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PLANT PROTECTION
Article

Quantification of resistance to *Meloidogyne incognita* in okra cultivars using linear and nonlinear analyses of growth parameters and nematode infestations

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ABSTRACT: One of the most effective and cost-efficient methods to manage plant parasitic nematodes is to use resistant cultivars. However, little information on the resistance of commercial okra cultivars grown in the country was available. Therefore, nine okra cultivars were evaluated for their comparative resistance against *Meloidogyne incognita*, the most damaging root-knot nematode. None of the tested okra cultivars showed high level of resistance. However, three cultivars viz. Pusa Swami, PB Selection and Green Star appeared as moderately resistant. Sabz Pari, Neelum and Tulsi were found moderately susceptible to the nematode. Two cultivars i.e. Ikra-1 and Ikra-2 were susceptible and Arka Anamika was highly susceptible. The okra cultivars differed significantly in their growth parameters depending on their response to *M. incognita*. Moderately resistant cultivars sustained little damage as compared to moderately susceptible or susceptible cultivars while the highly susceptible cultivar suffered the maximum damage by the nematode. Significant differences in the numbers of galls, eggmasses and reproductive factors of M. incognita on the nine okra cultivars were also observed. The highest numbers were found on Arka Anamika, followed by Ikra-1 and Ikra-2. The lowest numbers were found on the moderately resistant cultivars, followed by the moderately susceptible ones. High and positive correlations between the number of galls and eggmasses and the decreases in growth metrics were found using linear and non-linear regression analysis.

Key words: *Abelmoschus esculentus*, root-knot nematodes, reproductive factor, resistance, growth metrics.

INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) Moench), an important member of the Malvaceae family, is extensively cultivated in subtropical and tropical regions worldwide (Marin et al. 2017). It is a popular food source for humans, with its fruits containing significant amounts of carbohydrates, crude fibers, minerals, oil, proteins, and vitamins (Abd El-Kader et al. 2010). The top five okra-producing countries are India, Nigeria, Sudan, Mali, and Pakistan, which contribute 61.9%, 22.2%, 3.2%, 2.7%, and 1.3% respectively to the total global okra production. These countries also export okra to Afghanistan, the UAE, Iran, Malaysia, and several other nations.

In Pakistan, one of the world's top producers of okra, numerous varieties of okra are cultivated on thousands of acres throughout the year (Hussain and Mukhtar 2019). The total cultivated area for okra in Pakistan exceeds 14.5 thousand hectares, with an annual output of 109.3 thousand tons (Kassi et al. 2018). In the year 2019, okra was grown on approximately 1.55×10^4 hectares of land, resulting in a yield of 1.19×10^5 tons. However, the per-acre yield of okra in the country is relatively low compared to high-producing countries due to various abiotic and biotic constraints, such as diseases and insect pests (Hussain et al. 2016a; Mukhtar and Hussain 2019; Mustafa et al. 2023).

Among the gall-forming nematodes in okra, the most dangerous are the root-knot nematodes (*Meloidogyne* spp.) (Hussain et al. 2011, 2012; Mukhtar et al. 2013a). Root-knot nematodes are considered one of the top five most important phytopathogens worldwide and are at the forefront of the list of the world's ten most devastating and economically significant genera of phytopathogenic nematodes (Mukhtar et al. 2013b). Infestation by root-knot nematodes leads to slow and stunted growth, impaired root development, chlorosis, root galling, and wilting. In severe infestations, it often results in root destruction, poor growth, and reduced yield (Mukhtar et al. 2017). Root-knot nematodes are responsible for causing yield losses of up to 91% in crops and vegetables. In okra, *Meloidogyne* spp. have been found to cause yield losses of up to 27% (Sikora and Fernandez 2005).

Root-knot nematodes can also contribute to the formation of disease complexes (Kayani et al. 2018; Shahid et al. 2022, 2023). They can exacerbate the symptoms of damping off and vascular wilt diseases when they infect the roots of host plants along with *Pythium debaryanum, Rhizoctonia solani* and *Fusarium oxyspourm* (Naz et al. 2021; Singh and Singh 2020). Likewise, they can aggravate the occurrence and severity of bacterial wilt and crown gall induced by *Ralstonia solanacearum* and *Agrobacterium tumefaciens* respectively in various vegetable and fruit crops (Aslam et al. 2017 a, b, 2019; Asghar et al. 2020). Root-knot nematodes have also been implicated in breaking the resistance of fungal and bacterial wilt resistant cultivars in numerous crops and vegetables (Naz et al. 2021; Singh and Singh 2020). Moreover, they can reduce the tolerance to abiotic stresses in some plants as a consequence of their infections (Castillo et al. 2003).

A variety of strategies, such as biological, chemical, cultural, physical, and regulatory methods, are being employed for the management of phytopathogens and root-knot nematodes (Ahmed et al. 2021; Azeem et al. 2021; Khan et al. 2023a, b; Mehmodd et al. 2023; Shahbaz et al. 2023). However, each of these approaches has its own set of drawbacks (Afzal et al. 2023). Synthetic nematicides have emerged as one of the most popular and commonly used methods for root-knot nematode management. Nevertheless, the use of these harmful and potentially hazardous chemicals raises significant concerns in developed societies (Talpur et al. 2023). Additionally, nematicides are associated with limitations such as an unfavorable cost-effectiveness, limited accessibility for small-scale farmers, the development of nematicide resistance, environmental and groundwater pollution, as well as disturbance to soil microflora and fauna. These factors necessitate a reduction in reliance on these chemicals and the exploration of alternative approaches for effective root-knot nematode control.

Among the viable and practical alternatives for managing gall-forming nematodes instead of relying on harmful nematicides, the deployment of nematode-resistant or tolerant cultivars stands out as one of the most effective and cost-efficient methods (Hussain et al. 2014, 2016a). These resistant cultivars can also be integrated with other tools for nematode management (Mukhtar et al. 2013c, 2021). Furthermore, employing resistant cultivars to control plant parasitic nematodes can contribute to sustainable crop production, ensuring an ample food supply for the growing human population. Due to the limited and fragmented information on the resistance of commercial okra cultivars grown in the country, the current study was designed to evaluate the reproduction, growth, and resistance of different okra cultivars to *M. incognita* infection. The study also compared the performance of linear and nonlinear models in predicting galls and growth parameters of okra cultivars.

MATERIALS AND METHODS

Nematode inoculum

The root-knot nematode, *M. incognita*, used to assess the resistance of okra cultivars was extracted from the infested roots of okra. The nematode culture was initiated from a single eggmass on a highly root-knot nematode susceptible cultivar of tomato, "Money maker," and identified by its perineal pattern (Taylor and Nestscher 1974). The nematode was mass-produced on the same variety as described by Mukhtar et al. (2017). After the life cycle was completed, the eggmasses were collected from the infected roots and the eggs were extracted (Hussey and Barker 1973). The eggs were then processed through extraction trays and the juveniles were collected (Whitehead and Hemming 1965). The freshly hatched second-stage juveniles (J2s) were standardized and concentrated.

Okra germplasm

Seeds of seven okra cultivars viz. Arka Anamika, Sabz Pari, Tulsi, Neelum, Pusa Swami, Green Star and PB Selection were collected from the Federal Seed Certification and Registration Department, Islamabad while the seeds of two cultivars viz. Ikra-1, Ikra-2 were obtained from the National Agriculture Research Centre (NARC), Islamabad, Pakistan.

Screening assay

Okra cultivars were assessed for their comparative resistance against the root-knot nematode, *M. incognita*, using plastic pots of 20-cm-diameters containing 2.5 kg sterilized soil comprising 70% of sand, 22% silt, 8% clay with pH of 7.5. Three seeds of each okra cultivar were sown in each plastic pot. Ten days after emergence, single healthy seedling was kept in each pot from each test cultivar. The seedlings of each okra cultivar were then inoculated with 2500 freshly hatched J2s of *M. incognita*. The un-inoculated plants of each cultivar served as control of that cultivar. Each treatment (cultivar) was repeated tenfold and the experiment was performed twice. The pots of all the cultivars were arranged in a Completely Randomized Design under field conditions in an iron cage for 7 weeks. The pots were watered as per requirement.

Data collection

After the stipulated period of 7 weeks, the plants of each okra cultivar were carefully uprooted from the pots and their roots were cut from the shoots. The roots were carefully washed to remove the adhering soil and blotted dry. The fresh shoot and root weights and shoot and root lengths were recorded. The numbers of galls and eggmasses were counted on the roots of each cultivar under a stereomicroscope at a magnification of 4×. The eggs were extracted from the roots of individual plants (Hussey and Barker 1973) and the juveniles from the respective soil of each pot (Whitehead and Hemming 1965). The total number of eggs in the roots and nematodes in the soil constituted the final nematode population. The reproductive factor was calculated by dividing the final population by 2500. The percent increases and decreases in growth metrics were calculated relative to controls (Mukhtar et al. 2017). The level of resistance or susceptibility was assessed using the rating scale based on the number of galls (Taylor and Sasser 1978).

Statistical analysis

The trial was conducted twice. All the data were subjected to Analysis of Variance (ANOVA) using SPSS software. Homogeneity of variance of samples were calculated by Levene's statistic test at ($p<0.05$). Equality of means was checked by Welch test at ($p<0.05$). Comparison between the means were checked by Duncan's Multiple Range test at ($P=0.05$). Levene's test, Welch test and Duncan's Multiple Range test were interpreted in SPSS software. Regression analyses were used to check the relationship between growth metrics and number of galls and eggmasses.

RESULTS AND DISCUSSION

Response of okra cultivars to *M. incognita*

Okra cultivars showed variations in their response to *M. incognita* on the basis of number of galls. None of the tested okra cultivars showed high level of resistance (highly resistant or resistant). However, three cultivars viz. Pusa Swami, PB Selection and Green Star showed moderately resistant reaction. Sabz Pari, Neelum and Tulsi were found moderately susceptible to the nematode. Two cultivars i.e. Ikra-1 and Ikra-2 showed susceptible response while Arka Anamika was found the highly susceptible cultivar (Table 1).

Table 1. Response of okra cultivars to *Meloidogyne incognita*.

Rating Scale	No. of galls	Cultivars	Response to *M. incognita*
0	0	-	Highly Resistant
1	1-2	-	Resistant
2	3-10	Pusa Swami, PB Selection, Green Star	Moderately Resistant
3	11-30	Sabz Pari, Neelum, Tulsi	Moderately susceptible
4	31-100	Ikra-1 and Ikra-2	Susceptible
5	< 100	Arka Anamika	Highly Susceptible

Source: Elaborated by the authors.

Comparison of growth variables among okra cultivars

Okra cultivars, with different responses to *M. incognita*, exhibited significant differences in growth parameters. The cultivars varied significantly in relation to the fresh weight of roots. The increases in the weights of roots were the minimum in the case of moderately resistant cultivars (2.55-4.85%) followed by moderately susceptible cultivars (5.68-6.65%). The maximum increase in root weight was recorded with the highly susceptible cultivar (33.78%) followed by susceptible cultivars ranging from 11.76 to 14.40%. Similarly, the moderately resistant cultivars showed the minimum reductions in fresh shoot weights (2.99-5.08%) as compared to moderately susceptible cultivars (7.39-9.07%). The highly susceptible cultivar showed the maximum reduction of 48.12% followed by susceptible cultivars (Table 2). Similar trends were found with root and shoot lengths (Table 3). It is clear that moderately resistant cultivars sustained little damage by the nematode as compared to moderately susceptible or susceptible cultivars while the highly susceptible cultivar suffered the maximum damage. The decreases or increases in these growth parameters were in the order MR < MS < S < HS.

Table 2. Effect of *Meloidogyne incognita* on fresh root and shoot weights of okra cultivars.

Cultivar	Fresh root weight			Fresh shoot weight		
	Uninoculated	Inoculated	% Increase	Uninoculated	Inoculated	% Decrease
PB Selection	4.74 ± 0.20b	4.98 ± 0.14a	4.85 ± 2.68a	32.34 ± 15.28a	30.85 ± 14.32c	2.99 ± 4.33a
Green Star	4.88 ± 0.14c	5.01 ± 0.12a	2.55 ± 0.97a	35.12 ± 0.47a	33.33 ± 0.59cd	5.08 ± 2.06ab
Neelum	5.19 ± 0.02f	5.51 ± 0.31b	5.68 ± 4.90a	34.80 ± 0.50a	32.22 ± 0.43cd	7.39 ± 2.09bc
Tulsi	5.16 ± 0.06ef	5.54 ± 0.12b	6.65 ± 3.15a	35.09 ± 0.66a	32.09 ± 0.55cd	8.53 ± 1.60c
Arka Anamika	4.49 ± 0.10a	6.78 ± 0.27d	33.78 ± 1.85c	32.03 ± 0.76a	16.61 ± 0.59a	48.12 ± 2.01e
Pusa Swami	4.92 ± 0.09cd	5.17 ± 0.15a	4.74 ± 2.66a	39.41 ± 0.43a	38.04 ± 0.56d	3.47 ± 0.76a
Sabz pari	5.17 ± 0.03f	5.50 ± 0.11b	5.99 ± 1.78a	34.41 ± 0.42a	31.29 ± 0.74cd	9.07 ± 2.00c
Irka-1	5.03 ± 0.09de	5.88 ± 0.13c	14.40 ± 2.55b	35.05 ± 0.80a	24.06 ± 0.47b	31.34 ± 0.72d
Irka-2	4.99 ± 0.03cd	5.66 ± 0.27bc	11.76 ± 4.28b	34.80 0.40a	23.84 ± 0.75b	31.50 ± 1.86d
ANOVA	F= 37.30 Df = 8,36 P = 0.000	F= 24.69 Df = 8,36 P= 0.000	F = 51.74 Df = 8,36 P = 0.000	F = 0.84 Df = 8,36 P = 0.572	F= 9.01 Df = 8,36 P= 0.000	F= 280.82 Df = 8,36 P = 0.000
Levene statistic	F = 3.62 Df = 8,36 P = 0.003	F = 5.89 Df = 8,36 P = 0.000	F = 2.52 Df = 8,36 P = 0.027	F = 6.50 Df = 8,36 P = 0.000	F = 6.42 Df = 8,36 P = 0.000	F = 1.49 Df = 8,36 P= 0.192
Welch test	F = 28.62 Df = 8,14.92 P = 0.000	F = 34.60 Df = 8,14.72 P = 0.000	F = 11.20 Df = 8,14.69 P = 0.000	F = 54.57 Df = 8,14.92 P = 0.000	F = 469.10 Df = 8,14.93 P = 0.000	F = 525.5 Df = 8,14.71 P= 0.000

Values (± SD) are means of ten replicates. At $P < 0.05$, Levene's test is significant (Variances of statistical data are not equal). At $P < 0.05$, Welch test (Robust test of equality of means) is significant; it rejects the null hypothesis of equality of means. Same letter in every column of means indicate that there is no significant difference among means according to Duncan's Multiple Range test at P=0.05 Source: Elaborated by the authors.

Table 3. Effect of *Meloidogyne incognita* on root and shoot lengths of okra cultivars.

Cultivar	Root length			Shoot length		
	Uninoculated	Inoculated	% Decrease	Uninoculated	Inoculated	% Decrease
PB Selection	25.12 ± 0.80f	24.36 ± 0.44g	2.98 ± 1.55a	51.56 ± 0.65f	49.34 ± 0.42h	4.30 ± 1.17bc
Green Star	22.03 ± 0.67de	21.13 ± 0.72f	4.0 ± 5.10a	45.70 ± 0.40b	44.13 ± 0.55e	3.43 ± 0.83b
Neelum	20.89 ± 0.80bc	20.18 ± 0.64e	3.22 ± 5.76a	52.22 ± 0.37g	49.31 ± 0.45h	5.57 ± 0.37d
Tulsi	21.47 ± 0.66cd	20.41 ± 0.59e	4.83 ± 5.14a	43.23 ± 0.44a	41.29 ±0.28c	4.48 ± 0.42bc
Arka Anamika	22.85 ± 0.23e	13.14 ± 0.40a	42.47 ± 1.64d	47.29 ± 0.41c	35.23 ± 0.43a	25.49 ± 0.39g
Pusa Swami	22.56 ± 0.42e	21.74 ± 0.30f	3.59 ± 2.78a	49.26 ± 0.43d	48.29 ±0.56g	1.98 ± 0.58a
Sabz pari	19.63 ± 0.36a	18.64 ± 0.40d	4.99 ± 2.82a	49.85 ± 0.15e	47.26 ± 0.42f	5.19 ± 0.99cd
Irka-1	22.76 ± 0.42e	17.09 ± 0.39c	24.91 ± 1.88c	46.99 ± 0.09c	40.19 ± 0.39b	14.46 ± 0.91f
Irka-2	20.16 ± 0.73b	16.18 ± 0.43b	19.71 ± 1.02b	49.21 ± 0.41d	42.60 ± 0.36d	13.42 ± 1.06e
ANOVA	F= 37.55 Df = 8,36 P= 0.000	F = 224.41 Df = 8,36 P = 0.000	F = 78.29 Df = 8,36 P = 0.000	F = 242.85 Df = 8,36 P = 0.572	F = 602.61 Df = 8,36 P = 0.000	F = 452.81 Df = 8,36 P = 0.000
Levene statistic	F= 1.73 Df = 8,36 P = 0.123	F = 0.856 Df = 8,36 P = 0.561	F = 2.320 Df = 8,36 P = 0.040	F = 1.86 Df = 8,36 P = 0.097	F = 0.543 Df = 8,36 P = 0.816	F = 1.78 Df = 8,36 P = 0.113
Welch test	F = 40.34 Df = 8,14.77 P= 0.000	F = 247.32 Df = 8,14.94 P = 0.000	F = 189.52 Df = 8,14.74 P = 0.000	F = 267.12 Df = 8,14.40 P = 0.000	F = 481.98 Df = 8,14.95 P = 0.000	F = 1033.58 Df = 8,14.84 P = 0.000

Values (± SD) are means of ten replicates. At $P < 0.05$, Levene's test is significant (Variances of statistical data are not equal). At $P < 0.05$, Welch test (Robust test of equality of means) is significant; it rejects the null hypothesis of equality of means. Same letter in every column of means indicate that there is no significant difference among means according to Duncan's Multiple Range test at P=0.05 Source: Elaborated by the authors.

Nematode infestations

Significant differences were observed in numbers of galls, eggmasses and reproductive factors of *M. incognita* on nine okra cultivars (Table 4). The maximum numbers of galls, eggmasses and reproductive factors were found on Arka Anamika, the highly susceptible okra cultivar followed by Ikra-1 and Ikra-2 showing susceptible reaction. However, the minimum numbers were observed on moderately resistant cultivars (Pusa Swami, PB Selection and Green Star) followed by moderately susceptible ones (Table 4).

Table 4. Numbers of galls and eggmasses produced by *Meloidogyne incognita* on different okra cultivars.

Cultivar	Number of galls	Number of eggmasses	Reproductive factor
PB Selection	12.0 ± 0.70a	8 ± 1.58a	1.20 ± 0.15a
Green Star	18.8 ± 1.92b	13 ± 1.00b	1.53 ± 0.40b
Neelum	32.4 ± 1.14c	25 ± 1.30c	2.83 ± 0.33d
Tulsi	35.8 ± 3.11c	24 ± 0.70c	2.28 ± 0.20c
Arka Anamika	110.8 ± 5.97e	99 ± 1.58f	5.13 ± 0.18f
Pusa Swami	13.2 ± 1.92a	9.2 ± 0.83a	1.40 ± 0.11ab
Sabz pari	33.2 ± 2.77c	25.2 ± 0.83c	2.21 ± 0.20c
Irka-1	80.6 ± 4.03d	73 ± 1.58d	4.02 ± 0.02e
Irka-2	79.6 ± 9.63d	77.6 ± 3.91e	4.24 ± 0.16e
ANOVA	F = 326.50 Df = 8,36 P = 0.000	F = 1921.34 Df = 8,36 P = 0.000	F = 192.94 Df = 8,36 P = 0.000
Levene statistic	F= 8.96 Df = 8,36 P = 0.000	F = 6.26 Df = 8,36 P = 0.000	F = 3.31 Df = 8,36 P = 0.006
Welch test	F = 378.84 Df = 8,14.49 P = 0.000	F = 1855.42 Df = 8,14.88 P = 0.000	F = 460.24 Df = 8,13.74 P = 0.000

Values (±SD) are means of ten replicates. At $P < 0.05$, Levene's test is significant (Variances of statistical data are not equal). At $P < 0.05$, Welch test (Robust test of equality of means) is significant; it rejects the null hypothesis of equality of means. Same letter in every column of means indicate that there is no significant difference among means according to Duncan's Multiple Range test at P=0.05. Source: Elaborated by the authors.

Relationships between growth variables and numbers of galls and eggmasses

The R^2 values of linear and non-linear regression analyses showed positive and significant relationships between number of galls and increases in fresh root weights and reductions in fresh shoot weights, root and shoot lengths (Figs. 1 and 2). Likewise, R^2 values of linear regression analysis showed positive and significant relationships between number of eggmasses and increases and decreases in above mentioned growth parameters (Figs. 3 and 4).

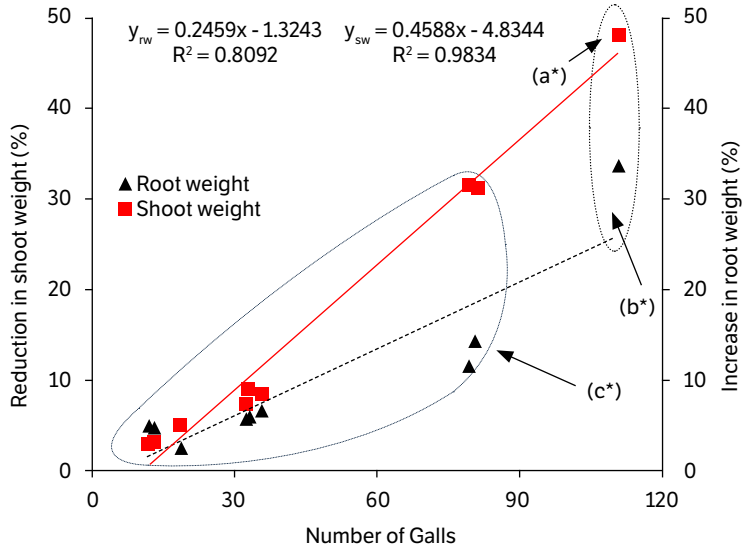

Figure 1. Linear Relationship between number of galls and % increase in fresh root weight and % reduction in fresh shoot weight (a) represents maximum reductions in shoot weight and (b) represents maximum increase in root weight (c) denotes different levels of reductions in shoot weight and increase in root weight. (----) and (——) linear trend lines show increase in root weight and decrease in shoot weight respectively. Y_{frw} and Y_{fsw} show regression equations of increase in fresh root weight and decrease in fresh shoot weight.

Source: Elaborated by the authors.

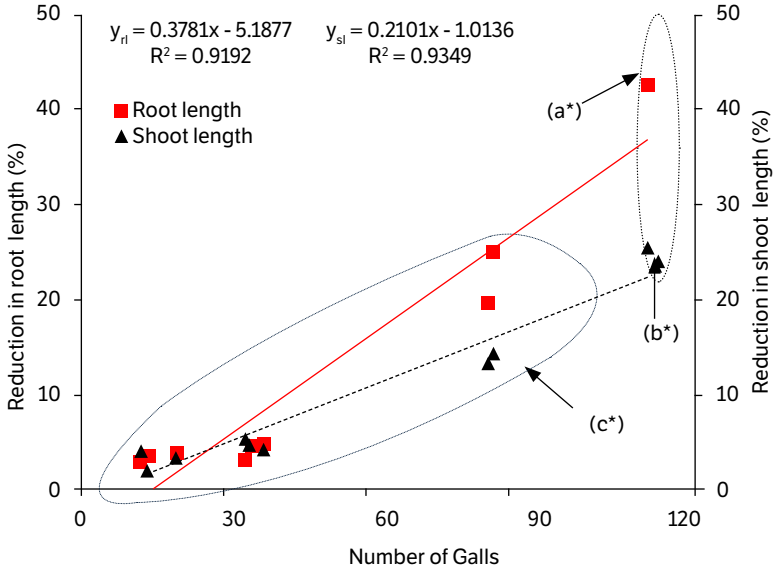

Figure 2. Linear Relationship between number of galls and % reductions in root length and shoot length (a) and (b) represent maximum reductions in root length and shoot length (c) denotes different levels of reductions in root length and shoot length. (——) and (----) linear trend lines show decrease in root length and shoot length respectively. Y_{rl} and Y_{sl} show regression equations of decrease in root length and shoot lengths.

Source: Elaborated by the authors.

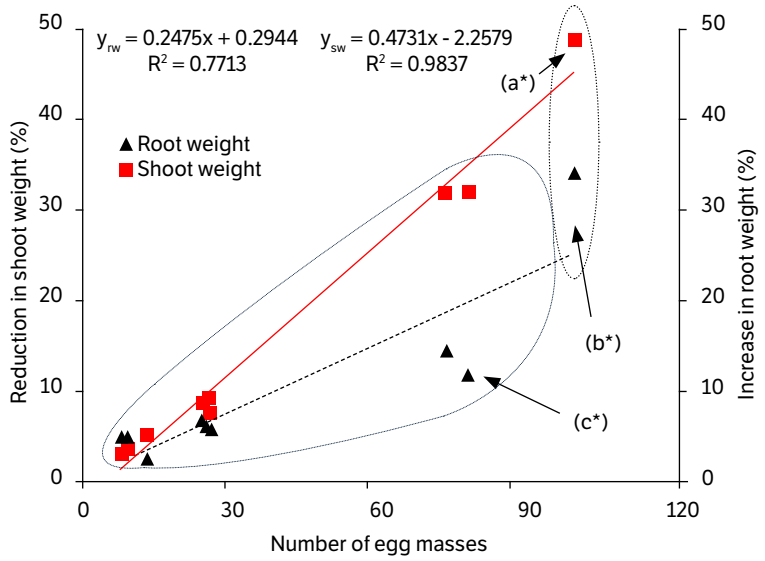

Figure 3. Linear Relationship between number of eggmasses and % increase in fresh root weight and % reduction in fresh shoot weight (a) represents maximum reductions in shoot weight and (b) represents maximum increase in root weight (c) denotes different levels of reductions in shoot weight and increase in root weight. (----) and (——) linear trend lines show increase in root weight and decrease in shoot weight respectively. Y_{frw} and Y_{fsw} show regression equations of increase in root weight and decrease in shoot weight.

Source: Elaborated by the authors.

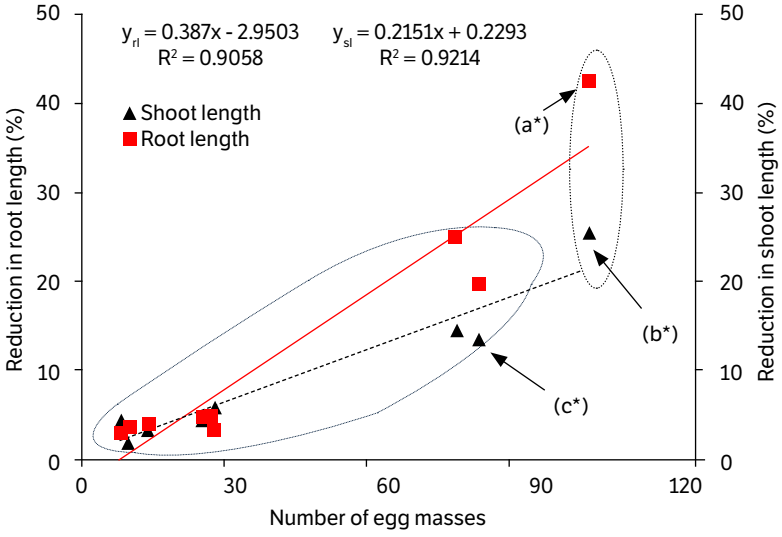

Figure 4. Linear Relationship between number of eggmasses and % reductions in root length and shoot length (a) and (b) represent maximum reductions in root length and shoot length (c) denotes different levels of reductions in root length and shoot length. (——) and (----) linear trend lines show decrease in root length and shoot length respectively. Y_{rl} and Y_{sl} show regression equations of decrease in root length and shoot length.

Source: Elaborated by the authors.

The use of resistant or tolerant cultivars for nematode management is gaining wider acceptance among growers due to the desire to avoid the harmful effects of nematicides. Numerous researchers have assessed different okra cultivars, lines, accessions, or rootstocks against root-knot nematodes and identified sources that showed resistance to the nematode (Hussain et al. 2016a; Kandouh et al. 2019; Marin et al. 2017; Mukhtar et al. 2014; Nacar and Ozarslandan 2021; Odeyemi et al. 2016). In the present study, we evaluated the resistance of okra cultivars to *M. incognita* and compared them in terms of reductions in growth metrics and nematode reproduction. The okra cultivars exhibited significant variations in their degrees of resistance against the nematode, as well as variations in growth parameter reductions. We found a significant positive relationship between the number of galls and eggmasses, and the decreases in growth metrics (Figs. 1, 2, 3, and 4).

The nematode formed galls and multiplied differently on nine okra cultivars. The variations in gall formation and nematode multiplication among different okra cultivars can be attributed to differences in their genetic makeup or the presence of genes that confer resistance or susceptibility (Trudgill 1991; Ye et al. 2017). When genes conferring resistance are present, the nematode is unable to infect or reproduce on non-host crops or resistant cultivars of the crop due to the absence of traits necessary for successful infection and parasitism.

To successfully establish feeding sites in the vascular tissues, the juveniles must be attracted to roots, enter into the epidermis and migrate through the cortical regions to reach the vascular bundles. The development of nurse cell system i.e. giant cells (feeding sites) in the vascular tissues is essential for continuous and incessant supply of nutrition for various nematode activities i.e. molting, development and production of eggs (Castillo et al. 2001; Di Vito et al. 2004; Favery et al. 2016). Resistant genes, in hosts or cultivars resistant to nematodes, involved in disrupting one or more essential stages required for nematodes to parasitize successfully are either stopped or suppressed (Ye et al. 2017).

The level of resistance or susceptibility to plant pathogenic nematodes is also determined by the reproduction potential of the latter on the hosts. In susceptible hosts, the nematodes penetrate, grow, develop and multiply normally and to the maximum degrees (Hussain et al. 2014). The current study revealed considerable differences in the rates of multiplication of *M. incognita* on all the cultivars of okra. The rate of multiplication of the nematode was found to be lower on moderately resistant cultivars as compared to moderately susceptible or susceptible cultivars. The rate of nematode multiplication was the highest on the highly susceptible cultivar Arka Anamika. The lower reproduction factor of *M. incognita* on moderately resistant cultivars is due to the decreased numbers of juveniles that penetrated the roots which subsequently suppressed the juveniles' development and resulted in lower multiplication rate (Hussain et al. 2016a). The highest multiplication of *M. incognita* was observed on the highly susceptible cultivar which revealed that the maximum number of juveniles entered into its roots and succeeded in completing their life cycles and produced the maximum eggmasses. Contrarily, moderately resistant cultivars only permitted a few numbers of *M. incognita* juveniles to enter the roots and complete their life cycles resulting in less production of eggmasses (Table 4).

Sasser (1954) reported that, the rate of invasion of roots of resistant plants by nematodes was not as rapid as was found in susceptible plants. Similar findings were made by Dropkin and Nelson (1960) who found that the number of completely developed nematodes in resistant cultivars is considerably lower than those in susceptible ones. The reduced invasion of resistant plants by juveniles is either caused by the hypersensitivity of plants and/or as a result of retarded growth of nematodes in the resistant plants resultantly there are few fully developed larvae (Dropkin 1969). The type of host has also been observed to influence juvenile development (Davide 1980). In susceptible hosts, juveniles grow fully and normally but in the case of resistant plants, the development of larvae is retarded, sluggish and delayed (Nelson et al. 1990).

Morphological alterations in the host viz. accumulation of lignin in the roots, reinforcement of cell wall, synthesis of toxic compounds, suppression of nematode feeding and resistance protein accumulation and stimulation of some transcription factors contribute to the resistance response to the nematode pests. In addition to structural modifications, many biochemical and molecular changes are also triggered in resistant cultivars following infection by nematodes. In the roots of resistant cultivars, after nematode infection, the activities of resistance associated enzymes viz. phenylalanine ammonia lyase and peroxidase increased greatly as compared to the susceptible ones (Ye et al. 2017).

Okra cultivars also differed significantly in their responses to *M. incognita* in terms of decreases in growth metrics. The nematode caused less damage to the moderately resistant cultivars while the greatest damage occurred to the highly susceptible cultivar. The decreases in growth metrics are due to injuries caused to roots as a result of nematode penetration and/or feeding, which impaired membrane permeability and badly affected the ability of roots to uptake water, nutrients and minerals. The infection of *Meloidogyne* spp. in the roots of plants causes development of nurse cell system (giant cells) in the stellar region for continuous supply of nutrition to the females thereby the xylem tissues are extensively disrupted which also hinders absorption of water (Castillo et al. 2001; Di Vito et al. 2004). In root-knot infected plants, the shift of photosynthates becomes more towards the infected roots and the foliar parts remain deficient or receive insufficient supplies of nutrition (Di Vito et al., 2004; Wyss, 2002). The inadequate supplies of nutrition, water, photosynthetic products, and energy negatively affect the growth and development of leaf tissue and its constituents, particularly chlorophyll pigments (Favery et al. 2016). The unthrifty growth of above ground parts results in reduced productivity.

The linear and non-linear prediction equations were developed to compare the findings of the current investigation with those of an earlier study. The earlier study considered various cultivars of okra to establish a linear relationship between the number of galls and the reduction in growth matrices (Hussain et al. 2016a). However, for the purpose of non-linear rational comparison, only four cultivars, namely Pusa Swami, Ikra-1, Ikra-2, and Arka Anamika, from the previous study (Hussain et al. 2016b), were chosen as they were comparable to the current study.

The linear fittings proposed in this study provided a more precise assessment of the resistance of okra cultivars to *M. incognita* based on growth parameters (Figs. 1, 2, 3 and 4). The correlation coefficients (R^2) for this study, representing the percentage reduction in growth parameters such as shoot weight, shoot length, and root length caused by *M. incognita*, were found to be 0.98, 0.93, and 0.91, respectively (Table 5 and Fig. 5). Conversely, in the previous study, the correlation coefficients (R^2) for the percentage reduction in the same parameters were found to be 0.95, 0.81, and 0.89, respectively (Table 5 and Fig. 5). Similarly, the non-linear fittings designed in this study can also assess the resistance of okra cultivars to *M. incognita* based on growth parameters better than the linear model designed in the previous study (Figs. 6 and 7). The linear and non-linear equations estimated from both studies are shown in (Table 6). As suggested by previous studies, the correlation coefficient higher than 0.8 demonstrate excellent prediction capability of an equation (Lee et al. 2023; Nawaz et al. 2022). Based on this rationale, the results confirm the improved prediction ability of the proposed correlations.

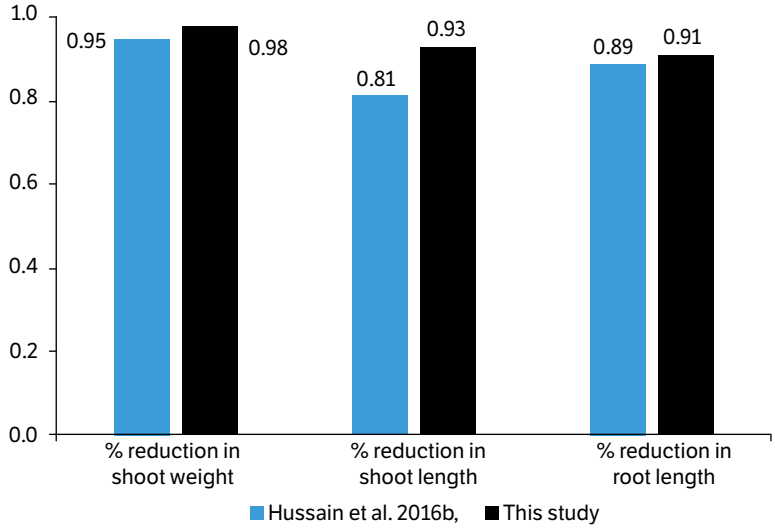

Figure 5. Comparison of the R^2 values of linear model proposed between the number of galls and growth parameters from previous and current study.

Source: Elaborated by the authors.

Table 5. Calculated R^2 values between the number of galls and growth parameters.

Parameters	Accuracy in statistical analysis (R^2)	
	Hussain et al., 2016	This study
Linear correlations		
Reduction shoot length	0.81	0.93
Shoot weight	0.95	0.98
Root length	0.89	0.91
Non-linear correlations		
Reduction shoot length	0.98	0.99
Shoot weight	0.97	0.99
Root length	0.99	0.98

Source: Elaborated by the authors.

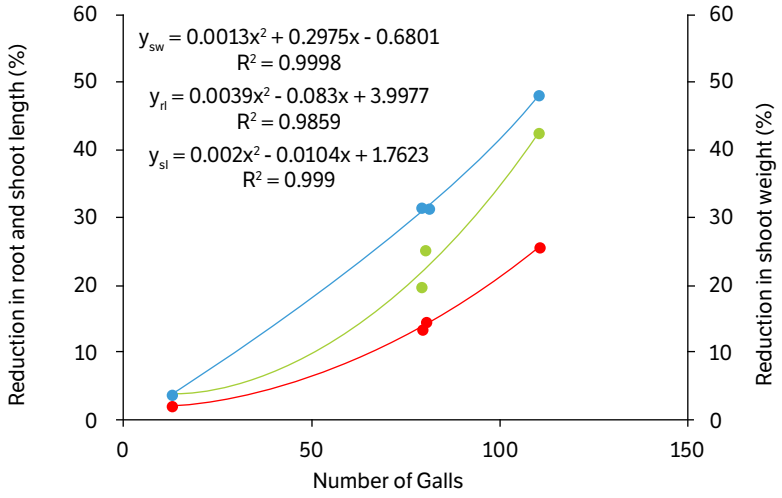

Figure 6. Non-linear relationships between number of galls and % reduction in root and shoot length and shoot weight in present study.

Source: Elaborated by the authors.

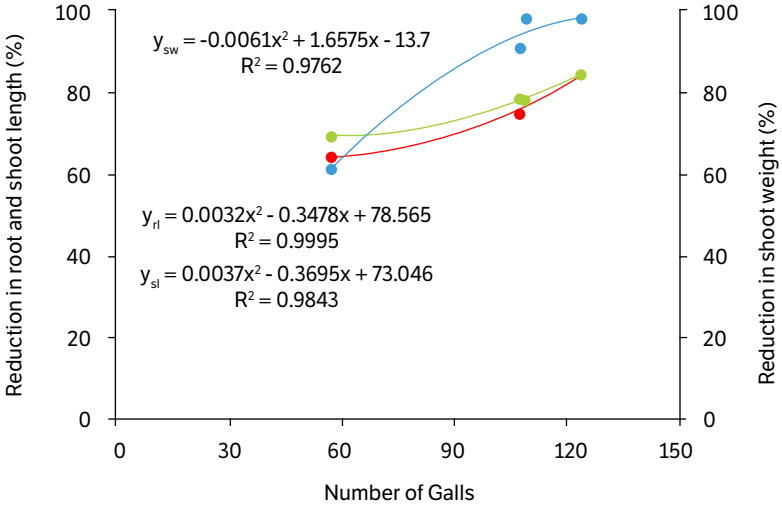

Figure 7. Non-linear relationships between number of galls and % reduction in root and shoot length and shoot weight in the previous study.

Source: Elaborated by the authors.

Table. 6. Estimated linear and non-linear equations between number of galls and growth parameters.

Parameters	Hussain et al., 2016	This study
Linear correlations		
Reduction shoot length	$R_{sl} = 11.465N - 28.834$	$R_{sl} = 0.2101N - 1.0136$
Shoot weight	$R_{sw} = 14.007N - 48.63$	$R_{sw} = 0.4588N - 4.8344$
Root length	$R_{rl} = 10.633N - 23.344$	$R_{rl} = 0.3781N - 5.1877$
Non-linear correlations		
Reduction shoot length	$R_{sl} = 0.0037N^2 - 0.3695N + 73.046$	$R_{sl} = 0.002N^2 - 0.0104N + 1.7623$
Shoot weight	$R_{sw} = 0.0061N^2 - 1.6575N + 13.7$	$R_{sw} = 0.0013N^2 - 0.2975N + 0.6801$
Root length	$R_{rl} = 0.0032N^2 - 0.3478N + 78.565$	$R_{rl} = 0.0039N^2 - 0.083N + 3.9977$

R_{sl} represents reduction in shoot length, R_{sw}= reduction in shoot weight, R_{rl}= reduction in root length. Source: Elaborated by the authors.

As discussed previously, no study has been conducted to propose non-linear estimation equations between the number of galls and reduction in growth parameters. Considering the non-linear nature of the data, this study suggests non-linear correlations. It was observed that non-linear fittings better captured the trends in the experimental dataset with higher accuracy. The correlation coefficients between the number of galls and growth parameters in the current study were found to be 0.99, 0.98, and 0.99, respectively (Table 5 and Fig. 8). In contrast, the previous study examined the correlation coefficients between the number of galls and growth parameters using linear equations.

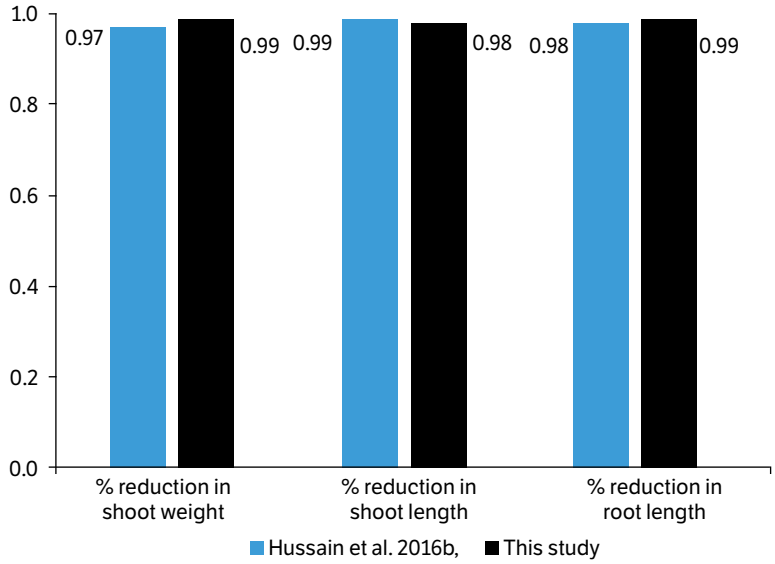

Figure 8. Comparison of the R^2 values of non-linear model proposed between the number of galls and growth parameters from previous and current study.

Source: Elaborated by the authors.

The results of this study show a significant improvement in prediction accuracy when compared to the previous study's linear correlations (Table 5 and 6, and Figs. 6, 7, and 8). To further support these findings, the percentage difference in the R^2 values between the number of galls and reduction in growth parameters was also calculated (Figs. 9 and 10). This analysis further justifies the necessity of determining the relationship between the number of galls and growth parameters using non-linear correlations.

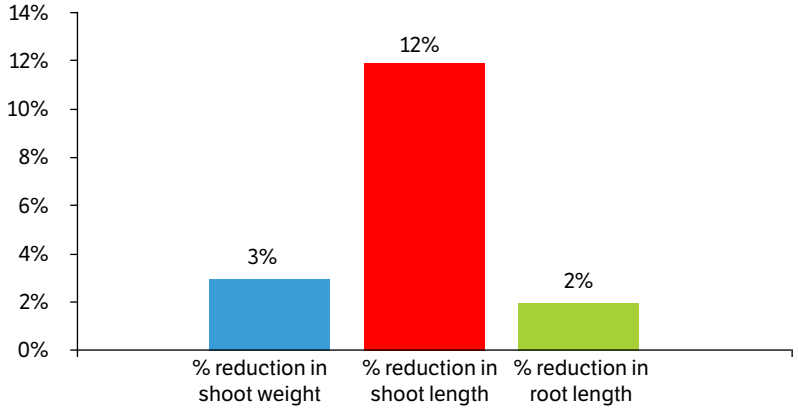

Figure 9. % difference in the R^2 values of linear model between the number of galls and growth parameters measured from previous and current study.

Source: Elaborated by the authors.

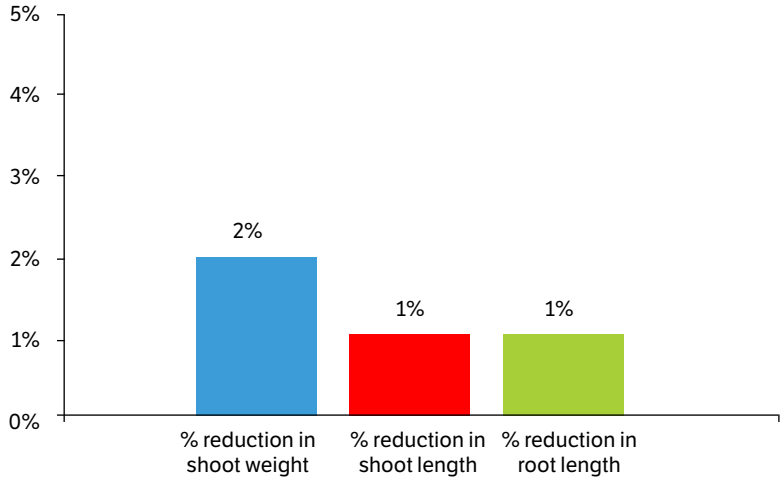

Figure 10. % difference in the R^2 values of non-linear model between the number of galls and growth parameters measured from previous and current study.

Source: Elaborated by the authors.

CONCLUSION

The findings of the current study suggest significant variations among okra cultivars regarding *M. incognita* reproduction, growth characteristics, and resistance response. In comparison to susceptible cultivars, moderately resistant cultivars exhibited slower and lower multiplication of the nematode. The moderately resistant cultivars were less affected by nematode damage, making them potential candidates for breeding programs aimed at developing nematode-resistant cultivars. Therefore, utilizing moderately resistant cultivars such as Pusa Swami, PB Selection, and Green Star in fields infested with root-knot nematodes can help reduce nematode reproduction, minimize environmental contamination, preserve biodiversity and agro-ecosystems, and enhance the efficiency and cost-effectiveness of management procedures. Additionally, the results of linear and non-linear fittings in this study provided the most accurate estimation of gall numbers and growth parameter trends. Consequently, future experiments evaluating resistance to *M. incognita* in okra cultivars should employ non-linear models for screening purposes.

AUTHORS' CONTRIBUTION

Conceptualization: Yaseen I. and Mukhtar T.; **Methodology:** Yaseen I., Mukhtar T. and Arshad B.; **Investigation:** Yaseen I., Mukhtar T. and Arshad B.; **Writing – Original Draft:** Yaseen I. and Mukhtar T.; **Writing – Review and Editing:** Mukhtar T. and Kim H.T.; **Resources:** Yaseen I. and Kim H.T.; **Supervision:** Mukhtar T.

DATA AVAILABILITY STATEMENT

Data will be made available on request.

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