% PRI = less susceptible, 41–60.9% PRI = moderately susceptible, and 61–100% PRI = highly susceptible. Also, rate of nematode build up (Pf/Pi) was calculated for each cultivar. The percent reduction in shoot length, shoot fresh and dry weights, and fresh weight of roots, number and weight of pods, and number of nodules were recorded. Also, the percent reduction potential of plant growth and yield for each cultivar was calculated by dividing the percent reduction of plant growth or yield of a given cultivar on the percent reduction of the highest one (cultivar) multiplied by 100.

In the second study, three cultivars of cowpea, *Vigna unguiculata* (Kafr El-Sheikh, Dokki and 331) were evaluated for their response to *M. incognita*. One week after germination, in May 20, 2017, each of the 2 plants were inoculated with 500 second-stage juveniles of root-knot nematode, *M. incognita* obtained from pure stock culture. There were three replicates for each cultivar. Another set of pots for each cultivar served as control (non-inoculated check). All pots were inoculated with Okadean (containing nitrogen-fixing bacterium of the genus, *Bradyrhizobium* spp.). Three months after nematode inoculation, in August 19, 2017, plants were uprooted. The same procedures were applied as mentioned before.

## Statistical analysis

This study was carried out on the basis of analysis of variance (ANOVA) procedures. Duncan's multiple range test as reported by Snedecor and Cochran (1989) was used for comparison among treatments at 5% level of probability. *T* test was carried out for comparison between each pair of treatments. This was done by Computer Statistical Package (COSTAT) User Manual Version 3.03, Barkley Co.

## Results

In the first study, data presented in Tables 1, 2, 3, and 4 indicate the host response of field pea cultivars against

**Table 1** Host suitability of different cultivars of field pea in response to root-knot nematode, *Meloidogyne incognita* infection

Pea cultivars	Nematode parameters						Rate of host resistance/susceptibility
	J <sub>2</sub> in soil/pot	No. of galls	No. of eggmasses	Final nematode population	Build up (Pf/Pi <sup>1</sup> )	Potential reproduction index (PRI)	
Cerdon	2660 cd	13 ab	10 ab	2670 cd	4.45	36.9	LS
Concessa	7200 a	17 a	12 a	7212a	12.02	100.0	HS
Diacole	3340 b	14 ab	9 ab	3349b	5.58	46.4	MS
Samantha	2400 d	10 b	7 b	2407d	4.01	33.4	LS
337	2800 c	12 ab	8 b	2808c	4.68	39.0	LS

Values are means of 5 replicates

Values with the same letter(s) are not significantly different according to Duncan's multiple range test at  $P \leq 0.05$ . Potential reproduction index was calculated by dividing final population of a given cultivar on final population of the highest one multiplied by 100. Where 21–40.9% PRI = less susceptible (LS), 41–50% PRI = moderately susceptible (MS), and 51–100% PRI = highly susceptible (HS)

Pf = final population, Pi = initial population

**Table 2** Plant growth of different cultivars of field pea in response to root-knot nematode, *Meloidogyne incognita* infection

Pea cultivars	Plant growth criteria								Average total %Red. of plant growth criteria
	Shoot length (cm)		Fresh shoot weight (g)		Dry shoot weight (g)		Fresh root weight (g)		
	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	
Cerdon	42.7 a	41.3 a (3.3%)	9.7 a	8.3 b (14.4%)	1.9 a	1.8 a (2.5%)	2.7 a	2.3 a (14.8%)	9.4
Concessa	44.4 a	35.7 b (20.0%)	5.3 a	4.7 a (11.3%)	1.7 a	1.1 b (35.3%)	1.8 a	1.7 a (6.0%)	18.2
Diacole	40.3 a	31.5 b (22.0%)	7.7 a	7.1 a (8.0%)	2.4 a	2.2 a (9.1%)	2.4 a	1.9 b (21.0%)	15.0
Samantha	44.7 a	40.4 a (10.0%)	9.3 a	6.6 b (29.0%)	1.7 a	1.3 a (23.5%)	2.6 a	1.7 b (34.6%)	24.3
337	48.3 a	42.3 b (12.4%)	6.9 a	5.4 a (22.0)	1.6 a	1.2 a (25.0%)	2.9 a	1.7 b (41.4%)	25.2

Values are means of 5 replicates

Values with same letter for comparison between healthy and infected plants for each criterion at the same row are not significantly different according to *T* test at  $P \leq 0.05$ . Red.=Reduction

Values between parentheses indicate the percentages reduction of plant growth criteria

root-knot nematode, *M. incognita* infection. There were significant ( $P \leq 0.05$ ) differences among the tested cultivars as Concessa cultivar had the highest significant numbers of nematode criteria and final nematode population compared to those on Diacole and 337. However, Samantha had the least significant final nematode population. Host resistance/susceptibility was rated on the basis of the potential reproduction index (PRI) of root-knot nematode (Table 1) as follows: Concessa was rated as highly susceptible; Diacole was rated as a moderately susceptible and Cerdon, Samantha and 337 were rated as less susceptible against *M. incognita*. On the basis of nematode build up, pea cultivars behaved the same trend against nematode infection.

The percent reduction of plant growth and yield of pea were calculated for each cultivar (Tables 2 and 3). There were significant ( $P \leq 0.05$ ) differences between healthy and infected plants in most cases. Host reaction was based on the average percent reduction potentials of plant growth and yield (Table 4) as follows: Cerdon, Concessa, Samantha, and 337 were rated as highly affected by root-knot nematode, but Diacole was rated as moderately affected.

As for number of nodules, the various field pea cultivars were differed in their number of bacterial nodules as affected by infection by root nematode, *M. incognita*. The highest non-significant reduction of the number of nodules (25%) was recorded on pea cv. 337 followed by cv. Concessa (20%), when comparison was conducted between healthy and infected plants. However, the number of nodules was not reduced on the infected field pea cv. Samantha (Table 3).

On the basis of combination host resistance/susceptibility and host reaction, these cultivars were grouped into three categories (Table 4) as follows: Concessa was categorized as highly susceptible cultivars after combining its host susceptibility (highly susceptible to nematode) and host reaction (growth and yield were highly affected). Similarly, Diacole was categorized as a moderately susceptible cultivar because its growth and yield that were moderately affected by nematode. Cerdon, Samantha, and 337 were categorized as intolerant cultivars because their growth and yields were highly affected by nematode.

In the second study, the same rating rules for cowpea cultivars response to root-knot nematode were applied

**Table 3** Yield and number of nodules of field pea as affected by inoculation with root-knot nematode, *Meloidogyne incognita* infection

Pea cultivars	Yield parameters						No. of bacterial nodules		
	No. of pods			Weight of pods (g)			Healthy	Infected	%Red.
	Healthy	Infected	%Red.	Healthy	Infected	%Red.			
Cerdon	5 a	3 a	40.0	8.5 a	4.9 b	42.4	6 a	5 a	16.7
Concessa	3 a	2 a	33.0	3.3 a	2.8 a	15.2	5 a	4 a	20.0
Diacole	3 a	3 a	00.0	6.1 a	4.2 b	31.1	9 a	8 a	11.1
Samantha	3 a	2 a	33.3	3.1 a	2.4 a	22.6	3 a	3 a	00.0
337	4 a	2 a	50.0	5.5 a	2.2 b	60.0	4 a	3 a	25.0

Values are means of 5 replicates

Values with same letter for comparison between healthy and infected plants for each criterion at the same row are not significantly different according to *T* test at  $P \leq 0.05$ . Red.= Reduction

**Table 4** Host reaction, host resistance/susceptibility, and host category of field pea as affected by root-knot nematode, *Meloidogyne incognita* infection

Pea cultivars	Average %Red. of yield	%Red. Potential of yield (A)	Average %Red. of plant growth	%Red. potential of plant growth (B)	Average %Red. potentials of A+B	Host reaction*	Host resistance/susceptibility	Host category**
Cerdon	41.2	74.9	9.4	37.3	56.1	HA	LS	Intolerant
Concessa	24.1	43.8	18.2	72.2	58.0	HA	HS	HS
Diacole	15.6	28.4	15.0	60.0	44.2	MA	MS	MS
Samantha	28.0	50.9	24.3	96.4	73.7	HA	LS	Intolerant
337	55.0	100.0	25.2	100.0	100.0	HA	LS	Intolerant

\*20–50.9% average %Red. Potential = moderately affected (MA); 51–100% = highly affected (HA)

\*\*HA or MA +HS = highly susceptible (HA); MA+MS = moderately susceptible (MS); HA+LS = intolerant. Percent yield reduction potential was calculated by dividing the percentage reduction of plant growth or yield of a given cultivar on the percent reduction of the highest one (cultivar) multiplied by 100.

Red.= reduction

(Tables 5 and 6). All cultivars were rated highly susceptible (Kafr El-Sheikh cultivar) and moderately susceptible (Dokki and 331 cultivars). The statistical analysis proved that Kafr El-Sheikh cultivar had the highest significant ( $P \leq 0.05$ ) numbers of nematode criteria and final nematode population compared to two other cowpea cultivars which were equal. Regarding the reduction in plant growth and yield for each cultivar, there were significant differences between healthy and infected plants in most cases. On the basis of the combination between host resistance/susceptibility and host reaction, these cultivars were grouped into two categories (Table 7) as follows: Kafr El-Sheikh cultivar was categorized highly susceptible to nematode (as rate of host susceptibility) and highly affected by nematode (as host reaction). Dokki and 331 cultivars were categorized as susceptible to root-knot nematode because their moderate infection and highly affected by nematode.

In terms of nodule numbers, the three examined cowpea cultivars differed in their bacterial nodule numbers as negative infection by root nematode. The highest significant ( $P \leq 0.05$ ) reduction of the number of nodules (61.7%) was recorded on cowpea cv. Kafr El-Sheikh followed by cv. 331 (4.2%). No nodule reduction appeared on the infected cowpea cv. Dokki (Table 6).

## Discussion

None of the tested cowpea and field pea cultivars was found to be resistant or immune to root-knot nematode, *M. incognita*. Subsequently, plant growth and yield of the tested cultivars seemed to be severely affected by root-knot nematode that may be attributed to less nutrient uptake by field pea and cowpea roots due to the nematode infection (Heffes et al. 1992). The highly susceptible and susceptible cultivars indicate that root-knot nematodes have an extra ability to modulate cellular processes of the host plants to promote their parasitism forming giant cells to serve as nutrient source for nematode which led to making galls on the roots (Lewis 1987). These galls are usually formed by the accumulation of auxin and cytokinin in host to promote the division and enlargement of cortical cells of roots (Hutangura et al. 1999). Because there is no resistant or immune cultivars in this study, more cultivars/accessions of cowpea and pea should be evaluated in the future studies against *M. incognita* infection. Masefield (1958) reported that bacterial nodulation on roots may be affected by formation of nematode galls occupying the same space on the root system and causing nutrient deficiency in host plants. Also, Sharma and Tiagi (1990) stated that *M. incognita* reduced number of bacterial nodules on roots of pea and interfered with symbiotic

**Table 5** Host suitability of three cultivars of cowpea to root-knot nematode, *Meloidogyne incognita* infection

Cowpea cultivars	Nematode parameters							Host resistance/susceptibility
	No. of J <sub>2</sub> in soil/pot	No. of J <sub>2</sub> in roots	No. of galls	No. of eggmasses	Final nematode population	Build up (Pf/Pi <sup>1</sup> )	Potential reproduction index (PRI)	
Kafr El-Sheikh	11190 a	50 b	42 a	38 a	11278a	22.56	100.0	HS
Dokki	7200 b	53 b	35 b	16 b	7269 b	14.54	64.5	MS
331	4560 c	267 a	31 b	18 b	4845 b	9.68	43.0	MS

Values are means of 5 replicates

Values with the same letter(s) are not significantly different according to Duncan's multiple range test at  $P \leq 0.05$ . Potential reproduction index was calculated by dividing final population of a given cultivar on final population of the highest one multiplied by 100. Where 21–40.9%PRI = less susceptible (LS); 41–60.9%PRI = moderately susceptible (MS), and 51–100%PRI = highly susceptible (HS)

Pf/Pi<sup>1</sup> Pf= final population, Pi = initial population

**Table 6** Plant growth, yield, and number of nodules of three cultivars of cowpea as affected by root-knot nematode, *Meloidogyne incognita* infection

Plant growth and yield criteria	Cowpea cultivars								
	Kafr El-Sheikh			Dokki			331		
	Healthy	Infected	%Red.	Healthy	Infected	%Red.	Healthy	Infected	%Red.
Length (cm)	43.0 a	39.3 b	9.0	56.7 a	48.3 b	14.8	75.3 a	48.7 b	35.3
Fresh shoot weight (g)	82.9 a	65.2 b	21.4	111.9 a	80.7 b	27.9	104.1 a	85.6 b	17.8
Dry shoot weight (g)	31.6 a	19.9 b	37.0	26.9 a	22.5 b	16.4	25.6 a	21.2 b	17.2
Fresh root weight (g)	15.5 a	14.7 a	5.2	13.1 b	18.6 a	-	17.5 b	19.1 a	-
Dry root weight (g)	4.6 a	3.7 b	35.0	3.8 a	3.3 a	13.2	5.0 a	3.4 a	32.0
No. of pods	8.0 a	7.0 a	13.0	7.0 a	5.0 a	28.6	8.0 a	6.0 a	25.0
Weight of pods (g)	10.9 a	10.3 a	6.0	8.4 a	6.3 b	25.0	10.6 a	8.1 b	23.6
Weight of 100 seeds (g)	16.6 a	13.8 b	16.9	14.7 a	13.7 a	7.0	17.0 a	14.4 b	15.3
Average total percent Red. of plant growth and yield %	-	-	17.9	-	-	16.6	-	-	20.7
No. of nodules and %Red.	47.0 a	18.0 b	61.7	41.0 b	60.0 a	-	47.0 a	45.0a	4.3

Values are averages of 5 replicates

Values with the same letter for comparison between healthy and infected plants for each criterion are not significantly different according to *T* test at  $P \leq 0.05$ .

Red.= reduction

nitrogen fixation. On this basis, higher number of galls was recorded on cowpea cv. Kafr El-Sheikh which led to formation of less number of nodules on its root system. However, less number of galls were formed on roots of the two other cowpea cultivars. As a result, more nodules were formed on their roots. In this study, the susceptible cultivars were highly compatible with *M. incognita* infection due to lack of resistant gene(s). Therefore, the careful integration of cultivating other resistant genotypes of cowpea and pea in the cropping sequencing system is essential for reducing crop losses caused by *M. incognita*. This procedure may help in keeping management processes more economical. Resistant cowpea cultivars were intercropped with susceptible maize which resulted in limiting disease complexes associated with root-knot nematodes and *Pratylenchus saaeensis* compared to monoculture (Egunjobi et al. 1986). Also, it is advised that the use of a breeding program (embryo, tissue culture, cell protoplast fusion, and somatic hybridization supported with genetic engineering) can be utilized to incorporate resistant gene(s) of resistant cultivars in the susceptible cultivars (Young 1998).

Interestingly, field pea cvs. Cerdon, Samantha, and 337 were rated as less susceptible to nematode on the basis of PRI and their growth and yield were highly affected by nematode. As a result, they were categorized as intolerant. Also, field pea cvs. Diacole was rated as moderately susceptible to root-knot nematode, but because that its growth and yield were moderately affected; it was categorized as moderately susceptible to root-knot nematode. However, Concessa pea was considered highly susceptible based on the same rules.

The same determination rules were applied to cowpea cultivars. This was because cowpea cv. Dokki and 331 were rated as moderately susceptible to root-knot nematode, *M. incognita* on the basis of PRI, but because that their growth and yield were highly affected by nematode, therefore, they were considered, in general, highly susceptible cultivars, while, Kafr El-Sheikh cultivar was categorized as highly susceptible based on the same rule.

According to Ammati et al. (1986), soil type and size, soil temperature, and other abiotic and biotic factors may be responsible for different responses of the tested cultivars. On the other hand, plant susceptibility/resistance could be attributed to the dominant nematode

**Table 7** Host reaction, host resistance/ susceptibility and host category of three cowpea cultivars as affected by root-knot nematode, *Meloidogyne incognita* infection

Cowpea Cultivars	Average total %Red. of plant growth and yield	Average %Red. potential	Host reaction*	Host resistance/ susceptibility	Host category**
Kafr El-Sheikh	17.9	86.5	HA	HS	HS
Dokki	16.6	80.2	HA	MS	S
331	20.7	100.0	HA	MS	S

\*20–50.9%Red. potential = moderately affected (MA); 51–100% = highly affected (HA).

\*\*HA+MS = susceptible (S); HA+HS = highly susceptible (HS). Red.= reduction

species or strain, and physiological and chemical status of the plant (Cook and Evans 1987; Mohamed et al. 1999) and resistance genes that suppress one or more critical steps in nematode parasitism and their reproduction rate (Banora and Almaghrabi 2019).

## Conclusions

None of the tested cowpea and garden pea cultivars was found to be resistant or immune to root-knot nematode, *M. incognita*. Subsequently, plant growth and yield of the tested cultivars seemed to be severely damaged by root-knot nematode that decreased nutrient uptake by field pea and cowpea roots as a result of nematode infection. Planting the highly susceptible or susceptible cultivars in sequence with resistant cultivars can limit or reduce these defects. More cultivars/accessions of cowpea and pea should be screened against root-knot nematode in future studies.

## Abbreviations

ANOVA: Analysis of variance; COSTAT: Computer Statistical Package; HA: Highly affected; HS: Highly susceptible; LS: Less susceptible; MA: Moderately affected; MS: Moderately susceptible; PRI: Potential reproduction index; Red.: Reduction; S: Susceptible

## Authors' contributions

The first and second authors carried out the experiment and wrote the manuscript. Both authors read and approved the final manuscript.

## Funding

There is no funding.

## Availability of data and materials

The tested cultivars are available in the Agricultural Research Center and were bred in the laboratory.

## Ethics approval and consent to participate

Not applicable

## Consent for publication

Not applicable

## Competing interests

The authors declare that they have no competing interests.

Received: 13 September 2019 Accepted: 12 November 2019

Published online: 09 December 2019

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