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Screening for resistance and susceptibility in some beetroot cultivars to root-knot nematode, *Meloidogyne javanica*

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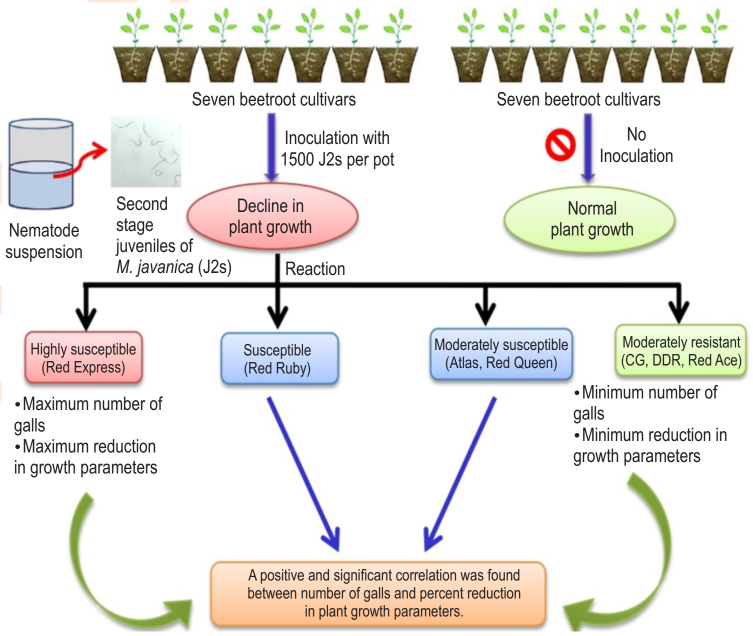
Abstract

Aim: The present study was conducted to identify the resistance and susceptibility of beetroot cultivars against root-knot nematode, *Meloidogyne javanica*.

Methodology: In this study, seven beetroot cultivars were screened against the root-knot nematode, *Meloidogyne javanica*, at the inoculum level of 1500 second-stage juveniles (J2s) per pot. Five replications of each cultivar of beetroot were used for screening and kept in a completely randomized design (CRD) at 25 ± 2 °C under greenhouse conditions. The data were subjected to ANOVA using SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA). According to Duncan's Multiple Range Test, the mean values were statistically compared and separated at $P \leq 0.05$.



Results: The results revealed that all seven cultivars of beetroot showed varying degrees of resistance and susceptibility to *M. javanica*. Among all the cultivars, three were moderately resistant, *i.e.,* CG, DDR and Red Ace with the minimum number of galls (4.0, 8.0 and 7.0), and two were moderately susceptible, *i.e.,* Atlas and Red Queen (28 and 22). Red Ruby (84) was found susceptible and Red Express was highly susceptible with the maximum galls (126). None of the cultivars were found to be highly resistant or immune.

Interpretation: A positive and significant correlation was found between the number of galls and per cent reduction in plant growth parameters. From the results, it can be recommended that the use of resistant cultivars is an environment-friendly approach that can be used to manage the nematode density below the economic threshold level.

Key words: *Meloidogyne javanica*, Resistance, Root-knot nematode, Screening, Susceptibility

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Introduction

Beetroot (*Beta vulgaris* L.) is a herbaceous vegetable crop that belongs to family Amaranthaceae. It is highly nutritious root vegetable that contain high concentration of vitamins, carotenoids, flavonoids, nitrates, minerals like calcium, potassium, phosphorous, copper, iron, magnesium, zinc, manganese, sodium, and water-soluble pigments like betacyanins and betaxanthins (Panghal *et al.,* 2018). Beetroot pigments exhibit antioxidant and anti-carcinogenic properties (Attokaran, 2011). Beetroot is an indigenous crop to Asia and Europe (Nirmal *et al*., 2021). It is found in temperate regions around the world (CABI Crop Protection Compendium, 2010). It is an easily cultivated vegetable crop and is consistently ranked as one of the top ten vegetables grown in India. Among three sub-species of beetroot such as *B. vulgaris, B. maritima* and *B. adanensis; B. vulgaris* sub sp. *vulgaris* is the most commercially and commonly grown beetroot (Arnaud *et al.,* 2010).

All over the world, the production of beetroot was 269,714 million tons in 2014 (Chhikara *et al.,* 2019). In India, it is easily cultivated in the northern and southern regions. In Tamil Nadu, during 2012- 2013, the area under beetroot cultivation was 1,308 hectares with a production of 39,383 tonnes. During 2012- 2013, in Tamil Nadu, an area under beetroot cultivation was 1,308 hectares with a production of 39,383 tonnes. Around the world, including India, the yields of vegetable crops are comparatively lower due to abiotic and biotic constraints. Vegetable fields that are heavily infested with plant-parasitic nematodes have been reported with 80% loss (Anamika *et al.,* 2011). Many biotic constraints, including plant-parasitic nematodes, limit the profitable production of vegetables qualitatively and quantitatively (Khan *et al.,* 2019). At global level, the estimated average annual yield loss of various crops by plant parasitic-nematodes is 8.8–14.6% of total crop production and 100-157 billion USD (Abad *et al.,* 2008; Atkinson *et al.,* 2012).

Among plant parasitic-nematodes, the root-knot nematodes are the most damaging and ubiquitous biotrophic parasites that infect over 3,000 plant species and cause excessive yield loss yearly (Huang *et al.,* 2014). In tropical parts of the country, an estimated 22% annual yield loss have been due to root-knot nematodes being considered as important production-limiting factors among all biotic constraints (Hussain *et al.,* 2011). Globally, an estimated annual loss of $157 billion was caused by *Meloidogyne* spp. (Ralmi *et al.,* 2016). Various nematicides have traditionally been used to control root-knot nematodes that cause severe damage in economically important agriculture crops (Jones *et al.,* 2017). Globally, in the past few decades, the frequent use of nematicides has been increased to control the diseases caused by plant-parasitic nematodes (Leng *et al.,* 2011). However, chemical nematicides are expensive, non-biodegradable chemical compounds and possess several detrimental effects on the environment human health and soil microbiota. Due to these disadvantages, most of the chemicals have been banned for the last two decades. Many alternative strategies have been developed to manage root-knot nematodes which includes cultivar resistance (Khan *et al.,* 2018; Asif *et al.,* 2018), organic soil amendments (Khan *et al.,* 2019; Khan *et al.,* 2021), biocontrol agents (Huang *et al.,* 2014). The use of resistant cultivars provide an efficient and environment-friendly safety check to reduce the nematode population (Changkwian *et al.,* 2019).

Natural resistance is frequently attributable to specific genes that might segregate within the host species. On the other hand, the nematode is unable to reproduce on non-host species or resistant cultivars due to lack of host traits essential for parasitism (Cui *et al.,* 2020; Alekcevetch *et al.,* 2021). Therefore, this research will help to identify the resistant cultivar and reduce the nematode population below the economic threshold; both are sustainable and environmentally compatible. The objective of the present study was to screen seven cultivars of beetroot for resistant and susceptible response to root-knot nematode, *Meloidogyne javanica* as an environment-friendly strategy in the nematode management methods.

Materials and Methods

Collection and identification of nematode inoculums: Root-knot nematode, *Meloidogyne* females, were collected from infected roots of eggplant and tomato fields and identified based on the perineal patterns of adult females and examined under a light microscope (Eisenback, 1985). *M. javanica* was maintained on eggplant in the greenhouse of Department of Botany, Aligarh Muslim University, India. Egg masses of *M. javanica* were collected in a Petri dish containing distilled water and left for hatching at 28°C. The juveniles hatched were collected after every 24 hrs and freshwater was added to Petri dish. The suspension of second-stage juveniles (J2) was standardized as per the requirement and used for plant inoculation.

Collection of beetroot cultivar: Seeds of seven beetroot cultivars, viz. Atlas, CG, DDR, Red Ace, Red Express, Red Queen, and Red Ruby were obtained from Agro Company Chola Beej Bhandar, Aligarh and Indian Agriculture Research Institute (IARI), New Delhi. Seeds of seven beetroot cultivars were surface sterilized with 0.1% mercuric chloride solution for 15 min, followed by risings with sterile water. Five seeds of each beetroot cultivars were sown in earthen pots containing 1 kg autoclaved soil. Two weeks after germination, thinning was done, leaving one healthy plant per pot with control.

Experimental design and inoculation technique: Five replications of each cultivar of beetroot were used for screening and kept in a completely randomized design at 25 ± 2 °C under greenhouse conditions. After two days of thinning, inoculation was done. All the plants were inoculated with 1500 freshly hatched second-stage juveniles (J2) of *M. javanica* per pot. Following pipett method, the inoculum suspension was poured into three holes around the root of seedlings. The other pots with uninoculated soil served as control. The plants were watered when needed and handled with proper care.

Data collection and observations: Three months after *M. javanica* juveniles' inoculation, plants were carefully uprooted from pots. The roots were gently rinsed in a bucket filled with water to remove adhering soil and debris. The roots were examined visually for root galls on each root system. The assessment was carried out based on the following plant growth parameters *viz.* fresh and dry shoot and root weight, shoot length and root length, bulb diameter, number of leaves, number of galls/root, egg masses/root, eggs/egg mass and population of nematodes /250 g of soil. Physiological parameters like chlorophyll content (MacKinney, 1941) and carotenoid content MacLachlan and Zalik (1963) were also estimated.

Categorization of cultivars for resistance: The susceptible or resistant behavior of all the cultivars were analyzed based on the degree of root-knot nematode infection, *i.e.,* root gall index, which was determined by the number of galls present on each root system as per Taylor and Sasser (1978).

Statistical analyses: The experimental data on plant growth parameters and root galling were subjected to One-way ANOVA using SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA). The mean values were statistically compared and separated according to Duncan's Multiple Range Test at P ≤0.05.

Results and Discussion

The present study aimed was to evaluate the resistance or susceptibility response of beetroot cultivars against *M. javanica* and in order grow healthy and high-quality beetroot in environmentally friendly methods. Variable effects of *M. javanica* were observed on plant growth and pathological parameters of all beetroot cultivars. All cultivars responded differently in terms of formation of galls or knots. None of the seven tested beetroot cultivars evaluated following the rating scale given by Taylor and Sasser (1978) were found highly resistant or immune to the root-knot nematode, *M. javanica*. Three cultivars, namely CG, DDR and Red Ace, were found to be moderately resistant with least number of galls, *i.e.,* 6.5, 8, and 7, respectively. Atlas and Red Queen cultivars were found to be moderately susceptible with 28 and 25. Cultivar Ruby Red was found to be susceptible with the number of galls 84, while cultivar Red Express was observed highly susceptible, having maximum number of galls 126 (Table 1). Maximum galls were observed on highly susceptible cultivars

Table 1: Effect of root-knot nematode, *Meloidogyne javanica* on different cultivars of beetroot in relation to nematode infestation parameters

Cultivar	Number of egg masses/ root system		Number of eggs/ egg mass		Nematode population / 250g soil		Number of galls /root system		Reaction
	C	I	C	I	C	I	C	I	
Atlas	-	56^{c}	-	132^{d}	-	1517^{d}	-	28^{c}	Moderately susceptible
CG	-	18^{e}	-	81^{e}	-	766^{g}	-	4^{e}	Moderately resistant
DDR	-	35^{d}	-	73^{ef}	-	847^{e}	-	8de	Moderately resistant
Red Ace	-	24de	-	64^{f}	-	816^{f}	-	7^{e}	Moderately resistant
Red Express	-	135^{a}	-	234^{a}	-	2265^{a}	-	126^{a}	Highly susceptible
Red Queen	-	63^{cd}	-	145^{c}	-	1664^{b}	-	22^{cd}	Moderately susceptible
Ruby Red	-	104^{b}	-	187^{b}	-	1928^{c}	-	84^{b}	Susceptible

Each value is mean of five replicates; Means in each column with different letters denote significant differences at P≤0.05 according to Duncan's Multiple Range Test; I– Inoculated; C – Control

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

Cultivar	Shoot length (cm)		% red. over control	Root length (cm)		% red. over control	Shoot fresh weight (g)		% red. over control	Root fresh weight (g)		% red. over control
	C	I		C	I		C	I		C	I	
Atlas	27.8^{de}	20.0^{de}	27.9^{c}	18.5^{cd}	12.9^{bc}	29.8^{c}	54.65^{e}	41.09^{c}	24.8^{d}	118.5^{d}	83.6^{c}	29.4^{d}
CG	30.5^{bcd}	24.6^{b}	19.2^{d}	19.2^{bcd}	14.9^{b}	22.2^{d}	56.75^{d}	45.0^{b}	20.6^{e}	123.2^{c}	92.5^{b}	24.9^{e}
DDR	26.0^{e}	21.4^{cd}	17.4^{e}	17.6^{d}	14.1^{bc}	19.7^{d}	52.22^{f}	42.0^{c}	17.9^{f}	115.1^{e}	92.3^{b}	19.8^{f}
Red Ace	34.4^{a}	29.4^{a}	14.3^{f}	21.5^{a}	18.1^{a}	15.6^{e}	62.80^{a}	53.4^{a}	14.9^{g}	127.6^{a}	107.1^{a}	16.1^{g}
Red Express	33.1^{ab}	18.6^{e}	43.6^{a}	20.8^{ab}	10.9^{c}	47.2^{a}	60.40^{b}	33.1^{e}	45.2^{a}	125.4^{b}	65.4^{e}	47.8^{a}
Red Queen	31.5^{bc}	22.3^{c}	29.2^{c}	20.1^{abc}	13.5^{bc}	32.5^{c}	58.38^{c}	41.8^{c}	28.4^{c}	124.9^{bc}	81.7^{c}	34.6^{c}
Ruby Red	29.3^{cd}	18.7^{f}	35.7^{b}	18.8^{cd}	11.0^{c}	41.2^{b}	55.59^{e}	35.8^{d}	35.5^{b}	120.3^{d}	72.0^{d}	40.1^{b}

Each value is mean of five replicates; Means in each column with different letters denote significant differences at P≤0.05 according to Duncan's Multiple Range Test; I –Inoculated; C – Control; % red. –Per cent reduction

Table 3: Effect of root-knot nematode, *Meloidogyne javanica* on different cultivars of beetroot in relation to plant growth parameters

Cultivar	Shoot dry weight (g)		% red. over control	Root dry weight (g)		% red. over control	Bulb diameter (cm)		% red. over control	Number of leaves/plant		% red. over control
	C	I		C	I		C	I		C	I	
Atlas	18.8^{ab}	13.8^{b}	26.2^{d}	33.2^{a}	23.8^{a}	28.1^{d}	7.1^{abc}	5.0^{bc}	28.6^{b}	18.0^{bcd}	13.5^{cd}	24.8^{d}
CG	13.5^{ef}	10.6^{c}	21.2^{e}	26.5^{bc}	20.0^{b}	24.5^{e}	8.7^{ab}	6.7^{ab}	22.4^{c}	20.5^{ab}	17.0^{ab}	15.2^{de}
DDR	20.8^{a}	17.3^{a}	16.6^{f}	23.4^{de}	18.2^{bc}	21.8^{f}	8.1^{abc}	6.5^{ab}	18.7^{c}	19.5^{bc}	15.6^{bc}	19.5^{d}
Red Ace	16.4^{cd}	14.1^{b}	13.5^{g}	28.2^{b}	23.0^{a}	18.4^{g}	9.2^{a}	7.8^{a}	14.2^{d}	22.0^{a}	19.2^{a}	12.6^{ef}
Red Express	12.8^{f}	7.2^{d}	43.8^{a}	20.7^{f}	11.2^{d}	45.9^{a}	6.2^{c}	3.8^{c}	35.2^{a}	10.5^{g}	4.2^{g}	40.0^{a}
Red Queen	15.2^{de}	10.7^{c}	29.5^{c}	22.6^{ef}	15.4^{c}	31.6^{c}	7.5^{abc}	5.4^{abc}	26.8^{b}	16.5^{cde}	11.5^{de}	30.3^{c}
Ruby Red	17.5^{bc}	11.8^{bc}	32.4^{b}	25.3^{cd}	16.2^{c}	35.7^{b}	6.8^{bc}	4.5^{bc}	32.5^{a}	13.0^{f}	8.0^{f}	35.7^{b}

Each value is mean of five replicates; Means in each column with different letters denote significant differences at $P \leq 0.05$ according to Duncan's Multiple Range Test; I – Inoculated; C – Control; % red. –Per cent reduction

Table 4: Effect of root-knot nematode, *Meloidogyne javanica* on different cultivars of beetroot in relation to physiological parameters

Cultivar	Chlorophyll content (mg g^{-1})		% red. over control	Carotenoid content (mg g^{-1})		% red. over over control
	C	I		C	I	
Atlas	1.24^{c}	0.86^{bc}	29.9^{d}	0.390^{ab}	0.285^{c}	26.8^{bcd}
CG	1.29^{bc}	0.97^{b}	24.6^{e}	0.388^{abc}	0.305^{b}	21.3^{cde}
DDR	1.21^{c}	0.98^{b}	18.2^{f}	0.398^{a}	0.324^{a}	18.4^{de}
Red Ace	1.40^{a}	1.2^{a}	13.6^{g}	0.377^{bcd}	0.331^{g}	12.1^{e}
Red Express	1.35^{ab}	0.78^{c}	41.8^{a}	0.382^{abc}	0.219^{f}	42.6^{a}
Red Queen	1.31^{abc}	0.87^{bc}	33.5^{c}	0.365^{c}	0.256^{d}	29.7^{bc}
Ruby Red	1.27^{bc}	0.79^{c}	37.2^{b}	0.371^{bc}	0.238^{e}	35.7^{ab}

Each value is mean of five replicates; Means in each column with different letters denote significant differences at $P \leq 0.05$ according to Duncan's Multiple Range Test; I – Inoculated; C – Control; % red. –Per cent reduction

and the minimum was recorded on moderately resistant cultivars. Similar results were found in the findings of Mukhtar *et al.* (2014) and Khan *et al.* (2018). Nematodes promote the formation of distinctive swelling structures called root-knots or galls on the host root and enhance their reproductive growth by interrupting the plant's developmental program of plant (Bartlem *et al.,* 2014).

The most significant number of egg masses per root system was obtained in cultivar, Red Express followed by Ruby Red, Red Queen, Atlas, DDR and Red Ace whereas minimum was recorded in CG, respectively (Table 1). Similarly, the maximum number of eggs/egg mass was found in beetroot cultivar Red Express followed by Ruby Red, Atlas, CG and DDR whereas Red Ace exhibited the minimum number of eggs/egg mass (Table 1). The highest number of egg masses per root system was found in highly susceptible cultivars whereas the least was recorded in moderately resistant cultivars. One of the mechanisms associated with the least number of egg masses is inhibition of reproduction of nematodes. It was found that the resistant cultivars reduced the development of juveniles on the root system. Therefore, there is a need to identify and develop strategies that can assist plants in defending from parasitic nematodes through genetic resistance (Regmi and Desaeger, 2020). Table 1 revealed that the final nematode population in 250 g soil was marked maximum in highly susceptible cultivar Red Express (2265) followed by susceptible cultivar Ruby Red (1928), moderately susceptible Red Queen (1664) and Atlas (1517), and moderately resistant cultivars DDR (847), Red Ace (816) and CG (766). These results are evident from work done previously by different researchers as they have investigated the reaction of different okra, carrot, tomato and lentil cultivars to root-knot nematodes (Mukhtar *et al.*, 2014; Khan *et al.,* 2018; Asif *et al.,* 2018; Ansari *et al.,* 2018) and reported varying levels of resistance or susceptibility. The population of nematodes in soil was significantly highest in highly susceptible and susceptible cultivars whereas it decreased in moderately resistant and resistant beetroot cultivars. Severe infection may change the permeability of root system, which leads to poor plant growth and crop production with significant yield losses.

One of the essential parameters for selecting cultivars is the reproductive factor of nematode. Cultivars with reduced reproductive characteristics are thought to be effective against root-knot nematodes (Kayani and Mukhtar, 2018). Resistance or

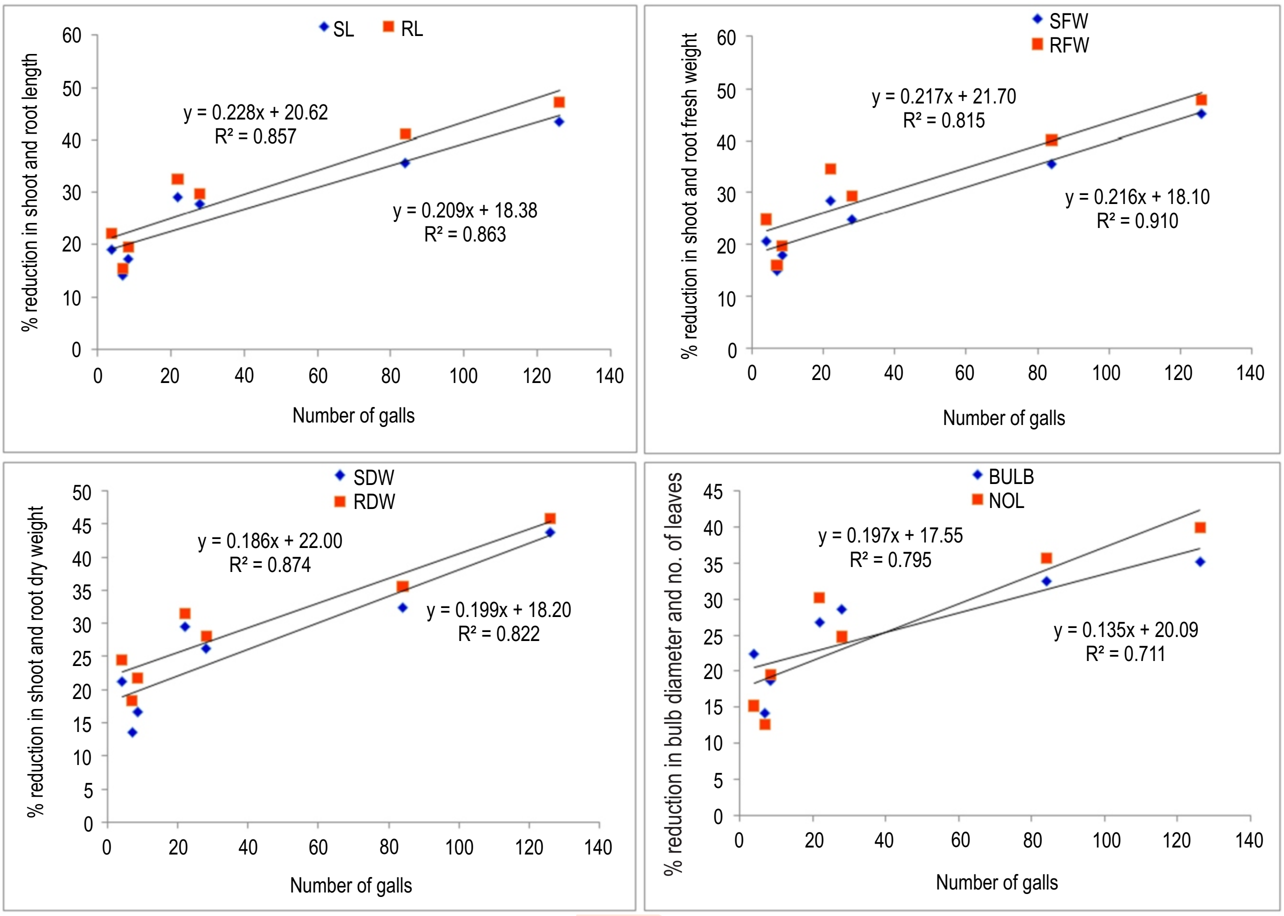

Fig. 1: Relationship between number of galls and percent reduction in different growth parameters of different beetroot cultivars.

susceptibility and genetic differences between nematode populations are determined by the genetic factor of the host (Petitot *et al.,* 2020; Haq *et al.,* 2021). The genetic differences of host affected several stages in the life cycle of nematode. The additive effects of multiple *M. incognita* resistance genes developed resistance in some cultivars (Chaudhary *et al.,* 2019; Hada *et al.,* 2021). The mechanisms of host-plant resistance described by Painter (1951) are antibiosis, antixenosis and tolerance. The word resistance is used to describe the ability of plant to suppress the development or reproduction of nematode. In the present study, among the moderately resistant cultivars, a wide range of tolerance was observed, which can be determined by the genetic makeup of host plant and many environmental factors that may influence the plant's growth.

Degrees of resistance and tolerance are not always same due to separate genetic control in some plant nematode interactions (Wille *et al.,* 2019; Gartner *et al.,* 2021). All the tested beetroot cultivars showed a significant decrease in plant growth parameters due to *M. javanica* infestation. Due to infection of root-knot nematode, the maximum reduction in shoot length and root length was 43.6% and 47.2%, respectively, in cultivar Red Express (Table 2). The decrease in shoot length and root length was found least pronounced with 14.3% and 15.6%, respectively, in the Red Ace cultivar, which was statistically lower than other susceptible cultivars (Table 2).

Due to nematode infection the maximum reduction in fresh shoot weight and fresh root weight was 45.2% and 47.8% in Red Express and the decrease in fresh shoot weight and fresh root weight were found least pronounced with 14.9% and 16.1% in Red Ace cultivar, respectively which was statistically lower than other susceptible cultivars (Table 2). The maximum reduction in dry shoot weight and dry root weight of beetroot cultivars were 43.8% and 45.9% in Red Express and the decrease in dry shoot weight and dry root weight were found least pronounced with 13.5% and 18.4%, respectively in Red Ace cultivar, which was statistically lower than other susceptible cultivars (Table 3). The maximum reduction in bulb diameter and the number of leaves/plants was 35.2% and 33.3% in cultivar Red Express whereas the least decrease in bulb diameter and number of leaves per plant were 14.2% and 14.6% in cultivar Red Ace, respectively, which was statistically lower than other susceptible cultivars (Table 3).

The reductions in growth parameters of highly susceptible cultivars were found more severe than susceptible cultivar. Similarly, the reduction in growth parameters of moderately susceptible cultivars were comparatively less severe than those observed in the susceptible and highly susceptible cultivars. Similar results were also found in carrot and tomato screening (Asif *et al.,* 2018; Khan *et al.,* 2018; Mukhtar *et al.,* 2018). The maximum reduction in growth and yield parameters of susceptible cultivars can be ascribed to severe root damage by penetration and development of nematode feeding sites, which resulted in impairment and disruption of root systems. *Meloidogyne* spp. induced gall formation and giant cells in the vascular region and thereby significantly reduced the absorption of water and nutrients from the soil and translocation of photosynthates to other parts of the plant (Siddique and Grundler, 2018; El-Sagheer, 2019). These obligate parasites can take over plant cell fate and differentiate root cells into specialized feeding structures called giant cells that provide essential nutrients for successful parasitism (Hewezi, 2020).

The results showed that the nematodes infected plant leaves showed a reduction in total chlorophylls and total carotenoids compared to healthy plants. The maximum reduction in beetroot cultivar's total chlorophylls and total carotenoids was 41.8% and 42.6%, respectively, in Red Express (Table 4). The decrease in total chlorophylls and total carotenoids were found least pronounced with 13.6% and 14.1%, respectively, in the Red Ace cultivar, which was statistically different from other susceptible cultivars (Table 4). A similar reduction in total chlorophyll and carotenoid content were also observed in the studies of Ansari *et al.* (2018) and Tsaniklidis *et al.* (2021) on lentil and *Cucumis melo* infested with *M. incognita* and *M. javanica*. The maximum damage caused to host plant resulted in physiological and biochemical changes caused by nematode feeding. The growth and development of leaf tissue and its constituents, especially chlorophyll pigments, are adversely affected by insufficient water, nutrients, and photosynthates (Khan and Khan, 1997). A positive and significant relationship was observed between root gall indices and the percent reduction in shoot length (R^2 = 0.86), root length (R^2 = 0.85), shoot fresh weight (R^2 = 0.91), root fresh weight (R^2 = 0.81), shoot dry weight (R^2 = 0.82), root dry weight (R^2 = 0.87), bulb diameter (R^2 = 0.71) and number of leaves (R^2 = 0.79) (Fig. 1). The relation was found positive because the increase in the number of galls resulted in increased percent reduction of plant growth parameters.

The results of this study provide valuable and useful information to beetroot growers and breeders working on nematode resistance breeding to ensure the crop's long-term viability. The present research evaluated significant variations (in plant growth and pathological parameters) found among all the screened cultivars in response to *Meloidogyne javanica*. The maximum development and multiplication of nematode were observed in highly susceptible cultivars whereas it was found least in the resistant ones. From this study, it can be concluded that the moderately resistant cultivars can be recommended for cultivation as an eco-friendly approach for the disease management programs in nematode infested fields.

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Add-on Information

Authors' contribution: A. Khan and M.A. Siddiqui: Designed the manuscript; **A. Khan and F. Khan:** Wrote the manuscript and carried out experimental work; **M.A. Siddiqui and M. Asif:** Supervised the manuscript; **M. Shariq and T. Ansari:** Edited the manuscript; **S. Fatima and M. Ikram:** Prepared figures/graphs; **F. Khan, A. Khan and M.A. Siddiqui:** Validated the data and finalized the manuscript.

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

Availability of data and material: The data provided in this study are available in the form of tables and figures.

Data from other sources: Not applicable.

Human and animal rights: No animals or humans were used in the present study.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology.*

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