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Nihal R
PhD Research Scholar, Department of Agricultural Entomology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Karan Singh Guleria
PhD Research Scholar, Department of Agricultural Entomology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Snehalatha N
M.Sc. Research Scholar, Department of Agricultural Entomology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Thakoor Pavan
PhD Research Scholar, Department of Agricultural Entomology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Correspondence
Nihal R
PhD Research Scholar, Department of Agricultural Entomology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Evaluation of tomato germplasms for resistance against root knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood race 2

Nihal R, Karan Singh Guleria, Snehalatha N and Thakoor Pavan

Abstract
The experiment was conducted during the year 2016 and 2017 at Department of Agricutural Entomology, BCKV, West Bengal to evaluate the germplasm resistance against root knot nematode. Fifty one cultivars of tomato were evaluated and the results obtained from the study on first experiment revealed that out of 51 genotypes, no genotype was highly resistant, genotypes (EC- 620394, EC- 620427, EC- 617047) were resistant having 1.1 to 2.0 root gall index, 10 genotypes exhibited moderately resistant reaction having root gall index between 2.1 to 3.0 and 20 were found susceptible and 18 lines were found to be highly susceptible. The correlation of root knot index with both root and shoot attributes conforms that the growth of root length, shoot length, fresh shoot weight and dry shoot weight is negatively correlated to the root knot index. While in case of egg mass, fresh root weight and dry root weight, increase was seen with increase in the root knot index.

Keywords: germplasms, resistance, root knot nematode

1. Introduction

Tomato (*Solanum lycopersicum*) is an important edible crop in India but productivity is very low (214.5 q/ha), compared to the developed countries, and this can be attributed to the vulnerability of tomato crop to various diseases including fungal, viral, bacterial and nematode diseases (Horna *et al*., 2006) [1]. Root knot nematode is one of the important pest of tomato. Their short life cycle of six to eight weeks enables them to survive well in the presence of a suitable host and their populations build up to a maximum usually as crops reach maturity (Shurtleff and Averre, 2000) [2]. Because of nematode parasitism root weight increases whereas shoot weight declines, shifting the root-shoot balance (Roberts, 1995) [3]. There is a need for development of alternative control strategies and long-term integrative approaches in order to replace chemical nematicides (Martin, 2003) [4]. Resistance in crops is one of the most effective and ecofriendly components of integrated pest management and inclusion of this property ensures increased crop yield in the presence of nematode (Khan and Mukhopadhyay, 2004) [5]. Genetic resistance in tomato against root knot nematodes is efficient in reducing their population densities and thereby, reducing the need for pesticide application. Host plant resistance has been prioritized over chemical, biological, cultural, and regulatory control components as a major goal for pest management because it provides an effective, sustainable and economical method for managing nematodes. Host plant resistance remains a very important potential component of a solution to many nematode problems of tropical agriculture especially, for the low input, small-scale farmers when used in combination with cultural techniques and traditionally grown crops (Luc *et al*., 2005) [6]. Keeping the above background information in view, the present study was undertaken.

2. Materials and Methods

The pot trial was conducted in the net house AICRP on Nematodes in Cropping Systems, Directorate of Research, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, during 2016-2017 to primarily investigate response of different tomato germplasms against root knot nematode. The net house is located at 22° 56' N latitude 88° 32' E and at 9.75 meter above mean sea level. 51 tomato germplasms were evaluated in three replications following Completely Randomized Design (CRD). 50 out of which were from Project coordinator AICRP on nematodes, ICAR (New Delhi) and one from local market.

The nematode inoculum used was obtained from heavily root-knot nematode infested tomato in net house conditions.

2.1 Extraction and counting of root-knot nematode eggs

Extraction of nematode eggs was done by using modified method of Hussey and Barker, (1973) [7]. Root-knot nematodes-infested tomato roots were washed, dabbed dry and then cut into pieces with a pair of scissors. The chopped roots were placed in a bottle and 0.5% sodium hypochlorite (NaOCl) solution was added to cover the roots and was agitated vigorously for four minutes. The mixture was collected and rinsed with tap water on 200 µm-pore mesh sieve over 500 µm-pore mesh sieve. Water was added to obtain the actual egg-water suspension. Root-knot nematode eggs were counted using a counting tray with the aid of a stereo microscope. Counting was done three times per entry.

2.2 Extraction and counting of root-knot nematode juveniles

Juveniles were extracted by using modified Baermann tray method (Whitehead and Hemming, 1965) [8]. The roots were chopped and 5g weight of each entry was placed in a plastic sieve lined with a two-ply tissue paper placed in a plastic plate. Tap water was poured gently into the plastic plate in which the sieve was placed until the tissue became moist. The set-up was left for 48h and the plates were then poured separately into beakers and left overnight for the juveniles to settle. Counting was done three times to obtain the mean number of juveniles.

2.3 Sterilization of potting medium

Potting medium was comprised of soil, sand and vermicompost in 3:1:1 ratio. The required amount of media was sterilized by 4 % formaldehyde solution. The treated media was covered by black polythene sheet and was removed after 7 days of treatment. The media was then pulverized regularly for a week to facilitate the removal of gas from the media.

2.4 Pot filling, sowing of crop and inoculation of pathogen

The potting media was ready to use after 15 days from the removal of polythene sheet. The earthen pots were filled with sterilized soil @ 1000 cc pot. Three seeds were sown in each pot. Only one plant per pot was kept after one week of germination. *M. incognita* race- 2 was selected as a test pathogen. Pure culture of *M. incognita* race- 2 was maintained on tomato roots in the net house. The egg masses were collected from the galled roots of tomato and allowed to hatch in Petri plates containing distilled water. The second stage infective juveniles (J_2) were collected from Petri dishes and counting of inoculums was done in counting dish under stereoscopic binocular using multichambered microscope. 1 J_2 per cc of soil i.e. 1000 J_2 /pot inoculation was done at 3-4 leaves stage of tomato germplasm. For the inoculation three to four holes to a depth of 3-5 cm were made with the help of glass rod near the rhizosphere of plant and the second stage juveniles (J_2) were inoculated with the help of 10 ml pipette and holes were subsequently covered with soil and pots were watered highly after inoculation.

2.5 Observations

The observations on shoot length, root length, fresh root weight, dry root weight, fresh shoot weight, dry shoot weight, root knot index (0-5 scale), and number of egg masses per 5g of root was taken. Counting of galls and egg masses were carried in the laboratory under stereoscopic binocular microscope. After counting roots as well as shoots were kept in paper packets for drying in dry air oven at 45ºC for 4-5 days. Then dry weight was taken. Root knot index was worked out as per the scale (1-5) proposed by All India Co-ordinate Project on Nematodes (Table 1).

Table 1: Root-knot index proposed by AICRP on nematodes.

Observation	Gall index	Reaction
0 no of gall	1	Highly resistant(HR)
1-10 no of galls	2	Resistant(R)
11-30 no of galls	3	Moderate resistant (MR)
31-100 no of galls	4	Susceptible(S)
Above 100 galls	5	Highly susceptible(HS)

2.6 Statistical Analysis

The critical difference (CD) at 5% level of significance was worked out from the data recorded during experiment and compared according to Duncan's Multiple Range Test at 5% level of probability; the data was analyzed in CRD.

3. Results and Discussions

Primary evaluation of fifty one tomato germplasms was conducted in search of source of resistance against southern root-knot nematode, *Meloidogyne incognita* race 2 in pots**.**It was observed that the tomato accession EC- 620427 was recorded for the greatest plant height (51.06 cm) and the lowest height (22.2 cm) was obtained in tomato accession EC-157568. 11 accessions were having no significant difference with EC- 620427 and 6 accessions had no significant difference with EC- 157568. Tomato accession EC- 620427 was recorded for the greatest fresh shoot weight (17.03 g) and the lowest weight (3.3g) was obtained in tomato accession EC-1643342 accessions were having no significant difference with EC- 620427 and 6 accessions had no significant difference with EC-164334. It was observed that the tomato accession EC- 620427 was recorded for the greatest dry shoot weight (3.13g) and the lowest weight (0.34g) was obtained in tomato accession EC-164334. 7 accessions had no significant difference with EC-164334. (Table 3)

With regard to root length, the tomato accession, EC- 620394 exhibited longest root length (7.9 cm) whereas the smallest root length (4.1 cm) was recorded with the accession EC-538153. 3 accessions and 12 accessions were statistically indifferent with the accessions EC- 620394 and EC- 538153 respectively. In respect to fresh root weight the accession EC-620410 was observed to record the greatest fresh root weight (5 g) and the accession EC- 620427 was noted to have roots of smallest weight. Five accessions were statistically at par with EC- 620427 and all other accessions were significantly different from both these two accessions. Performance trend of germplasms with regard to dry root weight of the plants was same as was noted in case of the fresh root weight. In reference to root-knot index, three germplasms were recorded resistant, 10 were moderately resistant and 20 germplasms were susceptible and 18 highly susceptible. However, interestingly six germplasms exhibited no statistically significant difference with the smallest value of root-knot index. It was further observed that number of egg masses in the roots of the germplasm EC- 165395 was lowest, 1 egg mass and this was statistically as par with no other germplasm. Maximum egg masses (40) were obtained in the roots of germplasm EC- 567305 which was statistically at par with the same recorded for the 9 germplasms. (Table 3)

The correlation of root knot index with both root and shoot attributes conforms that the growth of root length, shoot length, fresh shoot weight and dry shoot weight is negatively correlated to the root knot index. While in case of egg mass, fresh root weight and dry root weight, increase was seen with increase in the root knot index. (Table 2)

Root knot index indicates susceptibility of plant to root-knot nematodes where the plant is compelled to accommodate extra growth of cells and excess number of cells, produced in plant under the influence of enzymes secreted by the root-knot nematodes as a concomitant phenomenon during establishment of feeding site of the organism in the plant. This holds true as Khan (1994) [9] reported that the nematode resistance in host plant was manifested by reduced rates of nematode reproduction, egg masses and consequently, low nematode population densities than that of a susceptible one. Hussey and Boerma (1989) [10] reported that this in turn reduces the uptake and transportation of nutrients, which in turn affect their shoot weight. Khan (2000) [11] also reported that there is a general trend of increase in shoot parameters (plant height, number of leaves, fresh shoot weight) and decrease in root parameters (fresh and dry weight) with the increase in resistant level of cultivars. El-Sherif *et al*. (2007) [12] root-knot nematode increases root weight for the most susceptible cultivar compared to resistant cultivar. Fassuliotis (1979) [13] reported that because galling occurs in most susceptible plants infected with root-knot nematodes, this can often be the sole measurement of resistance during screening experiments. Our finding also corroborates the observation all the above research as the susceptible cultivars developed heavier root systems and resistant plants have shown more growth in shoot attributes.

4. Conclusion

Intense observation on the evaluation of genotypes for in tomato against root knot nematode revealed that out of 51 genotypes, no genotype was highly resistant, three genotypes (EC- 620394, EC- 620427, EC- 617047) were resistant, 10 genotypes (EC- 164863, EC- 165700, EC- 520078, EC-521067-B, EC- 620361, EC- 620387, EC- 620401, EC-620406, EC- 620431, EC- 620433**)** exhibited moderately resistant reaction and among the remaining genotypes, 20 were found susceptible and 18 lines were found to be highly susceptible. Susceptible cultivars developed heavier root systems because of root galling, compared to resistant cultivars. Similarly the resistant plants have shown more growth in shoot attributes. The correlation of root knot index with both root and shoot attributes conforms that the growth of root length, shoot length, fresh shoot weight and dry shoot weight is negatively correlated to the root knot index. While in case of egg mass, fresh root weight and dry root weight, increase was seen with increase in the root knot index.

Table 2: Correlation between Root knot index and other parameters

Parameters	Root length	Shoot length	Fresh root weight	Fresh shoot weight	Dry root weight	Dry shoot weight	Egg mass
Root-knot index	-0.867**	-0.917**	0.855**	-0.917**	0.761**	-0.925**	0.723**

Table 3: Shoot parameters, root parameters and number of egg masses in roots of tomato germplasms evaluated against root knot nematode.

S. No.	Germplasm	Shoot length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	Root knot index	Egg mass	Reaction
1	EC-3176	31.47	5.8	0.73	4.86	3.13	0.6	5	23	HS*
2	EC- 145057	26.27	5.46	0.63	4.2	2.9	0.43	5	25	HS
3	EC- 151568	31.6	5.2	0.63	4.87	3.3	0.45	5	21	HS
4	EC- 157568	22.27	4.2	0.47	4.5	3	0.47	5	26	HS
5	EC- 160885	30.6	3.8	0.38	4.8	2.8	0.4	5	19	HS
6	EC- 162601	31.2	4.8	0.67	4.8	2.4	0.33	4.4	13	HS
7	EC- 163605	35.37	6	0.77	5.16	1.6	0.21	4	18	S
8	EC- 164334	27	3.4	0.34	4.3	2.3	0.3	4.8	17	HS
9	EC- 164563	30.36	4.4	0.5	4.7	2.5	0.33	4.4	19	HS
10	EC- 164670	30.86	3.6	0.37	4.6	2.3	0.3	4.4	21	HS
11	EC- 164677	31.17	5.2	0.7	4.83	2.3	0.33	4.6	18	HS
12	EC- 164838	30.63	3.9	0.46	4.76	2.7	0.43	4.4	16	HS
13	EC- 164863	44.5	9.8	1.57	6.26	1.1	0.15	3	11	MR
14	EC- 165395	38.2	6.4	0.8	5.23	1.3	0.18	3.6	1	S
15	EC- 165690	39.33	8.1	0.87	5.4	1.7	0.25	3.8	15	S
16	EC- 165700	45.16	10.03	1.63	6.36	1.1	0.15	2.8	9	MR
17	EC- 249508	39.57	8.03	0.97	5.4	1.5	0.19	3.2	11	S
18	EC- 249514	29.07	5.16	0.56	4.5	2.4	0.33	4.8	18	HS
19	EC- 520078	46.26	10.2	1.72	6.1	0.66	0.13	3	8	MR
20	EC- 521067-B	45.3	10.76	1.78	6.4	1.1	0.13	2.8	9	MR
21	EC- 523851	33.83	8.66	1.16	5.7	2.13	0.27	3.6	17	S
22	EC- 528368	35.3	8.13	0.97	5.5	1.8	0.22	4	11	S
23	EC- 538153	23.5	3.63	0.47	4.1	2.6	0.38	4.2	18	HS
24	EC- 538156	33.53	6.83	0.8	5.1	2.3	0.37	4	23	S
25	EC- 549819	36.03	7.2	1.12	6.2	1.9	0.22	3.8	21	S
26	EC- 567305	40.63	7.06	1.04	6	2.3	0.3	4	40	S
27	EC- 617047	48.53	15.13	2.83	7.3	0.7	0.11	2	2	R
28	EC- 620343	39.26	9.36	1.47	5.9	2.1	0.25	3.2	5	S
29	EC- 620361	46.1	11.23	2.03	7.2	0.8	0.13	2.8	5	MR

30	EC- 620370	36.03	7.33	1.07	6	2	0.27	4	22	S
31	EC- 620372	30.63	5.16	0.7	5.3	2.9	0.47	4.4	35	HS
32	EC- 620373	42.4	9.73	1.33	6.2	1.7	0.27	3.4	7	S
33	EC-620382	40.7	8.8	1.23	6.3	1.7	0.25	4	13	S
34	EC- 620387	42.93	11.53	2.03	6.33	1.1	0.18	2.4	5	MR
35	EC- 620394	49.47	15.43	2.61	7.9	0.7	0.12	2	3	R
36	EC- 620395	37.2	8.1	1.1	5.6	1.9	0.23	3.8	13	S
37	EC- 620396	31.76	4.9	0.67	5	3.2	0.47	4.6	20	HS
38	EC- 620397	41.2	8.5	1.11	5.8	2.3	0.33	3.8	11	S
39	EC- 620401	47.23	13.9	2.26	6.8	1.3	0.17	2.6	7	MR
40	EC- 620406	46.43	14.3	2.32	7.2	1.1	0.15	2.8	5	MR
41	EC- 620410	32.26	5.9	0.73	5.5	5	0.9	5	19	HS
42	EC- 620417	42.2	9.73	1.58	6.3	2.2	0.28	3.2	6	S
43	EC- 620422	41.36	8.9	1.47	6.1	2	0.47	3.4	11	S
44	EC- 620427	51.07	17.03	3.13	7	0.6	0.12	2	2	R
45	EC- 620431	46.1	10.7	1.87	6.3	1	0.13	2.4	5	MR
46	EC- 620433	47.93	13.93	2.42	6.1	1.2	0.2	2.4	5	MR
47	EC- 631359	37.93	7.16	1.03	6.1	2.4	0.37	3.8	11	S
48	EC- 631369	28.3	5.43	0.74	4.7	2.6	0.42	4.4	10	HS
49	EC- 631376	34.23	6.3	0.87	5.5	1.8	0.3	3.8	15	S
50	EC- 631379	33.9	7.9	1.26	5.43	1.3	0.16	4	13	S
51	Check (Patharkuchi)	27.46	6.4	0.76	4.4	2.5	0.39	4.6	10	HS
	LSD(5%)	7.043	1.23	0.27	0.75	0.45	0.084	0.787	4.688	
	CV (%)	11.71	9.45	13.8	8.23	13.98	17.66	16.811	20.789	

*R=Resistant, MR= Moderately resistant, S= Susceptible and HS=Highly susceptible

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