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Root - knot nematode (*Meloidogyne incognita)* amelioration in tomato under protected conditions through rootstocks

Vibhuti Sharma, Pardeep Kumar, Parveen Sharma, Pawan K Sharma, Amar Singh and ND Negi

Abstract
Tomato yield and quality are considerably affected and diminished by root knot nematodes. The chemical measures and manipulations of agronomical practices are ineffective in controlling root knot nematodes and moreover, they have toxic effect and lead to escalation in production cost. Thus, grafting susceptible tomato scions on resistant rootstocks is one of the most appropriate approaches which provides resistance to soil-borne pathogens and improves yields and quality. In the present investigation, sixteen different rootstocks were screened for their resistance to *Meloidogyne incognita*. Out of total rootstocks, one rootstock of tomato 'Green Gourd' with RKI-1, one brinjal ' VI-034845' with RKI-5 and 'VI-047335' were found moderately resistant with RKI-16, two chilli rootstocks ' PI-201232' with RKI-2 and 'AVPP0205' with RKI-3 were found resistant to root knot nematodes. Rootstock Green Gourd and VI-34845 were found resistant for nematode incidence as well as for maximum yield per square metre.

Keywords: Grafting, second juvenile stage, root knot index, galls

Introduction

Tomato is one of the potential crops grown under protected conditions worldwide. Protected cultivation creates favorable environment for enhanced yield so as to harness or exploit its maximum potential even under adverse climatic conditions. Besides, this protected cultivation provides better quality of produce, higher productivity, better insect and disease control and resources are utilized more efficiently. Root-Knot nematodes are becoming a serious production problem under protected conditions due to favourable environmental conditions.

Root -knot nematodes (*Meloidogyne* sp.) are agricultural pests of economic importance. Root-knot nematodes have become an inhibiting constraint in the production of vegetables, particularly tomato (Anwar *et al*., 2007) [5]. Tomato is considered as favourable host for root knot nematodes and mainly the potential damage is caused by four species viz., *Meloidogyne incognita, Meloidogyne javanica, Meloidogyne arenaria and Meloidogyne hapla* in open as well as protected conditions (Sasser *et al*., 1983) [29]. They are polyphagous in nature and have great impact on health, yield and quality of the crop. The nematodes damage plants by inducing direct physical injury by their stylets into the plant roots, thereby, producing galls throughout the root system of infected plants (Thangamani *et al*., 2018) [32]. They further interfered with the plants metabolic activities like water and nutrient uptake thus, leading to stunted growth of plants and also drastic reduction in fruit size and quality and hence, plant loses vigor and heavy yield losses are incurred (Anwar *et al*., 2006) [4]. Damage is most severe when infestation occurs in early stages of plant growth. Infecting stage of nematode is Juvenile stage (J_2) which penetrates into the roots and further into the vascular tissues of plants forming permanent feeding site (Williamson and Hussey, 1996) [34]. In the tropical and sub-tropical climates, yield losses were estimated at 14.6% in the developing countries compared to 8.8% in developed countries with an average of 12.3%. In India, the annual estimated crop losses due to major plant parasitic nematodes were estimated to the tune of Rs. 242.1 billion. In India, an average annual loss 19.6% has been estimated due to plant parasitic nematodes. However, in protected cultivation, an overall average annual yield loss in major horticultural crops due to nematodes goes up to 60%. (Gowda *et al*., 2017) [12]. The use of resistant rootstocks reduces dependency on agrochemicals, so grafting is therefore, considered ecofriendly for Louws, 2008) [28].

Use of rootstocks can counter plant biotic responses by improving plant vigour through vigorous attainment of soil nutrients, avoidance of soil pathogens and tolerance to low soil temperature and salinity. The type of rootstock affects scion growth, yield and quality of fruits. Besides improving productivity, in terms of increased resistance against biotic and abiotic stresses rootstocks also help to ensure better fruit set during off-season. Changing rootsystem through grafting in vegetable crops is becoming an important tool not only to manage soil borne diseases but also to improve crop response to a variety of abiotic and biotic stresses.

Symptoms produced in plants as a result of damage by nematodes are characterized by yellowing of foliage wilting during hot dry periods particularly in broad leaved crops, necrosis, formation of galls and twisting of stem (Haq *et al*., 2011) [13]. It is a fact that still farmers from remote parts of the country are unable to use various modern tools to manage biotic stress. Poor access to tools and products of modern tool box of crop management is retrogressive to ambitions of growth in agriculture. Use of grafted seedlings became a modern tool to counter biotic as well as abiotic stresses on farmer's field, thus benefitting resource poor, less knowledgeable farmers and is gaining popularity in case of cucurbits, tomato, eggplant and pepper. (Pogonyi *et al*., 2005) [24].This technology is gaining importance in areas where land is intensive and continuous cropping is practiced (Khah *et al*., 2006) [16]. Commercial vegetable grafting is a new technique and the area under vegetable grafting is progressively increasing (Kumar *et al.*, 2018) [17]. Vegetable grafting is one of the alternative tools to slow breeding procedures which is further used for development of resistant varieties. Therefore, this study was undertaken to identify and evaluate sixteen different rootstocks for their resistance to *Meliodogyne incognita.*

Materials and Methods

Sixteen different rootstocks namely, Hawaii-7996, Hawaii-7998, Palam Pink, Palam Pride, LS-89, Green Gourd, Back attack, VI-045376 (EG-203), VI-047335 (EG-195), V1-034845, Arka Nidhi, Arka Keshav, *Solanum torvum* (Wild Brinjal), AVPP0205, PI-201232 and Local Pumpkin were screened for resistance to root knot nematodes in pot culture. The experiment was carried out during 2017-18 and 2018-19 at the Entomology Screen House, CSKHPKV, Palampur (India) in a Randomized Block Design (RBD).

Extraction of *Meloidogyne icognita* eggs from infested roots

Roots having nematode galls were collected from experimental plots laid under protected conditions in the Department of Vegetable Science and Floriculture, CSKHPKV, Palampur. Roots containing egg masses were dissolved in flask containing 25 ml of water and *Meloidogyne incognita* eggs were extracted from galled roots of tomato using 2.5 ml sodium hypochlorite (NaOCl) (Hussey and Barker, 1973) [14] and flask was then shaken for about 5 minutes to dissolve the egg masses from galled roots. The contents of the flask (roots + NaOCl) were passed through a 200 mesh sieve nested over a 500 mesh sieve. From 500 mesh sieve, the eggs were collected into a beaker by rinsing water. The contents of the beaker were finally transferred on a moulded wire mesh lined with six layers of tissue paper. The wire mesh along with these tissue paper kept over a Petri plate containing water filled up to the level that touches the layer of tissue paper on the wire mesh. After 24 hr, the contents of the Petri plate were examined for freshly hatched J_2 of the nematode.

Analysis of the samples for nematode

Soil samples of 200 cc each, were processed by Cobb's Sieving and decanting technique (Cobb, 1918) [9]. The volume of nematode suspension collected in a Petri plate after 24 hr of washing was made up to 100 ml. Next day larvae were counted in counting dish under sterezoom microscope by taking an aliquot of one ml drawn after gently agitating the suspension. Total population of *M. incognita* in the sample was determined by multiplying the count with 100.

Screening of rootstocks for resistance to *Meloidogyne incognita*

The preliminary screening was done in pots filled with sterilized soil mixed with well decomposed farmyard manure. Three to four seeds of each rootstock were sown in a pot, having 1 Kg of sterilized soil. After one week of germination, plants were thinned to one and J_2 of *Meloidogyne icognita* @ 1000/ pot/ plant were inoculated in the rhizosphere of plants as per method suggested by (Sasser *et al*., 1957) [30]. There were three replications for each rootstock. After 45 days of inoculation, plants were uprooted, washed gently under tap water, cut into small bits and examined under sterozoom microscope for the number of galls. Each rootstock was rated for their resistance/ susceptibility as per the following rating scheme given by (Gaur *et al*., 2001) [11]. No galls, no egg masses- Highly resistant (HR), 1-10 galls/ egg masses per plant Resistant (R), 11-30 galls/ egg masses per plant-Moderately resistant (MR), 31-100 galls/ egg masses per plant- Susceptible (S) and 101 and above egg masses per plant- Highly Susceptible (HS).

After Screening of rootstocks grafting was done and seedlings were transplanted in randomized block design

The different rootstocks used in the present studies were procured from world vegetable centre- Taiwan, CSKHPKV, Japan, Palampur and IIHR-Bengaluru.Whereas, scion of tomato was a commercial private sector hybrid from Golden Seeds, UPL Ltd. Total forty treatments comprising of thirteen rootstocks and Control non-grafted were used. The grafted seedlings were transplanted in a Randomized Block Design (RBD) having three replications in a modified naturally ventilated quonset polyhouse of the size 25 m × 10 m at a spacing of 70 x 30 cm.

The scion variety GS-600 was grafted on various rootstocks using cleft grafting on attaining graftable height of 15-20 cm with stem thickness of 5-10 mm to ensure higher grafting success rate and compatibility. Scion seedlings were grafted on various rootstocks on 24th, 26th and 27th August 2017, while transplanting was done on 12th September 2017. Whereas, during 2018 seedlings were grafted on 12th, 14th and 15th April 2018 and transplanting was done on 24th May, 2018. Graft union was secured with a grafting clip or plastic tape to ensure good vascular connection and to ensure complete healing of grafted portions.

Immediately after grafting the plants were sprayed with water and were kept inside grafting chamber (healing chamber) for 3-4 days. Water was sprayed on grafted plants during day once or twice depending on weather conditions so as to avoid wilting and ensure complete healing. For successful healing of grafted seedlings reduced light intensity, moderate temperature (25-30^0 C) and high relative humidity (85-90%) are essential to establish good vascular connection and continue to grow as single plant.

On an average tomato took 2-3 days, brinjal 3-5 days and chilli 5-7 days for complete and strong vascular union when such conditions were maintained and care taken fully for a specific period of time. After completion of healing processes the plastic clips were removed from graft union so as to avoid cessation and stunted growth of plants. For acclimatization grafted seedlings were taken outside the healing chamber and kept under sunlight so as to provide hardening prior to transplanting and to reduce transplanting shock. On an average grafted seedlings took three to four days for complete acclimatization and later they were transplanted in well prepared beds inside naturally ventilated poly house. Observations were recorded on following traits: Total number of marketable fruits were calculated by adding the number of marketable fruits harvested in each picking. The average fruit weight was calculated by dividing the total marketable yield of five selected plants by total number of fruits. The fruits were harvested at different intervals of 5-7 days till last harvesting. Fruit yield per plant was calculated by adding yield of all pickings. Yield per square metre area was calculated by counting the number of plants per square metre area and multiplied by yield per plant.

Data Analysis: Data was analyzed as per the methods suggested by Panse and Sukhtame, 1984 [23].

Results and Discussion

The root gall rating ranged from 1 to 157. The lowest gall ratings were observed on Green Gourd (RKI-1), PI-201232 (RKI-2), AVPP0205 (RKI-3), VI-034845 (RKI-5), and VI-047335 (RKI-16) and the highest were recorded on LS-89 (RKI-157) (Table 1). Resistant cultivars tolerated some root knot nematode reproduction and were significantly lower in resistant cultivars as compared to susceptible and highly susceptible plants. Rootstocks screened had different response for root knot nematodes. Grafting susceptible tomato scions with desirable horticultural traits onto cultivars that confer resistance to root-knot nematodes was a viable management technique for tomato growers.Total sixteen rootstocks were screened for nematode resistance, out of seven rootstocks of tomato one rootstock Green Gourd showed resistance, whereas, out of six brinjal rootstocks one rootstock VI-034845 exhibited resistance, four were susceptible and one rootstock VI-047335 showed mild resistance. Two chilli rootstocks viz., AVPP0205 and PI-201232 were found resistant. One rootstock of local pumpkin was recorded as susceptible to ***Meliodogyne incognita.*** Susceptible host plants did not tolerate root knot nematode attack. They enter plant roots, start reproduction and produce severe root galling (Karssen and Moens, 2006) [15]. The resistant cultivars as rootstocks reduced nematode reproduction compared to susceptible cultivars as suggested by (Owusu *et al*., 2016). These rootstocks, can serve as potential rootstocks in nematode management under protected conditions.

Table 1: Reaction after inoculation of *Meliodogyne incognita* during 2017-18 and 2018-19 under pot conditions

Sr. No.	Crop	Rootstocks	RKI after 45 days of inoculation	Reaction
1.	Tomato	Hawaii-7996	78	Susceptible
2.	Tomato	Hawaii-7998	37	Susceptible
3.	Tomato	LS-89	157	Highly Susceptible
4.	Tomato	Green Gourd	1	Resistant
5.	Tomato	Back Attack	35	Susceptible
6.	Tomato	Palam Pink	93	Susceptible
7.	Tomato	Palam Pride	39	Susceptible
8.	Brinjal	VI-034845	5	Resistant
9.	Brinjal	Arka Nidhi	35	Susceptible
10.	Brinjal	Arka Keshav	31	Susceptible
11.	Brinjal	VI-047335 (EG-195)	16	Moderately Resistant
12.	Brinjal	VI-045376 (EG-203)	62	Susceptible
13.	Brinjal	*Solanum torvum*	75	Susceptible
14.	Chilli	AVPP0205	3	Resistant
15.	Chilli	PI-201232	2	Resistant
16.	Pumpkin	Local Pumpkin	56	Susceptible

Out of sixteen rootstocks only thirteen rootstocks were found compatible with scion GS-600, whereas three rootstocks viz., PI-201232, AVPP0205 and Local pumpkin did not show compatibility. However, initially they showed some growth but later on their growth was ceased. Therefore, only one parameter i.e. plant height was observed on plants grafted on these rootstocks, whereas other parameters could not be recorded due to poor stock-scion compatibility.

Number of marketable fruits per plant

Number of fruits is an important factor which contributes remarkably for total yield on a plant or per hectare basis. To obtain higher yield number of fruits per plant should be more with marketable quality and fruit weight.

Rootstocks significantly affected the number of fruits per plant as evident from the (Table 2). Plants grafted on rootstock Green Gourd produced maximum number of marketable fruits per plant (24.33) during 2016-17 which was statistically at par with Palam Pride (22.67). Whereas, in 2017-18 highest number of marketable fruits per plant were also observed in plants grafted on rootstock Green Gourd (23.00) which were at par with Arka Keshav (22.67), Back Attack (22.33), VI-45376 (22.33), *Solanum torvum* (22.67), LS-89 (22.67), Palam Pride (21.33) and VI-47335 (21.00).

Pooled analysis of data also showed maximum number of fruits per plant in plants grafted on rootstock Green Gourd (23.67) followed by *Solanum torvum* (21.67), Arka Keshav (21.17), and VI-47335 (21.17). Non-grafted plants recorded 32.40% less number of marketable fruits than grafted. The increased number of marketable fruits in grafted plants as compared to non- grafted was due to use of vigorous rootstocks which led to improvement of cytokinin content in scion which ultimately improved fruit load on the plants. Similar findings were also reported by Fernandez *et al* (2013)

[10], Kumar *et al* (2017) [18], Rahmatian *et al* (2014) [26], Tamilselvi and Pugalendhi, (2017) [31] and Velkov and Pevicharova, 2016 [33].

Table 2: Effect of rootstocks on yield and other contributing traits in tomato under protected conditions

Rootstocks	Number of marketable Fruits/plant			Average Fruit Weight (g)			Marketable fruit yield/plant (kg)		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
Back Attack	21.33	22.33	21.83	77.85	79.00	78.43	1.66	1.76	1.71
Palam Pride	22.67	21.33	22.00	88.66	85.85	87.25	2.01	1.83	1.92
Palam Pink	21.67	19.33	20.50	83.06	80.70	81.88	1.80	1.56	1.68
Hawaii-7998	19.67	20.33	20.00	76.76	80.67	78.72	1.51	1.64	1.58
Green Gourd	24.33	23.00	23.67	90.83	92.17	91.50	2.21	2.12	2.16
Hawaii-7996	20.00	18.33	19.17	82.00	78.01	80.00	1.64	1.43	1.54
LS-89	19.00	22.67	20.83	78.42	81.15	79.79	1.49	1.84	1.67
VI-34845	19.33	18.00	18.65	79.67	76.67	78.17	1.54	1.38	1.46
Arka Nidhi	20.67	17.33	19.00	70.15	75.00	72.58	1.45	1.30	1.38
Arka Keshav	19.67	22.67	21.17	86.43	84.25	85.34	1.70	1.91	1.80
Solanum torvum	20.67	22.67	21.67	73.00	75.98	74.49	1.51	1.72	1.61
VI-47335 (EG-195)	21.33	21.00	21.17	72.67	74.76	73.72	1.55	1.57	1.56
VI-45376 (EG-203)	22.00	22.33	22.17	82.72	77.93	80.33	1.82	1.74	1.78
Control (GS-600)	15.33	16.67	16.00	69.79	68.98	69.48	1.07	1.15	1.11
CD (0.05)	1.78	2.23	1.49	2.22	1.95	1.60	0.03	0.02	0.02
CV (%)	6.33	7.87	5.28	1.96	1.75	1.42	1.43	1.18	1.02

Average fruit weight (g)

Rootstocks had significant effects on average fruit weight of tomato during both the years (Table 2). Plants grafted on rootstock Green Gourd recorded highest average fruit weight (90.83 g) followed by rootstock Palam Pride (88.66 g) during 2016-17. Whereas, plants grafted on rootstock Green Gourd also recorded highest average fruit weight (92.17 g) during 2017-18 followed by Palam Pride (85.85 g), Arka Keshav (84.25 g), LS-89 (81.15 g), Palam Pink (80.70 g) and Hawaii-7998 (80.67 g). Similarly, pooled analysis of data also showed maximum average fruit weight in plants grafted on rootstock Green Gourd (91.50 g) which was followed by Palam Pride (87.25 g), Arka Keshav (85.34 g), Palam Pink (81.88 g) and VI-45376 (80.33 g). Non-grafted plants recorded 24.06 % less fruit weight in comparison to grafted ones.

Higher average fruit weight in grafted plants might be due to interactions between rootstocks and scion which influenced more efficient uptake of minerals, water and nutrients throughout the plant system. Results are supported by the conclusions drawn from the findings of Fernandez *et al*. (2013) [10], Moncada *et al*. (2013) [20], Rahmatian *et al* (2014) [26] and Riga 2015 [27].

Marketable fruit yield per plant (kg)

It is apparent from the data presented in the (Table 2) that different rootstocks affected the fruit yield per plant significantly. Plants grafted on rootstock Green Gourd produced maximum fruit yield per plant (2.21 kg) followed by Palam Pride (2.01 kg), VI-45376 (1.82 g) and Palam Pink (1.80 g). In the year, 2017-18 maximum fruit yield was also recorded in plants grafted on rootstock Green Gourd (2.12 kg) which was followed by Arka Keshav (1.91 kg), LS-89 (1.84 kg) and Palam Pride (1.83 kg). Pooled analysis of data showed that plants grafted on rootstock Green Gourd resulted in maximum yield per plant (2.16 kg) followed by Palam Pride (1.92kg), Arka Keshav (1.80kg) and VI-45376 (1.78 kg). Thus, grafted plants produced 48.61% more yield than non-grafted.

The differences in yield response observed may be due to method of grafting, different growth characteristics of cultivars and different response to grafting, growth period and compatibility of rootstock and scions. Higher yield in grafted plants is attributed to resistance provided by the rootstocks against soil borne diseases (Bacterial wilt & Nematodes), better absorption and translocation of phosphorus, nitrogen, magnesium and calcium which leads to improved nutrient uptake as rootstocks have well developed and strong root systems which release more cytokinins into the xylem sap resulting in increased yield. Similar findings were reported by Aloni *et al* (2010), Bogoescu and Doltu (2015), Blestos *et al* (2003), Lee (1994), Marsic and Osvald (2004) and Pulgar *et al* (2000) [1, 8, 7, 21, 25].

Marketable fruit yield per square metre (kg)

From the Data presented in the Table 3 it is inferred that rootstocks exerted significant influence on yield per square metre. During 2016-17, Maximum yield per square metre was obtained in plants grafted on rootstock Green Gourd (26.52 kg) followed by Palam Pride (24.12), VI-45376 (21.84 kg), Palam Pink (21.60 kg) and Arka Keshav (20.40 kg). In the year, 2017-18 maximum yield per square metre was also reported in plants grafted on rootstock Green Gourd (25.44 kg) followed by Arka Keshav (22.92 kg), LS-89 (22.08 kg), VI-45376 (20.88 kg) and *Solanum torvum* (20.64 kg).

Table 3: Effect of rootstocks on marketable yield /square metre in tomato under protected conditions

Rootstocks	Marketable fruit yield/sq.m (kg/m^2)		
	2016-17	2017-18	Pooled
Back Attack	19.92	21.12	20.52
Palam Pride	24.12	21.96	23.04
Palam Pink	21.60	18.72	20.16
Hawaii-7998	18.12	19.68	18.90
Green Gourd	26.52	25.44	25.92
Hawaii-7996	19.68	17.16	18.42

LS-89	17.88	22.08	20.04
VI-34845	18.48	16.56	17.52
Arka Nidhi	17.40	15.60	16.50
Arka Keshav	20.40	22.92	21.66
Solanum torvum	18.12	20.64	19.38
VI-47335 (EG-195)	18.60	18.84	18.72
VI-45376 (EG-203)	21.84	20.88	21.36
Control (GS-600)	12.84	13.80	13.32
CD (0.05)	0.96	1.18	0.74
CV (%)	5.04	6.25	3.90

Pooled analysis of data also showed maximum yield per square metre in the rootstock Green Gourd (25.92 kg) followed by Palam Pride (23.04 kg), Arka Keshav (21.66 kg), VI-45376 (21.36 kg), Back Attack (20.52 kg) and LS-89 (20.04 kg). Grafted plants produced 48.61% more yield per square metre than non-grafted. The higher marketable yield obtained by grafting was due to an improvement in water and nutrient uptake by the vigorous rootstocks more efficiently, prolonged harvest duration, earliness in flowering and fruiting, increased fruit weight, number of fruits per plant, rootstock scion combinations, or due to low sunlight and low carbon-dioxide content in greenhouses during winter months.. These results are in conformity with the findings of Alvarado *et al* (2017), Al-Harbi *et al* (2016), Kyriacou *et al* (2017), Rahmatian *et al* (2014) [2, 3, 19, 26] and Turkmen *et al* (2010).

Conclusions

Rootstock Green Gourd and VI-34845 were found resistant for nematode incidence as well as for maximum yield per square metre. But, Green Gourd had higher marketable yield per square metre than VI-34845. Rootstock Green Gourd, VI-034845, AVPP0205 and PI-201232 were found resistant to root- knot nematode incidence. Therefore, they can be used for managing biotic stresses caused by nematodes efficiently under protected conditions. Nematode-resistant rootstocks can be further utilized as a potential source of resistance in reducing nematode infestations under polyhouse conditions. Thus, grafting of susceptible tomato scions on resistant rootstocks was proven as an effective management strategy in combating RKNs besides reducing cost of production, improving yield and quality. Resistant rootstocks proved as an alternative strategy to reduce dependency on agrochemicals which can be regarded as one of the most appropriate approaches for nematode management

Therefore, selection and appropriate use of scion/rootstock combinations that possess desirable horticultural traits in combination with resistance to root knot nematodes can be considered as innovative tool in comparison to long term breeding procedures. Overall, grafting susceptible cultivars onto resistant rootstocks came up as potential and as a practical component for root-knot nematode control under mid hill conditions of Himachal Pradesh (India). Thus, adoption of resistant rootstocks, as an alternative control for root-knot nematodes can also prove non-hazardous to environment besides remaining farmers driendly.

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