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RESEARCH ARTICLE

Growth, physiological and biochemical variations in okra infected by root-knot nematode

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Abstract

Nematode infection causes significant change in the physiology of the plant. So, the study was conducted to investigate the impact of root knot nematode on okra. Six cultivars of okra were evaluated against root knot nematode; out of these, one was found to be resistant, two were moderately resistant and three were susceptible cultivars with the highest number of galls. In present study, the chlorophyll content of the susceptible cultivar was less than the moderately resistant and resistant cultivar. Maximum reduction was found in inoculated Utkal Gaurav 31.64 % as compared to Chota bana bhendi (18.44 %). In Chota bana bhendi, the chlorophyll b content was decreased from 5.4 to 4.6 mg/g of fresh leaf. Similarly, maximum reduction was seen in the case of susceptible cultivar Utkal Gaurav, i.e., 25.35 % and 22.34 % in Chota Bana Bhendi. The phenol content of inoculated roots of okra was increased by 15.40 %, 17.95 % and 34.91 % in cultivars Utkal Gaurav, Jhar bhendi and Chota bana bhendi respectively, over uninoculated plants. Whereas the proline content in roots of inoculated plants of okra was increased by 27.63 %, 21.51 % and 19.31 % in Utkal Gaurav, Jhar bhendi and Chota bana bhendi cultivars respectively. In all cultivars percentage macronutrient content significantly increased over the uninoculated plants, but the maximum increase recorded in Utkal gaurav shown Nitrogen (2.52 %), Phosphorus (1.6 %) and Potassium (3.43 %). The lowest concentration of Nitrogen (2.14 %) and Phosphorus (1.14 %) and Potassium (3.06 %) were observed in inoculated resistant cultivar. So, the identified resistant cultivar recommended for commercial cultivation.

Keywords: biochemical parameters; okra; resistant; root-knot nematode

Introduction

Okra is an important biannual crop with two growing seasons of tropical countries. It belongs to the family Malvaceae and genus *Abelmoschus* (*Abelmoschus esculentus* L. Moench). Although its origin in the tropics of Afro-Asian countries, this vegetable has become essential in the Indo-Pak subcontinent due to its high nutritional value. It is high in protein and the most common elements discovered in it are potassium, magnesium, sodium, calcium, iron and vitamins (1). Among various biotic stresses, root-knot nematodes cause both primary infection and facilitate other soil microorganisms to cause disease such as bacterial wilt, collar rot and fusarium wilt (2). Root-knot nematodes (*Meloidogyne* spp.) are the most prevalent group of plant-parasitic nematodes, resulting in significant losses in a variety of economically valuable crops (3, 4). Over 100 root-knot nematode species have been reported worldwide (5). Among all nematodes affecting okra, the most dangerous are the root-knot nematodes (*Meloidogyne* spp.) (6-8). These disguised biotic stresses severely affect okra growth, production and quality in tropical and subtropical climates (9). They cause a yield loss of approximately 19.6 % in vegetable crops across India (10). 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 % (11). Excessive use of chemical nematicides can degrade soil health and the ecosystem (12), despite their effectiveness for management of nematodes. So, host plant resistance provides a key to managing root knot nematodes which is environment friendly and economically viable (12, 13). Plants create enormous amounts of secondary metabolites involved in plant defence mechanisms, such as phenols and prolines. A series of biochemical and physiological reactions that occur in host plants in response to root-knot nematode infection results in disease occurrence. To improve our knowledge of the interaction between plant nematodes and to choose an appropriate management approach, a thorough analysis of these biochemical and physiological processes is necessary. The purpose of the current investigation was to find possible candidate biochemical substances that give resistance considering this background information.

Materials and Methods

Isolation and preparation of nematode inoculum

The experiment was conducted out in Department of Nematology, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar using statistical design CRD with four numbers of replication. Earthen pots were sterilised with 1 % formalin and filled with soil, sand and FYM (2:1:1). *Meloidogyne incognita* egg masses were collected and propagated on a susceptible tomato variety (Pusa Ruby). Different species of root knot nematode were identified according to perennial pattern suggested by previous researchers (14). From tomato plant, galled roots were collected. With the aid of tweezers, egg masses were removed from galled roots and spread out over the wire, gauze and paper assembly that was placed on the petridish. *Meloidogyne incognita* second stage juveniles that had just hatched were isolated and surface sterilised using mercurochrome (0.1 %) for 30 min to prevent contamination. The average number of 1000 J_2 per 10 mL of sample was standardised. Initially the seeds of the okra were surface sterilized for 2 min. in 2.5 % sodium hypochlorite solution. In each pot, seeds were sown and light irrigation for made the soil wet. After germination when seeds, kept one seedling per pot.

Screening and evaluation of okra cultivar against root knot nematode, *M. incognita*:

After 40 days of inoculation, the plants were uprooted smoothly and growth parameters like shoot length (cm), root length (cm), shoot weight (g), root weight (g), final nematode population (number) and okra cultivars were categorized as per the root-knot index scale (Table 1) given below (15).

Table 1. Root-knot index 1-5 Scale

Gall index	Observations	Reactions
1	No egg mass/galls/plant	Highly Resistant (HR)
2	1-10 egg masses/galls/plant	Resistant(R)
3	11-30 egg mass/galls/plant	Moderately Resistant (MR)
4	31-100 egg mass/galls/plant	Susceptible(S)
5	> 100 egg masses/galls/plant	Highly Susceptible (HS)

Physiological and biochemical analysis of okra roots

Estimation of chlorophyll content

100 mg of leaves from each treatment were collected and macerated with 10 mL of 80 % acetone and kept it dark for 24 hr. Then the content was filtered and observation taken at 645 nm and 663 nm by the help of spectrophotometer. The chlorophyll content (a, b and total) was calculated by formulae suggested by previous researchers (16).

Estimation of phenol and proline content

For estimation of phenolic content 100 mg of root sample were grounded with 80 % of 10 mL ethanol and centrifuged at 5000 rpm for 20 min. Aliquot of 0.5 mL was pipetted out from the extract and add 0.5 mL of 1N folin-Ciocalteu was added. Then 2mL of 20 % Na_2CO_3 was added after 3 min. Absorbance taken at 650 nm and total phenol was determined by standard prepared using catechol (10-100 µg mL^{-1}) (17).

Total proline content was estimated using procedure described previously (18). Root sample was grounded with 10ml of 3 % sulfo-salicylic acid and centrifuged for 10 min at 3000 rpm. 2 mL of GAA and 2 mL of ninhydrin added and boiled for 1hr at 100 ^{0}C. The reaction was stopped by cooling down in ice bath. Tolune (4 mL) was mixed and shaken vigorously. Later the toluene layer was separated using separating funnel at bottom and absorbance taken at 520 nm. The proline content was estimated by using fresh proline as standard.

Analysis of nitrogen, phosphorus and potassium content

Root samples of each treatment were dried, powdered and digested with triple acid extract and the extracts were estimated for total nitrogen by Kjeldahl method described by former researchers (19). For estimation of phosphorus and potassium content method described previously (20) and the content were expressed in percentage.

Results

Screening and evaluation of okra cultivars

Root knot nematode infection was recorded at 40 days after inoculation with nematode using root knot index scale and results were shown in Table 2. Out of the six screened okra cultivars, one was found to be resistant (R), with the lowest no. of galls (10 galls per plant), while two cultivars, Jhar bhendi and Nayagada local, were found to be moderately resistant (MR), three cultivars were found to be susceptible (S), with the 41–73 galls per plant. It was noticed that the shoot and root growth parameters decreased as root knot nematode susceptibility increased. Furthermore, it was discovered that the plant's growth parameters were less hampered in resistant cultivars. The highest average shoot length and fresh shoot weight were found in okra local cultivar Chota bana bhendi with 14.13 cm and 16.27 g respectively while the declined in shoot length and fresh weight was more severe in susceptible cultivars. Maximum decrease in shoot length and fresh weight 9.10 cm

Table 2. Screening and evaluation of okra cultivars against root-knot nematode, *M. incognita*

Sl. No.	Cultivars	Fresh shoot weight# (g)	Fresh root weight# (g)	Shoot length# (cm)	Root length# (cm)	No. of galls#	Final Population#	Root knot index (1-5 scale)	Reaction
1	Utkal gaurav	10.62± 0.095	3.163 ± 0.083	9.10 ± 0.231	2.83 ± 0.145	73.67± 5.81	2.109* ± 0.008	4	S
2	Chota bana bhendi	16.27± 0.070	6.323± 0.066	14.13 ± 0.203	4.47 ± 0.186	10.31± 2.91	2.02* ± 0.003	2	R
3	Nayagada local	14.22± 0.044	5.757 ± 0.055	13.23 ± 0.24	3.70 ± 0.173	29.33± 5.04	2.04* ± 0.002	3	MR
4	BO-13	13.57± 0.072	5.223± 0.058	11.10 ± 0.173	3.33 ± 0.233	41.67± 5.04	2.065* ± 0.002	4	S
5	V-33	11.27± 0.084	4.127± 0.023	10.30 ± 0.231	3.00 ± 0.1	53.33± 3.18	2.086* ± 0.001	4	S
6	Jhar bhendi	14.50± 0.071	5.993± 0.073	13.67 ± 0.176	3.53 ± 0.233	23 ± 4.36	2.034* ± 0.002	3	MR
	CD (<0.05)		0.195	0.657	0.575	14.06	0.011		
	SE(m) ±		0.063	0.211	0.185	4.51	0.004		

#Mean ± SE(m) of three replications; *log transformed value

S -Susceptible, R -Resistant, MR- Moderately resistant; CD: Critical difference; SE(m): Standard Error of mean.

and 10.62 g respectively in cultivar Utkal gaurav. Among the two moderately resistant cultivars cultivar Jhar bhendi had the shoot weight and shoot length 14.50 g and 13.67 cm respectively. Fresh shoot weight and length of susceptible varieties were found to be negatively impacted by the root knot nematode *M. incognita*. Nematode infection also affects the root growth parameters severely. The maximum reduction average root length of the okra cultivar Utkal gaurav was 2.83 cm whereas Jhar bhendi shown less reduction in root length around 4.47 cm that was more pronounced than that of another susceptible cultivar. The fresh root weight of susceptible cultivar Utkal Gaurav was 3.163 g which was less as compared to other resistant and moderately resistant cultivars. The final nematode population in both soil and root was also varied among different varieties. Maximum population of nematode found in susceptible cultivars like Utkal Gaurav, V-33 followed by BO-13.

Estimation of chlorophyll content

In the present study it was noted that the chlorophyll 'a' content in the inoculated decreased as compared to uninoculated cultivars. Maximum percent reduction was found in inoculated susceptible check Utkal Gaurav 31.64 % followed by Jhar bhendi (23.55 %) and Chota bana bhendi (18.44 %) over uninoculated plants (Table 3, Fig. 1). Again, the chlorophyll 'b' contents in the nematode inoculated plants decreased as compared to uninoculated plants. Maximum reduction was seen in susceptible cultivars (Utkal Gaurav) i.e 25.95 %. In resistant cultivar (Chota bana bhendi) the chlorophyll b content was decreased from 5.4 to 4.6 mg/g of fresh leaf (Table 3, Fig. 2) over uninoculated plant. Similarly, the total chlorophyll content was decreased in all the cultivars after inoculation. But maximum reduction was seen in case of susceptible cultivar Utkal Gaurav i.e 25.35 % followed by 24.01 % and 22.34 % in Jhar bhendi and Chota bana bhendi respectively (Table 3, Fig. 3).

Estimation of phenol and proline content

Resistance cultivar, Chota bana bhendi produced considerably more phenolic substances than the susceptible Utkal gaurav. The phenol content of inoculated roots of okra cultivars was increased by 15.40 %, 17.95 %, 34.91 % in cultivars Utkal Gaurav, Jhar bhendi and Chota bana bhendi respectively over uninoculated plants (Table 4, Fig. 4). There is significant difference between inoculated and uninoculated plants. Whereas the proline content in roots of inoculated plants of okra cultivars were increased by 27.63 %, 21.51 % and 19.31 % in Utkal Gaurav, Jhar bhendi and Chota bana bhendi cultivars respectively over uninoculated plants. Moreover, due to infection of root knot nematode the proline contents of these varieties increased significantly over uninoculated plants (Table 4, Fig. 5)

Estimation of macronutrient (N, P, K) content

Macronutrients like Nitrogen, Phosphorus and Potassium content were significantly influenced after inoculation of nematode. In all cultivar's percentage Nitrogen, phosphorus and potassium content increases over the control but maximum increase recorded in susceptible cultivar Utkal gaurav showed the maximum nitrogen (2.52 %), phosphorus (1.6 %) and potassium (3.43 %) followed by inoculated moderately resistant cultivar. in contrary, the lowest concentration of nitrogen (2.14 %) and phosphorus (1.14 %) and potassium (3.06 %) were observed in roots of inoculated resistant cultivar i.e. Chota bana bhendi (Table 5, Fig. 6-8).

Table 3. Chlorophyll (a, b, total) in the roots of okra

Cultivars	Chlorophyll a			Chlorophyll b			Total chlorophyll		
	I	UI	% Change	I	UI	% Change	I	UI	% Change
S	4.63	6.77	-31.64	5.54	7.48	-25.95	12.40	16.61	-25.35
MR	3.98	5.21	-23.55	5.33	6.35	-16.03	11.71	15.41	-24.01
R	3.65	4.47	-18.44	4.60	5.40	-14.81	11.09	14.28	-22.34
C.D. (<0.05)	0.34	0.30		0.41	0.21		0.24	0.21	
SE(m)±	0.10	0.09		0.13	0.06		12.40	16.61	

I = Inoculated; UI = Uninoculated

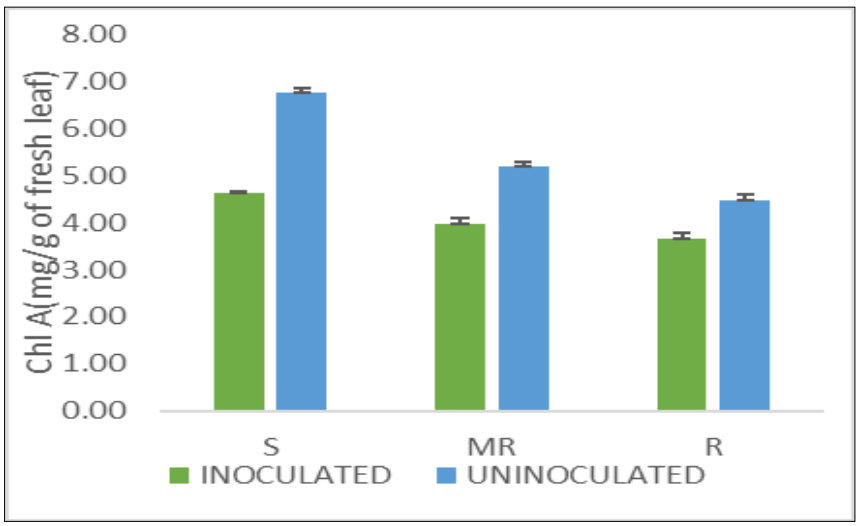

Fig. 1. Effect of *M. incognita* in chlorophyll content in three of okra cultivars - Chl A.

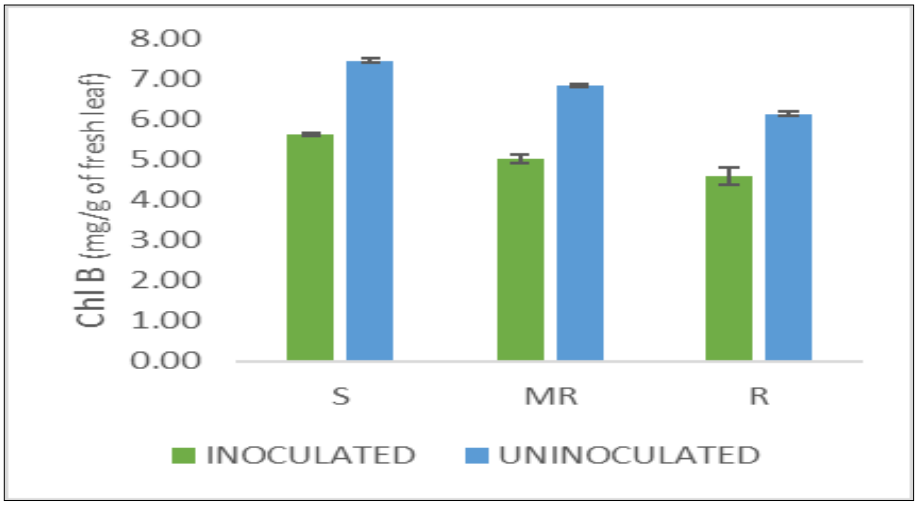

Fig. 2. Effect of *M. incognita* in chlorophyll content in three of okra cultivars - Chl B.

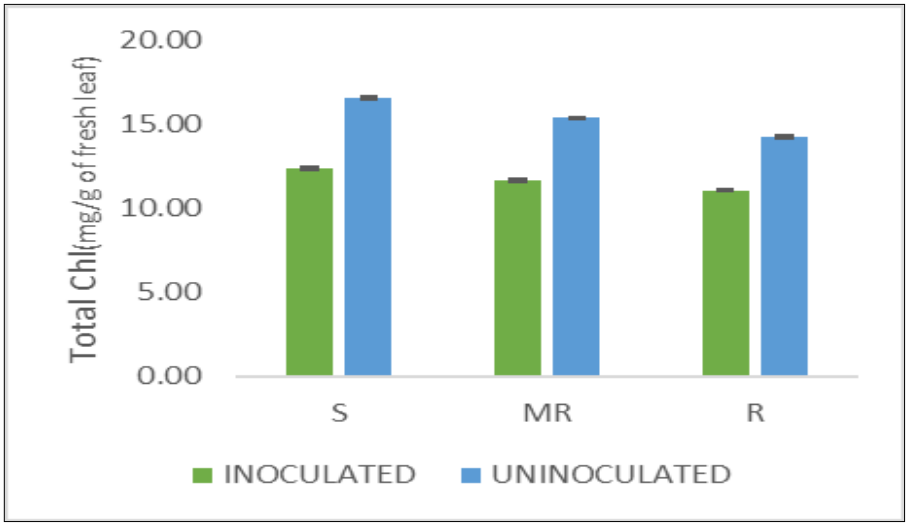

Fig. 3. Effect of *M. incognita* in chlorophyll content in three of okra cultivars - Total Chl.

Table 4. Phenolic and proline content in the roots of okra

Cultivars	Phenol content (mg/g)			Proline content (mg/g)		
	I	UI	% Change	I	UI	% Change
S	2.68	2.33	15.40	1.61	1.26	27.63
MR	2.91	2.46	17.95	1.29	1.06	21.51
R	3.39	2.52	34.91	1.21	1.01	19.31
C.D. (<0.05)	0.25	0.09		0.10	0.08	
SE(m)±	0.08	0.03		0.03	0.03	

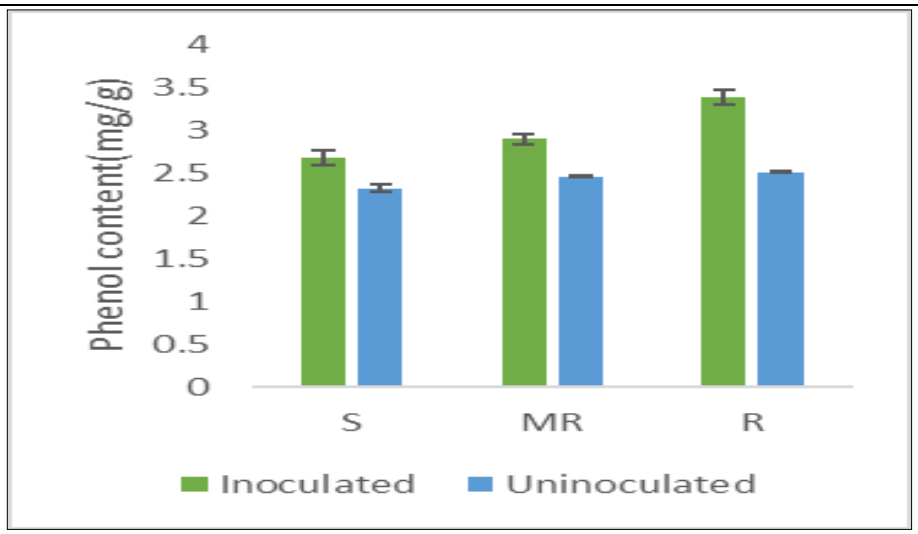

Fig. 4. Effect of *M. incognita* in phenol content.

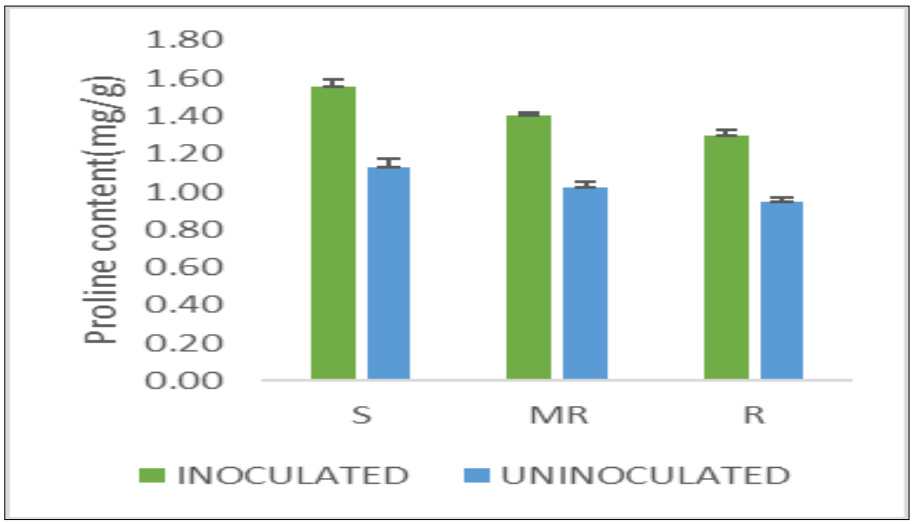

Fig. 5. Effect of *M. incognita* in proline content.

Table 5. Macronutrient (Nitrogen, Phosphorus, Potassium) content in the roots of okra

Cultivars	Nitrogen content			Phosphorus content			Potassium content		
	I	UI	% Change	I	UI	% Change	I	UI	% Change
S	2.52	2.12	19.01	1.60	1.04	54.13	3.43	2.95	16.37
MR	2.30	1.97	17.05	1.33	0.91	46.96	3.20	2.84	12.78
R	2.14	1.86	14.92	1.14	0.82	38.27	3.06	2.77	10.58
C.D. (<0.05)	0.15	0.11		0.14	0.15		0.10	0.08	
SE(m)±	0.05	0.04		0.04	0.05		3.43	2.95	

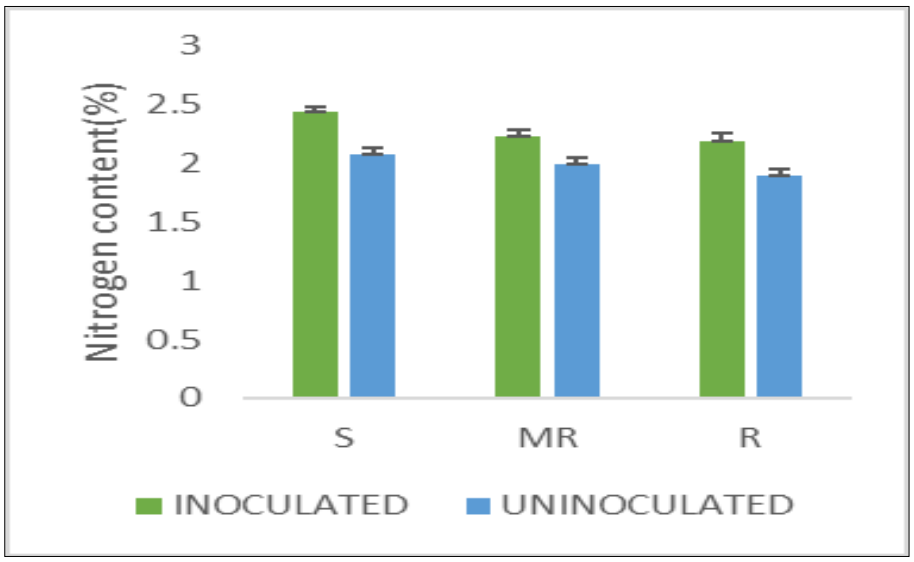

Fig. 6. Effect of *M. incognita* in Nitrogen content.

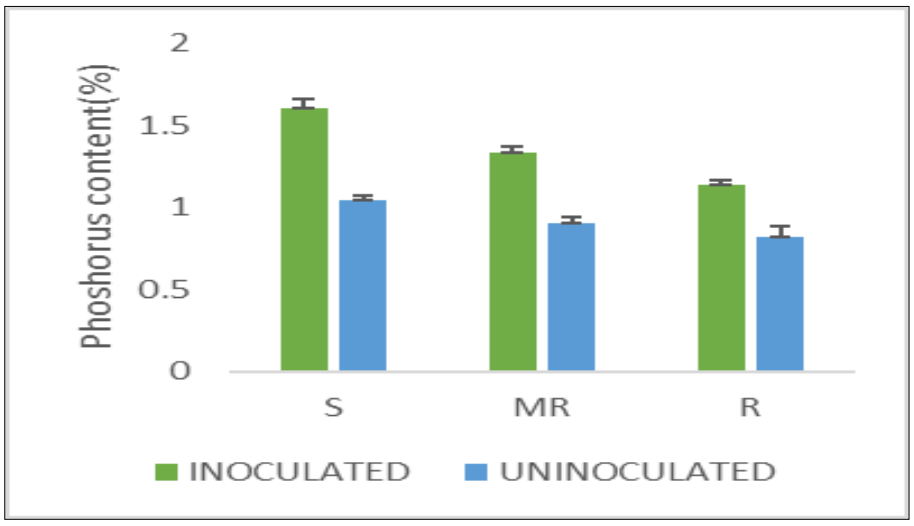

Fig. 7. Effect of *M. incognita* in Phosphorus content.

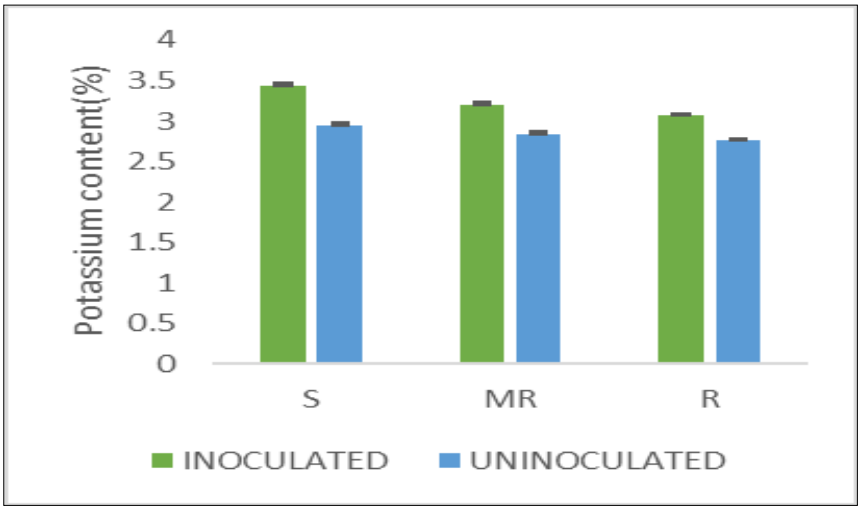

Fig. 8. Effect of *M. incognita* in Potassium content.

Discussion

The use of resistant cultivars is regarded as a crucial nematode management option for the future. In the current study, six okra cultivars were assessed for resistance or susceptibility to *M. incognita* using growth metrics and nematode reproduction. There were significant differences between okra cultivars responded to the nematode. The okra cultivars varied significantly in terms of resistance and growth parameter reductions. We discovered a substantial positive correlation between the number of galls and nematode population and decrease in growth parameters. The nematode produced galls and multiplied differently in six different okra cultivars. Variations among okra cultivars can be linked to genetic differences or the existence of genes conferring resistance or susceptibility (21, 22). When resistance genes are present, the nematode cannot infect or reproduce on non-host crops or resistant cultivars of the crop because the features required for effective infection and parasitism are absent. The result of different growth parameters showed that nematode infection negatively affects growth parameters, which was expected because the nematode draws the nutrients by forming the giant cell and blocking the free flow of nutrients through xylem tissue.

In the present study we noted that there was significant change in chlorophyll content after inoculation of nematode in comparison with uninoculated check. In case of three reaction maximum decrease in susceptible then moderately resistant followed by resistant reaction. Similar findings noted previously Similar results were also obtained by previous results (23-25), reported that, infestation with RKN markedly decreased photosynthetic pigment contents and altered several photosynthetic traits (WUE and photosynthesis rate). They also revealed that the pictures of photosynthetic pigments, content and composition, are considered the poignant markers for nematode infection. Moreover, the decrease in photosynthetic values in plants infected with nematodes was also accompanied by proteolytic enzyme activities (26).

Increase in total phenol contents in okra, mung bean, different babchi varieties due to RKN infection has been reported in previous studies (27-29). This increase in phenolic content can be explained as a resistance strategy of the plant to create lignins by breakdown of bound phenols or shifting of phenols to alternative pathways (30). There was increase in proline content of susceptible cultivar roots than the resistant cultivars. Similar result found earlier in tomato after inoculation with nematode (31).

Nutrients are essential elements for plant growth and development and are essential for various physiological processes (28). Compared to uninoculated control plants, nematode inoculation increased root N, P and K concentrations and maximum increase was noted in susceptible. These findings previously noted in bitter gourd where macro nutrients content was increased due to decreased flow of nutrients to shoots and all nutrients accumulated in infected roots (32).

Conclusion

The study found significant variations within okra cultivars in terms of *M. incognita* reproduction, growth and resistance response. Resistant and moderately resistant cultivars displayed slower and lower nematode proliferation than susceptible cultivars. The moderately resistant cultivars were less susceptible to nematode damage, making them good candidates for breeding operations focused at creating nematode-resistant cultivars. Study about different biochemical and physiological parameters gave clear idea about different parameters that involved in imparting resistant after nematode infection.

Future scope

Root knot nematode is a sedentary endoparasite and resistance can be affected within the soil to the root environment. The information generated from this investigation can be manipulated through advanced biotechnological research for suitable management strategies and that is the important implications for future research who wish to increase agricultural yields by utilising genotypes of okra that are resistant to nematodes. Further using the implications of the research helping for development of nematode resistant variety.

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Authors' contributions

AS and DKN contributed equally to conducting the research and drafting the manuscript. SKB participated in the research and data collection. AKS was responsible for statistical analysis and preparation of the final draft. ND contributed to the study design and coordination. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: The authors declared no conflict of interest.

Ethical issues: None

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