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Comparative evaluation of root knot nematode, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 resistance in tomato (*Lycopersicon esculentum* L.)

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Abstract
An *in vitro* screening method was developed for evaluating tomato accessions under lab condition and compared with normal screening method under glasshouse conditions. Thirty genotypes of tomato were evaluated for resistance against root knot nematode, *Meloidogyne incognita* (Race 3) under glasshouse and laboratory conditions. The lab method was done using Petri dish and germination paper which was named as early detection method. Early detection method is the simple method, where the susceptible varieties can be easily identified within a week after inoculation of root knot juveniles. At 45 days after inoculation of second stage juveniles, the seedlings were uprooted and washed to observe the presence of galls and egg masses. The accessions were ranked based on Root Knot Index (RKI). Accordingly, the reactions of tomato genotypes were indexed. Among 30 accessions screened against root knot nematode, *M. incognita* two accessions (EC 631364, EC 164863) were identified as highly resistant, eight accessions (EC 620394, EC 617051, EC 620288, EC 145615, EC 636874, EC 151568, EC 163606, EC 620498) were identified as resistant. The use of resistant plants is one of the cost effective alternate method to chemicals to manage the plant parasitic nematode incidence.

Keywords: Root knot nematode, *M. incognita*, classical screening, early detection assay (EDA), tomato (*Lycopersicon esculentum* L.)

1. Introduction
Tomato is one of the most frequently used vegetable crops in Indian cusine which provides constant economic support to farmers. Most of the Solanaceous crops are susceptible to pest, diseases and nematodes. Among them, root knot nematode plays a major role in causing economic losses through reduction in crop yield. The frequently occurring root knot nematode species are *M. incognita*, *M. areneria*, *M. javanica* and *M. hapla* (Sasser and Taylor, 1978) [12]. The root knot nematode, *M. incognita* alone causes yield loss of 30 - 75% in tomato (Jain, 1991) [9] by inducing large number of galls throughout the roots thereby damaging the root cells that intercept nutrient uptake from soil leading to day wilting (Anwar, 2010) [2]. The *in vitro* screening method followed in this study was aimed to reduce the screening time and to increase the precision compared to classical screening. Various approaches such as physical, cultural, chemical and biological practices have been used to manage the incidence of root knot nematode. Cultural practises such as soil solarisation and crop rotation show limited value to manage nematodes, due to its broad host range (Ntalli *et al*., 2010) [10]. Cultivation of resistant varieties is one of the strategies to manage nematodes, which is non-chemical, most economical, environmentally safe and easy for the management of root knot nematode, *M. incognita.*

2. Materials and Methods
2.1 Pure culture maintenance of root knot nematode, *M. incognita* (Race 3)
Root knot nematode, *M. incognita* collected from an infested tomato field and the species was confirmed morphologically by Posterior Cuticular Pattern (PCP) as *M. incognita* and egg masses incubated in water for hatching with frequent aeration. The second stage juveniles (J2) were inoculated in to the pots filled with sterile pot mixture (Red earth: FYM: Sand @ 2: 1: 1 ratio) containing susceptible tomato variety (PKM 1) and maintained under glasshouse conditions as pure culture of root knot nematode, *M. incognita*.

2.2 Classical screening of tomato germplasms against root knot nematode, *M. incognita*

The seeds of each tomato accession were sown in portray and maintained in glasshouse with required optimum moisture and temperature (26-30ºC). Pots were filled with sterilised pot mixture and 15 days old tomato seedlings were transplanted in each pot with regular watering. Five replications of each accession were maintained. After seedling establishment, the plants were inoculated with freshly hatched juveniles @ 2 J2/ g of soil. After 45 days of inoculation, the plants were uprooted and numbers of galls on the root were recorded based on Root Knot Index (RKI) (Hartmann and Sasser, 1985) [7].

Table 1: Root knot index, (Hartmann and Sasser, 1985)

Gall Index	No. of galls / egg masses/ plant	Reaction
1	No gall	Highly resistant
2	1 – 10	Resistant
3	11 – 30	Moderately resistant
4	31 – 100	Susceptible
5	>100	Highly susceptible

2.3 Early detection of root knot nematode resistance in tomato

An alternate concept was carried to improve the method developed by Ho *et al*., 1992 [8] with minimum modifications. Germination sheets were cut in to 9cm diameter circular discs. Two layers of such discs were kept within the Petri dish with sufficient moisture. Five surface sterilized (70 % ethanol for 2 – 3 minutes) seeds per accession were placed over the circular disc and the plates were kept in a plant growth chamber adjusted to 25±1°C. After initiation of roots, the root tips were inoculated with 15 second stage juveniles per seedling. The inoculated plates were placed in a plant growth chamber with a photoperiod of 16hrs light, 8hrs dark and 70% relative humidity. Observations were made at regular intervals from 24hrs up to 168hrs. The localized necrosis or swelling in the root tips were observed under advanced zoom stereoscopy (Labomed, CZM6, USA). The susceptible genotypes displayed typical swellings at the root tips within 120hrs of inoculation which indicated host susceptible interaction with root knot nematode (Fig., 2.1) whereas absence of development of galls with necrotic (brown) lesions indicating plant defence against nematode resistant reactions represented swellings or necrotic lesions on roots (Fig., 2.2). Further, the roots were stained using acid fuchsin lactophenol and destained using plain lactophenol. The stained roots were observed under compound microscope for the presence of second stage juveniles within the roots or their point of entry within the roots.

3. Results and Discussion

Fig 1a: Susceptible variety (PKM 1) with well developed galls

Fig 1b: Resistant accessions with absence of galls, denser roots with increased root length

Fig 1: Identification of resistant and susceptible accessions of tomato against *M. incognita* by classical screening method

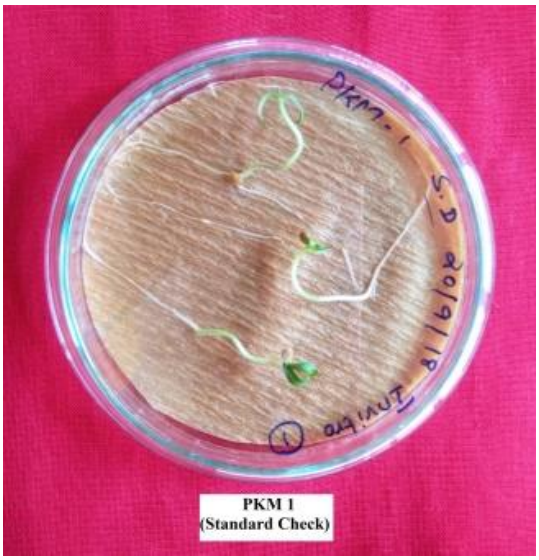

Fig 2.1.a: Petri plate with *M. incognita* J_2 inoculated cotyledons

Fig 2.2a: Petri plate with *M. incognita* J_2 inoculated cotyledons

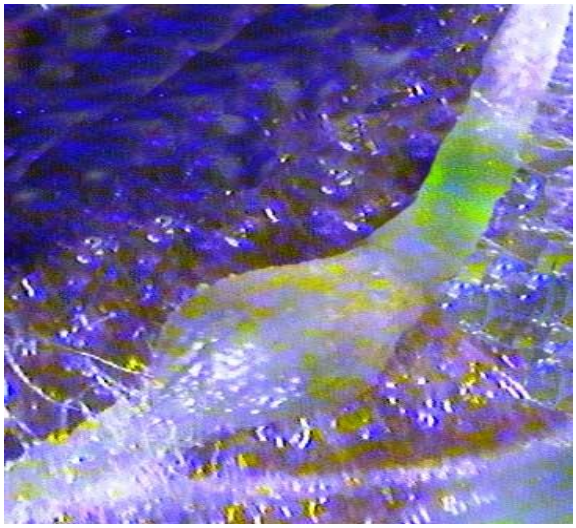

Fig 2.1.b: Well developed galls in suceptible cotyledons

Fig 2.1: Susceptible plant with development of galls

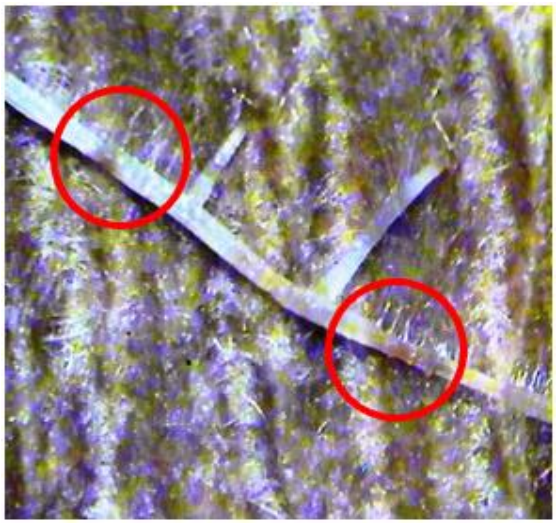

Fig 2.2b: Brown lesions on roots of resistant cotyledons

Fig 2.2: Resistant plant with brown lesions

Fig 2: Identification of resistant and susceptible accessions of tomato against root knot nematode *M. incognita* by Early Detection Assay (EDA)

Table 2: Comparative study of classical screening and early detection assay

S. No	Particulars	Classical Screening	Early Detection Assay
1.	Time period	45 days	7 days
2.	Inoculum level	2000 J_2/plant	10 J_2/ plant
3.	Seed requirement	More	Less
4.	Temperature	30 - 35°C	26±2°C/room temperature
5.	Water Consumption	More (5 – 6 litres)	Less (< 50 ml)
6.	Space requirement	Requires more space	Requires less space
7.	Influence of other factors	Influenced by other pests and diseases	Less influence

Table 3: Detection of root knot nematode, *M. incognita* resistance in tomato (*Lycopersicon esculentum* L.) by classical and early detection screening method

SI. No	Tomato accessions	*In vitro* screening			*In vivo* screening	Response of tomato accessions
		Number of galls/plant	Number of egg masses/plant	Root Knot Index (RKI)	Presence / absence of galls	
1.	EC 326141	138	66	5	+	HS
2.	EC 620394	8	-	2	-	R
3.	EC 165700	123	58	5	+	HS
4.	EC 567305	53	10	4	+	S
5.	EC 620385	45	12	4	-	S
6.	EC 164334	36	7	3	+	MR
7.	EC 161245	64	33	4	+	S

8.	EC 164838	82	39	5	+	HS
9.	EC 617051	11	1	2	-	R
10.	EC 162601	38	11	3	+	MR
11.	EC 620288	8	3	2	-	R
12.	EC 631364	No galls	No egg masses	1	-	HR
13.	EC 238308	39	8	3	+	MR
14.	EC 164334	57	9	4	+	S
15.	EC 620370	61	11	4	+	S
16.	EC 257751	45	6	3	+	MR
17.	EC 362959	35	12	3	+	MR
18.	EC 368799	31	4	3	+	MR
19.	EC 321425	35	1	3	+	MR
20.	EC 362944	39	10	3	-	MR
21.	EC 164863	No galls	No egg masses	1	-	HR
22.	EC 326146	39	3	3	+	MR
23.	EC 368786	62	15	4	+	S
24.	EC 163606	2	No egg masses	2	-	R
25.	EC 620498	7	No egg masses	2	-	R
26.	EC 136894	187	29	5	+	HS
27.	EC 338716	31	4	3	+	MR
28.	EC 145057	104	13	5	+	HS
29.	EC 157568	77	18	5	+	HS
30.	PKM 1 (Standard Check)	184	48	5	+	HS

HR=Highly Resistant; R=Resistant; MR=Moderately Resistant; S=Susceptible; HS=Highly Susceptible (+) = Presence of galls, (-) = Absence of galls.

In classical screening, the resistant and susceptible accessions were identified based on the presence of number of galls/plant and number of egg masses/plant. Among 32 accessions screened by classical and early detection assay, two accessions (EC 631364, EC 16486, Fig., 1.a and 1.b) were identified as highly resistant and eight accessions (EC 620394, EC 617051, EC 620288, EC 145615, EC 636874, EC 151568, EC 163606, EC 620498) were identified as resistant and nine accessions were identified as moderately resistant and Eleven were susceptible (Table 3). In Early detection assay, the susceptible accessions showed presence of galls (Fig. 2.1) where in the resistant accessions showed the absence of galls with brown lesions due to accumulation of defence compounds (Fig. 2.2)

The comparative study showed that Early Detection Assay requires less time period (7 days), less inoculum level (10 J_2 per plant), less water consumption (< 50 ml) and also requires only minimum space where as Classical screening required more time period (45 days), high inoculum level (2000 J_2 per plant) and water consumption was high (5 – 6 litres) and also requires more space. The major factor, that the plants which get influenced by other factors such as pests and diseases were completely avoided in early detection assay to that of classical screening. The results from the observation concluded that no galls developed in highly resistant and resistant plants, whereas in susceptible plants, galls were developed even within a short period of time than the classical screening (Table 2).

The presence or absence of galls is the best parameter for assessing the nematode infestations in glasshouse experiment (Aalders *et al*., 2009) [1] (Table 1). The result of the current study is in agreement with the findings wherein, the resistant and susceptible accessions were identified by classical screening of tomato genotypes under glasshouse conditions based on the presence of number of galls / plant and number of egg masses / plant. Depending on the ability of nematode reproduction, the resistance and susceptibility in plants were expressed (Cook and Evans, 1987) [6].

The nematode infestation in susceptible plants results in plant growth reduction due to severe galling and reduced root system (Sujatha *et al*., 2017) [11]. The susceptible plant showed development of galls within a week of inoculation of second stage juveniles. Thus, the efficacy of the galled root leads to cellular changes which results in modification in uptake and translocation of water to aerial parts of the plant that results in chlorosis and stunted growth (Bala *et al*., 1984) [3]. This reduced root system due to nematode infestation leads to incompatible interaction between soil and the plants for uptake of water and other nutrients (Clark *et al*., 2003) [5]. By analysing the *M. incognita* infested field, the commercially available tomato genotype has showed a wide range of susceptible reactions (Sujatha *et al*., 2017) [11]. The presence of variable reactions in susceptible genotypes caused by *M. incognita* infestation might be due to some genetic differences (Brown *et al*., 1997) [4]. Hence, this experiment reveals that genetic differences may occurs due to absence of nematode resistant genes (*Mi*gene) leading a way to nematode penetration and their greater infestation. In addition to that, absence or decrease of several biochemical compounds or enzymes such as phenols, peroxidases, polyphenol oxidases, acid phosphatases due to nematode infestation are also responsible for susceptibility in plants.

The resistant plants showed brown lesions at the point of infestation due to accumulation of defence compounds such as phenolic compounds which inhibits the entry of nematodes within the roots or prevent the development of feeding sites results in hypersensitive reaction (HR) i.e., rapid and localized cell death (Williamson *et al*., 2006) [13]. Hence, incompatible interaction takes place between plants and the nematodes which may be due to the presence of resistant genes which defend the plants from nematode infestation.

The observation of the current study revealed that the reaction of resistant and susceptibe accessions of tomato showed similar results in comparison with both *in vitro* and *in vivo* assays. The susceptible accession showed development of large sized galls with reduced root (Fig. 1.a) and shoot growth whereas, the resistant plants shows absence of galls with increased root length (Fig. 1.b).

In conclusion, the present investigation proved that early detection method is viable, economically feasible and also scientifically reliable method. This method is suitable to screen susceptible accessions within a short period of time. Hence, this method is highly useful to identify the susceptible accessions at an early stage of the crop and resistant accessions alone can be forwarded for further resistant breeding programme against root knot nematode, *M. incognita*.

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