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Evaluation of 14 Tomato Genotypes for Yield and Root Knot Nematode Resistance Parameters

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ABSTRACT

A study was conducted for two seasons to assess the mean performance of yield, yield contributing traits and root knot nematode resistance in 14 genotypes (CLN 2026C, CLN 2026E, CLN 1466J, CLN 1466S, CLN 1464A, PT 4671A, PT 4716A, CO 3, LE 812, Arka Ahuti, Hisar N_1, Hisar N_2, Patriot and SL 120) of tomato (*Lycopersicon esculentum* Mill.). PT 4716A, Hisar N_1, SL 120, Patriot, Hisar N_2 and LE 812 showed superior yield and other yield-related characters but lower root weight. PT 4716A, LE 812, Hisar N_1, Hisar N_2, Patriot and SL 120 did not show any root knot nematode infestation. Total phenol and orthodihydroxy phenol content were highest in SL 120 and LE 812. This evaluation study showed that LE 812, CLN 2026C, CLN 2026E and CLN 1464A showed best yield and root knot nematode resistant characters.

Keywords: fruit weight, orthodihydroxy phenol, total phenol

INTRODUCTION

World production of tomato (*Lycopersicon esculentum* Mill.) is about 100 million tons of fresh fruit produced on 3.7 million ha produced by 144 countries (FAOSTAT Database 2004), the major country being China in both ha of harvested production (1,255,100 ha) and weight of fruit produced (30,102,040 Mt). The two leading countries in fruit yield/ha are the Netherlands (4,961,539 Hg/ha) and Belgium (4,166,667 (Hg/ha) (FAOSTAT Database 2004). According to FAOSTAT, the top producers of tomatoes in 2007 were China, United States, Turkey, India and Egypt.

Root-knot nematodes (RKN; *Meloidogyne* spp.) are one of the major pathogens of tomatoes worldwide and limit fruit production (Sikora and Fernandez 2005). RKN population density also affects yield loss and tolerance levels of different tomato cultivars (Singh Sunil and Khurma Uma 2007). Yield loss in tomato due to RKN had been estimated at 61% (Nirmala Devi and Tikoo 1992). The *Mi* gene originally found in wild tomato species *Lycopersicon peruvianum* is one of the best characterized nematode resistance genes and has been genetically engineered into many commercial tomato varieties (Nono-womdim *et al*. 2002; Abad *et al*. 2003).

RKN cause characteristic galls on roots; galls may be up to 1 inch in diameter, but are usually smaller (**Figs. 1, 2**). These galls interfere with the flow of water and nutrients to the plant; infected plants appear less vigorous than healthy plants, may be yellowed, are prone to wilt in hot weather, and respond poorly to fertilizer. Damage areas usually appear as irregular patches and are frequently associated with lighter-textured soils (Roberts 2008).

There are more than 90 described species in the genus *Meloidogyne* but the four most commonly occurring species are *Meloidogyne incognita*, *M. arenaria*, *M. javanica* and *M. hapla* (Sasser and Taylor 1978; Karssen 2000; Hunt *et al.* 2005). The aim of the present study was to evaluate the mean performance of tomato genotypes, which would be useful in further breeding programmes.

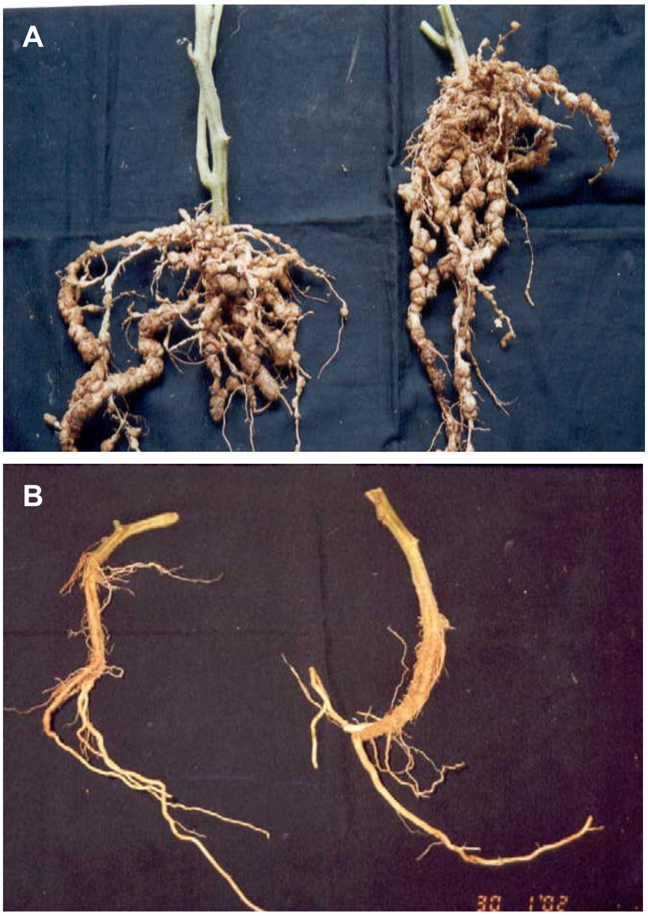

Fig. 1 Root knot symptom in susceptible roots (A) and healthy roots (B).

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Fig. 2 Characteristic symptoms of root knot nematode infestation. 1. Yellowing of leaves 2. Stunted growth 3. Reduced yield.

MATERIALS AND METHODS

In the present study, seeds of 14 tomato genotypes of diverse origin collected from distinct geographical regions *viz*., CLN 2026C, CLN 2026E, CLN 1466J, CLN 1466S, CLN 1464A, PT 4671A, PT 4716A, CO 3, LE 812, Arka Ahuti, Hisar N_1, Hisar N_2, Patriot and SL 120 were sown and nursery-raised at the College orchard, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore in two seasons, January-May and June-October. When the seedlings were 30 days old, they were transplanted at a distance of 60 × 45 cm. Each genotype was replicated thrice in a randomized block design. The harvesting of fruits was started from two months. At the peak of the harvest season a representative composite sample of red-ripe fruits were collected for each replicate and transferred to the laboratory for analysis.

Inoculation

The inocula for resistance were prepared following the method suggested by Bailey (1941). Eggmasses of *M. incognita* were removed from tomato roots with forceps, placed in Petri dishs containing distilled water and incubated for three days under laboratory conditions. The hatched larvae were collected and the nematode population in the suspension was adjusted to a known number by the addition of water. The nematode inoculum was pipetted into a 2-cm deep depression made in the soil around the plants and then covered with sterile sand. Each pot was inoculated at a rate of 1 larva (J_2)/g of soil 25 days after sowing (**Fig. 3**).

Fig. 3 Artificial inoculation of *Meloidogyne incognita* inoculum at the rate of 1 larva (J_2)/g of soil 25 days after sowing under pot culture.

Assessment of nematode population in roots

The roots were harvested 15, 30 and 45 days after inoculation and washed free of soil, stained in boiling acid fuschin lactophenol and cleared in plain lactophenol for 48 hrs. The number of galls and eggmass/root system was assessed by viewing under a binocular microscope. The nematode larvae were dissected out of root galls and mounted on micro slides under a cover slip for microscopic examination to determine the number and sex of different larval stages. The number of eggs present in five egg masses/root system were counted after dispersal in a drop of clear lactophenol and the number of eggs present/root system was arrived at by calculation (Bailey 1941).

Screening for RKN resistance

The test plants were bombarded by planting susceptible COTH-1 variety in rows on all four sides of the plants at a distance of 60 × 45 cm. The weeds around the experimental plots were not removed and no application of any nematicides was done throughout the study.

Root gall indexing

The plants were removed 15, 30 and 45 days after inoculation with the entire root system intact and washed free of soil. The number of root knot galls and egg masses were assessed replication wise. The genotypes were indexed based on the method suggested by Heald *et al.* (1989) from 1 to 5 (1, no galls; 2, 1–25 galls; 3, 26–50 galls; 4, 51–75 galls; 5, >75 galls). Root length was measured from the base of the plant to the tip in 10 randomly selected roots and mean was expressed in cm.

All the genotypes included in the field trials were used for biochemical studies to assess and determine the defense mechanisms. The physical characteristics like plant height (cm), number of fruits/plant, average fruit weight (g), yield/plant (kg) and RKN resistant characters like root length (cm), root weight (g), root gall index, number of females/g root, number of eggmasses/g root, number of eggs/eggmass and Biochemical basis of RKN resistance characters like total phenol (µg/g) (Bray and Thorpe 1954), ortho-dihydroxy phenol (µg/g) (Johnson and Schaal 1957), IAA oxidase (µg/100 mg) (Sadasivam and Manickam 1997), chlorogenic acid (µg/g) (Arnow 1937) and ascorbic acid (mg/100 g) in root was estimated following the procedure given in A.O.A.C. (1975).

The mean data obtained for each character was tabulated and statistically analysed (GENRES statistical software, TNAU, Coimbatore) and the means were compared at a probability of 5% and 1% level (Fisher and Yates 1925).

RESULTS AND DISCUSSION

In the present work, plant height varied significantly in all the 14 investigated genotypes. Plant height ranged from 49.50 to 106.85 cm in CLN 1464A and PT 4716A, respectively. The number of fruits/plant had considerable influence on total fruit yield. The highest number of fruits/plant was recorded in PT 4716A and the lowest number in CLN 1466S. Fruit weight is yet another important trait contributing directly to yield. CLN 1466S had a significantly higher fruit weight than other varieties followed by CLN 1466J and CLN 2026E. The lowest fruit weight was recorded in PT 4716A. The highest yield of fruits/plant was in LE 812 and PT 4671A gave the lowest value. Fruit yield in tomato is determined by fruit weight and number of fruits (Dudi and Kalloo 1982). Yield is a complex character and is

Table 1 Mean performance of selected genotypes for yield and root knot nematode resistance characters (Season I).

Genotypes	Plant height (cm)	№ of fruits/ plant	Fruit weight (g)	Yield/plant (Kg)	Root length (cm)	Root weight (g)	Root knot nematode gall index	№ of females/g root
CLN 2026C	70.10	28.73	51.96	1.49	11.92	18.57	3.3	14.13 (3.82)
CLN 2026E	52.85	29.33	54.44	1.60	14.03	21.27	2.7	11.80 (3.51)
CLN 1466J	52.00	21.04	55.97	1.18	16.00	17.88	3.1	13.90 (3.79)
CLN 1466S	51.55	17.11	74.31	1.27	19.61	19.49	3.3	17.93 (4.29)
CLN 1464A	49.50	34.39	41.79	1.44	16.26	16.19	2.3	7.40 (2.80)
PT 4671A	52.35	55.72	18.87	1.05	19.11	20.03	2.1	7.15 (2.76)
PT 4716A	106.85	113.25	10.75	1.22	23.55	11.59	1.0	0.00 (0.71)
CO 3	74.45	32.43	48.92	1.59	26.13	12.08	2.0	6.70 (2.67)
LE 812	61.95	42.59	42.43	1.81	26.39	14.40	1.0	0.00 (0.71)
Arka Ahuti	66.85	42.41	38.93	1.66	17.73	18.04	2.2	12.12 (3.55)
Hisar N_1	51.95	54.77	20.71	1.14	23.10	13.07	1.0	0.00 (0.71)
Hisar N_2	54.05	23.70	52.20	1.24	25.86	14.06	1.0	0.00 (0.71)
Patriot	61.15	40.63	27.84	1.13	23.95	14.35	1.0	0.00 (0.71)
SL 120	67.95	21.77	79.98	1.74	27.27	13.46	1.0	0.00 (0.71)
Mean	62.40	39.85	44.22	1.40	20.78	16.03	1.93	6.51 (2.25)
SE	1.551	1.552	1.460	0.080	1.621	1.177	0.287	0.140
CD (0.01)	4.144	4.148	3.903	0.215	4.333	3.146	0.767	0.375
CD(0.05)	3.112	3.114	2.930	0.162	3.253	2.361	0.576	0.282

(Values in the parenthesis indicate transformed values)

Table 1A Mean performance of selected genotypes for yield and root knot nematode resistance characters (Season I).

Genotypes	№ of eggmasses/g root	№ of eggs/ eggmass	Total phenol (µg/g)	Ortho dihydroxy phenol (µg/g)	IAA oxidase (µg/100 mg)	Chlorogenic acid (µg/g)	Ascorbic acid in roots (mg/100 g)
CLN 2026C	10.80 (3.36)	158.30 (12.58)	78.50	32.50	37.25	24.00	28.60
CLN 2026E	8.98 (3.07)	137.75 (11.75)	62.50	23.50	31.70	21.50	25.60
CLN 1466J	11.65 (3.49)	99.28 (9.98)	46.00	16.38	21.50	13.50	31.00
CLN 1466S	15.95 (4.03)	166.00 (12.90)	44.00	20.75	47.00	15.75	14.65
CLN 1464A	4.75 (2.29)	99.80 (10.02)	62.50	36.75	38.75	27.75	28.60
PT 4671A	5.83 (2.52)	91.99 (10.24)	67.00	39.50	77.25	26.88	14.05
PT 4716A	0.00 (0.71)	0.00 (0.71)	118.50	60.75	33.88	68.75	52.73
CO 3	5.15 (2.36)	105.00 (10.27)	79.00	25.75	37.25	26.70	31.75
LE 812	0.00 (0.71)	0.00 (0.71)	109.00	63.50	81.25	64.00	42.25
Arka Ahuti	5.65 (3.02)	98.18 (9.93)	62.50	20.56	20.63	17.75	33.90
Hisar N_1	0.00 (0.71)	0.00 (0.71)	113.50	50.58	75.50	59.58	46.60
Hisar N_2	0.00 (0.71)	0.00 (0.71)	112.50	53.00	68.50	63.63	55.85
Patriot	0.00 (0.71)	0.00 (0.71)	114.00	56.88	72.50	74.75	58.50
SL 120	0.00 (0.71)	0.00 (0.71)	129.00	64.25	83.75	77.75	60.10
Mean	4.91(2.03)	68.31(6.57)	85.61	40.33	51.91	41.59	37.44
SE	0.162	0.283	4.739	3.650	1.404	1.660	0.793
CD (0.01)	0.435	0.757	12.662	9.754	3.752	4.437	2.120
CD(0.05)	0.326	0.568	9.506	7.323	2.817	3.331	1.592

(Values in the parenthesis indicate transformed values)

dependent on its component traits and their inheritance. Any change in these would reflect on yield. Considering the yield, the mean expression of the parent LE 812 was high (**Table 1, 1A, 2, 2A**).

RKN resistant characters

Root length is a good indication of growth of tomato resisting RKN infestation. The highest root length (27.27 cm) was recorded by SL 120 and the lowest (11.92 cm) was by CLN 2026C. The mean performance for root length revealed superiority of SL 120, LE 812 and Hisar N_2.

Root weight is yet another trait that indicates the resistance or susceptibility of plants. RKN infested plants show higher root weight because of galls. The resistant plants show normal fresh root weight. The highest fresh root weight (21.27 g) was recorded by the genotypes CLN 2026E and PT 4716A showed the lowest root weight of 11.59 g. The mean performance of genotypes indicated that the genotypes PT 4716A, Hisar N_1 and SL 120 recorded the lowest fresh root weight.

The susceptibility of a plant to RKN depends on the ability of RKN juveniles to penetrate the roots of the plant and cause the formation of giant cells which appear as knots (galls) on the roots (Chen and Dickson 2004). Evaluation of tomato for its resistance or susceptibility to RKN by various methods includes root gall count. Taking this into consideration in most cases rating is done into different classes depending upon the intensity of gall formation as suggested by Heald *et al*. (1989). Differential susceptibility to species/ populations of *Meliodogyne* has been reported in tomato (Netscher 1977; Viglierchio 1978). The *Mi* gene confers resistance by localized tissue necrosis around the region where the juveniles penetrate, thus juveniles are unable to establish feeding sites resulting in their death or migration out of the roots (Milligan *et al*. 1998; López-Pérez *et al*. 2006). In the present investigation, PT 4716A, LE 812, Hisar N_1, Hisar N_2, Patriot and SL 120 which produced no galls were scored as '1' under field conditions in both seasons. The genotypes were indexed for RKN galls on a 1–5 scale. Among the genotypes PT 4716A, LE 812, Hisar N_1, Hisar N_2, Patriot and SL 120 were scored as 1, i.e. rated as highly resistant to RKN.

With respect to the number of females/g root the highest number (17.93) was recorded by CLN 1466S hile CO 3 recorded the lowest number (6.70). Hisar N_1, Hisar N_2, Patriot and SL 120 showed no galls and were scored as 1.

Infection index based on number of eggmasses was suggested by Kushman and Machmer (1947) as one of the methods of scoring resistance to RKN. Usually galling is

Table 2 Mean performance of selected genotypes for yield and root knot nematode resistance characters (Season II).

Genotypes	Plant height (cm)	№ of fruits/ plant	Fruit weight (g)	Yield/plant (Kg)	Root length (cm)	Root weight (g)	Root knot nematode gall index	№ of females/g root
CLN 2026C	72.25	30.70	52.72	1.61	12.75	21.30	3.1	13.70
CLN 2026E	81.59	31.88	54.27	1.73	15.68	23.53	2.8	11.23
CLN 1466J	50.55	25.10	44.48	1.12	14.80	20.55	3.8	19.50
CLN 1466S	48.26	19.30	51.06	0.97	17.75	21.85	3.1	15.38
CLN 1464A	51.30	38.78	51.82	2.01	17.67	19.50	2.4	7.77
PT 4671A	42.38	46.69	20.03	0.93	24.37	22.85	2.2	7.20
PT 4716A	110.54	105.23	11.51	1.21	26.73	13.10	1.0	-
CO 3	71.20	40.98	51.36	2.10	30.21	14.37	2.3	14.15
LE 812	61.26	48.30	44.88	2.17	27.83	15.93	1.0	-
Arka Ahuti	68.25	36.49	32.30	1.18	17.45	17.72	1.0	-
Hisar N_1	47.48	28.98	25.16	0.73	25.38	14.22	1.0	-
Hisar N_2	58.38	41.48	35.34	1.47	27.00	15.57	1.0	-
Patriot	61.40	38.97	25.92	1.01	25.77	15.55	1.0	-
SL 120	70.28	27.78	63.57	1.77	30.88	15.00	1.0	-
Mean	63.94	40.05	40.32	1.43	22.45	17.93	19.07	6.35
SE	0.803	0.569	1.192	0.109	0.872	0.770	0.086	0.013
CD (0.01)	2.134	1.512	3.169	0.291	2.315	2.044	0.228	0.033
CD(0.05)	1.604	1.136	2.382	0.219	1.740	1.536	0.172	0.025

(Values in the parenthesis indicate transformed values)

Table 2A Mean performance of selected genotypes for yield and root knot nematode resistance characters (Season II).

Genotypes	№ of eggmasses/g root	№ of eggs/ eggmass	Total phenol (μg/g)	Ortho dihydroxy phenol (μg/g)	IAA oxidase (μg/100 mg)	Chlorogenic acid (μg/g)	Ascorbic acid in roots (mg/100 g)
CLN 2026C	10.13	147.56	40.75	24.13	28.83	31.10	32.27
CLN 2026E	9.02	136.40	31.54	21.15	30.38	30.30	28.42
CLN 1466J	16.65	145.40	40.33	14.75	18.50	15.75	34.00
CLN 1466S	13.88	132.15	38.73	18.38	42.25	13.50	20.65
CLN 1464A	6.38	106.02	31.33	18.97	24.73	21.80	29.12
PT 4671A	5.53	92.80	28.58	12.47	40.50	22.27	20.03
PT 4716A	-	-	92.58	33.80	71.00	71.45	49.93
CO 3	9.37	135.46	41.73	28.90	53.97	32.10	30.57
LE 812	-	-	114.57	51.48	79.00	75.30	41.80
Arka Ahuti	-	-	52.50	30.15	25.63	18.50	40.90
Hisar N_1	-	-	102.75	41.98	71.00	60.97	48.47
Hisar N_2	-	-	106.48	49.58	74.97	66.10	57.40
Patriot	-	-	103.58	51.20	70.82	79.57	61.37
SL 120	-	-	132.42	66.72	80.10	82.45	63.72
Mean	5.07	55.27	68.42	33.12	50.83	44.37	39.90
SE	0.011	0.005	1.805	1.856	0.791	0.862	1.234
CD (0.01)	0.030	0.013	4.796	4.929	2.100	2.291	3.356
CD(0.05)	0.022	0.010	3.605	3.705	1.579	1.721	2.523

the response of the host to root knot infestation, while egg production indicates the ability of the nematode to complete its life cycle or, in other words, a host's suitability to the invading parasite. The susceptible genotype CLN 1466S recorded the highest number of eggmasses/g root (15.95) and CLN 1464A recorded the lowest number of eggmasses/ g root (4.75). The mean performance showed that the resistant genotypes produced no eggmasses.

Number of eggs/eggmass is yet another RKN resistant trait in tomato. The resistant genotypes Hisar N_1, Hisar N_2, Patriot and SL 120 recorded zero value for number of eggs/ eggmass indicating their resistant behaviour (Das *et al.* 2008). The highest number of eggs/eggmass (166.00) was recorded by the susceptible genotype CLN 1466S and the lowest number (91.99) was recorded by susceptible genotype PT 4671A.

Biochemical characters for resistance

Among the biochemical parameters total phenol content in roots indicates the degree of resistance to RKN (Masood and Husain 1976; Ramesh Kumar *et al*. 2008). The total phenol content was higher in SL 120 followed by PT 4716A. The total phenol content in the root samples ranged from 44.00 in CLN 1466S to 129.00 μg/g in SL 120. In resistant genotypes active phenols may be released from glycosides by increased activities of β-glucosidases and later get oxidized; this is the principal factor responsible for browning and necrotic tissues (Acedo and Rohde 1971).

Orthodihydroxy phenol, a specific group of phenols, is responsible for imparting resistance to pathogens, insects or nematodes in plants. SL 120 recorded the highest orthodihydroxy phenol content followed by CLN 2026C and PT 4716A. The increased levels of orthodihydroxy phenols might have resulted as a means of defensive reaction following RKN infestation since orthodihydroxy phenols are known to be reactive and upon oxidation yield quinones which are still more toxic to invading organisms. These results agree with the observation made by Farkas and Kiraly (1962), Lakshmanan (1981) that plants have a wide range of phytochemicals which impart protective action against nematodes. Among these an aromatic ring bearing a hydroxyl substituent called phenolic substituent has antifungal, antibacterial and antiviral activities. Accumulation of phenolic compounds in host parasite reaction is the general phenomenon of resistance and breakdown of these compounds determined the degree of resistance.

An increase in IAA oxidase activity of plant tissue was found to be a resistant mechanism to RKN. SL 120, LE 812, Hisar N_2 and PT 4671A had a high mean; IAA oxidase activity ranged from 20.63 in Arka Ahuti to 83.75 μg/100 mg in SL 120.

The invasion of tomato roots by RKN results in accumulation of chlorogenic acid, which is subsequently oxidized by the action of the host or nematode polyphenol oxidase resulting in the formation of brown coloured melanin in injured areas. Such compounds might inhibit nematode activity and prevent RKN larvae from penetrating the endodermis into tissues suitable for giant cell production (Singh and Choudhury 1973). Among the genotypes, SL 120 and Patriot showed higher chlorogenic acid content. A similar finding was also reported in pepper, *Capsicum annuum* CM334 by Pegard *et al*. (2005) in which resistance of CM334 to root-knot nematodes was associated with unidentified factors that limited nematode penetration and with post-penetration biochemical responses, including the hypersensitive response, which apparently blocked nematode migration and thereby prevented juvenile development and reproduction. High-performance liquid chromatography analysis suggested that phenolic compounds, especially chlorogenic acid, may be involved in CM334 resistance.

Ascorbic acid content of root tissue is yet another biochemical indicator for resistance mechanism against RKN. Increase in ascorbic acid oxidase activity after nematode infestation in tomato roots was reported by Pankaj *et al.* (1998) and Ramesh Kumar *et al*. (2008). The resistant genotypes showed more ascorbic acid content in roots than the susceptible genotypes. The ascorbic acid content in roots ranged from 14.05 in PT 4671A to 60.10 mg/100 g in SL 120.

CONCLUSION

From the results obtained in our work, it can be concluded that yield, yield-contributing traits and RKN resistant traits of the investigated tomatoes varied significantly. Among these 14 genotypes, CLN 2026C, CLN 2026E, CLN 1464A and LE 812 recorded high mean for yield, yield contributing traits and resistance to RKN (*M. incognita*) in both seasons. So these four genotypes can be exploited in a hybridization programme to develop high-yielding hybrids with resistance to RKN. The susceptibility of the different tomato varieties has important implications on the yield and economic returns thus information on susceptibility to RKN can be useful to farmers while selecting the variety for planting on RKN infested fields.

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