PHENOTYPING OF TOMATO GERMPLASM FOR ROOT KNOT NEMATODE RESISTANCE

by

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(2018-11-051)

THESIS

Submitted in partial fulfilment of the

requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT BREEDING AND GENETICS

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM – 695522

KERALA, INDIA

2020

DECLARATION

I, hereby declare that this thesis entitled "**Phenotyping of tomato germplasm for root knot nematode resistance**" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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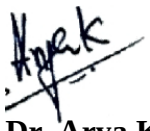

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Acknowledgement

First and foremost, thanks to my family, for everything that happens to me…

With great respect and devotion, I would like to record my deep sense of gratitude and indebtedness to my major advisor ***Dr. Jayalekshmy V. G.****, RC, Professor and Head, Department of Seed Science and Technology for her valuable guidance, constant inspiration, abundant encouragement, critical scrutiny of the manuscript and valuable suggestions which render me to accomplish the research work successfully. I extend my sincere gratitude for her care and affection bestowed on me throughout the study period which helped me to achieve a research fellowship at World Vegetable Center, Taiwan.*

It gives me immense pleasure to express my deep sense of gratitude to ***Dr. Arya K,*** *Professor and Head, Department of Plant Breeding and Genetics, for her valuable advices right from the beginning, which helped me in the successful completion of the thesis.*

I convey my heartfelt gratitude to ***Dr. Nisha. M S****, Assistant professor Department of Nematology for her generous and timely help, unstinting support, suggestions and passionate approach rendered during the period of research work.*

I consider it as my privilege to express my deep-felt gratitude to Dr. ***Radhika N S****, Assistant Professor, Department of Plant Pathology, for her incessant motivation, valuable suggestions and technical advices throughout the research work.*

I am deeply grateful to my beloved teachers of Plant Breeding and Genetics, ***Dr. C. Lekha Rani*** *(Rtd.),* ***Dr. Mareen Abraham****,* ***Dr. Beena Thomas*** *and* ***Dr. Gayathri. G.*** *for the generous support, timely advice, constructive criticisms and constant encouragement rendered to me during the course of my study and research work.*

No choice of words will suffice to express thanks to my friends Reshma, Roshin, Darshana, Smera and Bhoomika for being with me from the beginning of course study till now and played a big part in the completion of the research programme.

Words are inadequate to express my thanks to all the seniors, batch mates and juniors whose cooperation, love, support and affection helped me through many hardships.

I am thankful to Kerala Agricultural University for the technical and financial assistance for carrying out my study and research work.

It would be impossible to list out all those who have helped me in one way or another in the successful completion of this work. I once again express my heartful thanks to all those who helped me in completing this venture in time.

Bikkasani Mythri

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LIST OF ABBREVATIONS AND SYMBOLS USED

%	Percentage
@	At the rate of
>	Greater than
µm	Micro meter
200 cc $soil^{-1}$	Per 200 cubic centimeter of soil
5 g $root^{-1}$	Per 5 gram root
Anon.	Anonymous
ANOVA	Analysis of Variance
Cc	Cubic centimeter
cc $soil^{-1}$	Per cubic centimeter of soil
CCSHAU	Chaudhary Charan Singh Haryana Agricultural University
CD	Critical Difference
cm	Centimeter
CRD	Completely Randomized Design
CSKHPKV	Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya
CTCRI	Central Tuber Crops Research Institute
Cv	Cultivar
DMRT	Duncan's Multiple Range Test
EC	Exotic Collection
Egg $mass^{-1}$	Per egg mass
et al.	And others

etc	And so on; and other people/things
F_1	First filial generation
Fig.	Figure
FYM	Farm Yard Manure
g	Gram
g $soil^{-1}$	Per gram of soil
Ha	Hectare
i.e.	In other words
IARI	Indian Agricultural Research Institute
ICAR	Indian Council of Agricultural Research
IIHR	Indian Institute of Horticultural Research
IIVR	Indian Institute of Vegetable Research
J_1	First stage juvenile
J_2	Second stage juvenile
J_3	Third stage juvenile
J_4	Fourth stage juvenile
KAU	Kerala Agricultural University
Kg	Kilo gram
kg $soil^{-1}$	Per kg soil
LOS	Level of Significance
m ha	Million hectares
m t	Million tonnes
ml	Milliliter
NBPGR	National Bureau of Plant Genetic Resources

No.	Number
ºC	Degree Centigrade
PAU	Punjab Agricultural University
Pf	Final nematode population
Pi	Initial nematode population
$Plant^{-1}$	Per plant
$Pot-^{1}$	Per pot
Rf	Reproduction factor
S.E(m)	Standard Error of the Mean
Sl.	Serial
sp. or spp.	Species (singular and plural)
t	Tonnes
tha^{-1}	Tonnes per hectare
TNAU	Tamil Nadu Agricultural University
UGD	UDP-glucose dehydrogenase
USD	United States Dollar

INTRODUCTION

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important solanaceous vegetable crop grown worldwide under both open field and protected cultivation. Originating in the Andes, tomato was imported to Europe in 16th Century and reached India through Portuguese explorers. It is a versatile vegetable owing to its high nutritive value and diversified use.

Tomato, considered as "Poor Man's Apple" is one of the most important vegetable crop in India. It is an important source of Vitamin A, Vitamin C and other minerals. The red pigment in tomato (Lycopene) is now considered as the "world's most powerful natural antioxidant" (Meena *et al.*, 2013). It is also a protective supplementary food and considered as important commercial and dietary vegetable crop (Pedapati *et al.*, 2013).

Worldwide production of 182 m t of fresh produce from 4.76 m ha is observed in tomato (FAOSTAT, 2020). In India, Tomato is cultivated in an area of 0.78 m ha with an average annual production of 19.8 m t and productivity of 25 t ha^{-1}. India ranks second in production next to china with a world's share of 10.4 per cent. Kerala produces 0.013 m t of tomatoes annually from 640 ha with a productivity of 19.8 t ha^{-1} which is relatively very less over national production. Andhra Pradesh, Madhya Pradesh, Karnataka, Gujarat and West Bengal are the major tomato producing states in India (Anon., 2018).

However, tomato production is severely hindered by both biotic and abiotic stresses. Root knot nematode is one of the major devastating and economically important plant pathogen of tomato in both open field and protected cultivation for small and commercial producers. Yield losses ranging from 25 – 100 per cent are reported due to root knot nematodes in tomato (Jablonska *et al.*, 2007; Seid *et al.*, 2015).

Root knot nematodes are obligate endoparasites and the disease is characterized by the presence of galls or root knots on infected plants. Symptoms often include

wilting, stunted growth, poor fruit yield, reduced number of harvests and susceptibility to other pathogens thus increasing the risk of disease complexes. The above ground symptoms of disease caused by nematodes can be difficult to detect and may be confused with symptoms of bacterial wilt and nutrient deficiency. However, in moist fertile soils or during cool weather conditions the above ground plant parts does not show symptoms.

Narrow or limited genetic variation in the crop makes it much more vulnerable to risk of disease and pest outbreaks. Cultural practices like crop rotation, early-season cropping, root destruction, soil solarisation, soilless media, bio-fumigation are available but are tedious and not worthy on a commercial scale. Chemical control although available, is usually not effective in case of root knot nematodes (Haydock *et al.,* 2006).

In view of damage potential caused by these hidden enemies and with due emphasis on non-chemical agricultural management, incorporating nematode resistance is a key component in modern tomato breeding.

The tomato varieties released by KAU are yet to be screened for root knot nematode resistance. Therefore the current study has been proposed with the objective of screening tomato germplasm including the released varieties of KAU and wild tomato species for natural resistance against *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood by artificial inoculation method.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 SCENARIO OF ROOT KNOT NEMATODES

2.1.1 World

Root knot nematodes are one among the top five plant pathogens affecting World's food production. They are adapted to parasitize on large number of plants and over 3000 species of wild and cultivated plants are reported to be affected causing average worldwide annual yield loss of up to USD 100 billion (Ghule *et al.*, 2014).

More than 97 known species of root knot nematodes have been recorded and only 14 known species of *Meloidogyne* are reported in India (Khan *et al.*, 2014).

2.1.2 India

Meloidogyne incognita (Kofoid and White, 1919) Chitwood is a warm weather root knot nematode species occurring in regions with annual temperature of 15-33ºC and observed in almost all parts of India, at least in some part of the year. The optimum temperature for development of *M. incognita* is 25-30ºC (Kamra and Sharma, 2000).

In India, estimated crop yield losses are reported to an extent of 27.2 per cent, 21.35 per cent, 18.20 per cent, 16.67 per cent, 14.10 per cent and 10.54 per cent in tomato, jute, cucurbits, brinjal, okra, and rice respectively accounting for an annual loss of 242.1 billion rupees due to root knot nematodes (Jain *et al*., 2007).

2.1.3 Kerala

Tomato is not cultivated anywhere in Kerala State except three panchayats in Palakkad district of Kerala, since farmers in Kerala are feared of sensitivity of tomato to many pathogens like bacteria, fungi and nematodes which is a common phenomenon

in coastal states. The state imports its entire tomato requirement from Tamil Nadu and Karnataka (Padre, 2017).

2.2 IDENTIFICATION OF PATHOGEN

Plant parasitic nematodes are invisible burdens of crop production and productivity. These nematodes are associated to most of the important agricultural and horticultural crops and are thus associated with risk of global food security (Bernard *et al.*, 2017). Plant parasitic nematodes are ubiquitous microscopic soil pests that feed on plant root system which is unnoticeable due to hidden nature and non-specific symptoms that often mimics the symptoms of drought, nutrient deficiency and other biotic stresses.

Among the plant parasitic nematodes, sedentary nematodes constitute the most harmful species since they establish a permanent feeding site in plant host to obtain the required nutrients. Sedentary nematodes possess a benefit over migratory type due to complex method of host cell transformation in developing suitable feeding structures. Among all the reported plant parasitic nematodes, only a few causes significant economic damage (Koenning *et al*., 1999).

Cetintas and Yarba (2010) reported that *M. incognita* is responsible for around 95 percent of annual yield loss in tomato. Thies and Fery (2003) mentioned that cucurbit and solanaceous vegetable production is limited in United States and worldwide due to *M. incognita.*

2.2.1 Symptomatology

M. incognita infected plants exhibit signs of nutrient deficiency such as yellowing of leaves, stunted growth and wilting during day time. Heavy infection on older plants leads to sudden wilting and dies off soon. Nematode infestation induce expansion of root cells and swellings or galls develop on the roots. Root knots produced on root system damages the vascular tissue of infected plant and thus interferes with

translocation of nutrients and water within the plant affecting the normal growth and development of plant.

The infection points serve as gateways to many other pathogenic fungi and bacteria thus making the host plant more vulnerable to a complex of diseases (Rahman, 2003). Root galls are easy to be identified with naked eye. However a stereomicroscope is required to identify the nematodes (Ralmi *et al.*, 2016).

The nematode infection causes mechanical injury and thus develops a host-parasite relation leading to physiological changes in host tissue as a result of substances secreted by nematodes and plant in response to infection. A giant cell is formed by group of cells at point of infection in host plant and possess dense cytoplasm enclosing several nuclei and nucleoli. Hypertrophic and hyperplasic reaction in stellar region is formed as a result of infection. Other effects such as suppressed cell division, root pruning and root proliferation are exhibited by host plant. Stunted growth, poor yield, low quality and reduced number of harvests are the generalized symptoms of root knot nematode infection (Perry and Moens, 2006).

Luc *et al.* (2005) reported root knot nematode infection in plants as non-specific. General symptoms appear as irregular distribution patches of plants in field with stunted growth, sparse and yellow foliage.

According to Khan and Reddy (1993), incipient wilting is exhibited by root knot nematode infested plant despite proper soil moisture where the plants wilt in day and recover back at nights. The roots invaded by nematodes are weakened and are prone to many other pathogens leading to root decay. Root rot, root lesions, root galls and cessation of growth occurs (Khan, 2008).

2.2.2 Life Cycle of Root Knot Nematode, *M. incognita* in Tomato

Berkeley in 1855 first reported root knot nematodes causing damage in cucumbers. *M. incognita* begin their life stage from eggs which upon hatching develop

into J_1 (first stage juvenile), which resides entirely in the translucent egg case and molts to J_2 (second stage juvenile). The motile J_2 is the only stage which can cause infection by attacking growing root tips and enter the roots intercellularly, behind the root cap region. The J_2 then move to area of cell elongation and initiate feeding site upon injecting esophageal gland secretions into root cells. These secretions lead to transformation of parasitized cells into giant cells. The J_2 do not carry any reproductive organs and molts to J_3 and J_4 stages.

Juveniles are usually 500 µm in length and 15 µm in width. Progression from J_4 stage juveniles to globose adult females (400-1000 µm in length) or to vermiform adult males (1100-2000 µm in length) turns clearly visible with distinct lips and strongly developed stylets. A single female nematode can produce varied number of eggs ranging from 500 to 1000 in egg masses.

Babu and Narayana (2019) reported morphological and morphometric characterization of *M. incognita* populations collected from major vegetable crops in Thiruvananthapuram, Idukki and Thrissur districts of Kerala. Intraspecific morphological variations like shape of females, tail characters, length and position of neck, rectum dilation and perineal pattern morphology. These variations in morphological characters are probably due to geographical, eco phenotypic or host induced. Nematode populations collected from tomato plants recorded minimum body length 392.52 µm, maximum head to median bulb (H-MB) value (50.82 µm), minimum length/tail length (7.61), minimum tail length/anal body width (4.72) and maximum tail length (52.51 µm).

The life cycle duration varies with temperature, race, *etc*. which can be as short as two weeks. In cooler regions longer life cycles are observed. Egg masses remain on root surface or may be released to soil matrix. Eggs hatch at random and under favorable conditions root knot nematodes eggs have been reported to survive for at least one year.

Sinha *et al.* (2012) studied the life cycle of *M. incognita* in tomato and brinjal seedlings during different months. Inoculated seedlings raised in the month of June

showed second stage juveniles which started invading within 40-48 hours of inoculation. Maximum penetration was recorded at fifth day after inoculation. The life cycle of *M. incognita* was completed within 16 days in the month of June *i.e*. from second stage juveniles to second stage juveniles. Similar trend was followed in the months of July and August but the life cycle was fond to be reduced by one day in the month of September and increased by two days in the month of October in comparison with June. In November, it took 25 days to complete one generation. In December and January, although the roots are infected with second stage juveniles there is no further development due to extremely low temperature.

2.3 Natural Resistance in Tomato against Root Knot Nematodes

Resistance in tomatoes to root knot nematodes was first observed by Bailey in the wild species (*Lycopersicon peruvianum* Mill.), P.I. 128657 in 1941.

Gene *Mi*, which confers resistance to several species of root knot nematodes, *Meloidogyne* sps. is present in many modern and commercial tomato cultivars (Williamson, 1998). According to many scientists, this gene is the only source of resistance against the pest in modern tomato cultivars. The resistance gene, which is naturally present in *Solanum peruvianum* was introgressed to modern tomato cultivar in 1940's by embryo culture technique (Smith, 1944).

Cloning of this gene revealed that it encodes a member of the plant resistance protein family characterised with presence of putative nucleotide binding site and a leucine rich repeat (Williamson, 1998). Further, analysis also revealed that *Mi* gene also confers resistance to potato aphids.

Williamson *et al.* (1994) reported that *Mi* gene although effective under many conditions, fails to confer resistance under high soil temperatures. Resistance genes that differ from the Mi gene in properties could be identified for broadening the base of root knot nematode resistance in tomato.

The International *Meloidigyne* Project (IMP) provided a world view of root knot nematodes, their origin and distribution. As evident from data obtained by IMP, a crop having resistance to *M. incognita* and *M. javanica* would be resistant to 82% of other *Meloidogyne* populations in world. Jones (2013) reported that tomato cultivar carrying resistance to *M. incognita*, *M. javanica* and *M. arenaria* would be resistant to 90% of other root knot nematodes.

The *Mi* gene, discovered long ago in P.I. 128657, an accession of *Solanum peruvianum* (Mill.), a wild relative of edible tomato (Cap *et al*., 1993). The gene was transferred and expressed in F_1 plants derived from a cross between *Solanum lycopersicum* 'Michigan State Forcing' by Smith in 1944. The gene is located on short arm of chromosome 6 and has been mapped in considerable detail initially (Messequer *et al*., 1991; Williamson *et al*., 1994).

2.4 FACTORS INFLUENCING THE RESISTANCE

2.4.1 Temperature

Temperature plays a key role in nematode survival, distribution, embryogenesis, hatching, migration and penetration, development and symptom expression in host plants (Joubert and Rappard, 1971). Temperature varies between nematode population thermotypes and with each host-parasite combination (Ritter, 1973; Thomason and Lear, 1961).

Under environmental stress nematode reproduction was found to be high and the highest temperature for hatching was reported at 27ºC (Dropkin *et al*., 1969). Nematode life cycle completes faster at high temperatures and thus more generations are produced. In addition, fewer males are produced at higher temperatures (David and Triantaphyllou, 1967). The differential plant responses to nematodes at high temperatures are probably an effect of quantitative differences in enzymatic reactions.

Many factors alter the expression of resistance. Genetic resistance to *Meloidogyne* species is sensitive to soil temperatures above 28ºC. Dropkin (1969) reported loss of resistance in tomato, sweet potato and beans at elevated temperatures. High soil temperatures served to be the reason behind loss of root knot resistance in Florida, United States of America (Walter, 1967)

Reproduction of *M. incognita* in elevated soil temperatures may be race dependant and that race 4 reproduces better on resistant tomato genotypes at 32.5ºC than race 1 (Arujo *et al*., 1983)

2.4.2 Tissue culture

Exogenous application of kinetin to tomato seedlings alters expression of resistance (Dropkin *et al*., 1969). Loss of resistance to root knot nematodes in the plants regenerated through tissue culture techniques from resistant cultivars may be observed (Fassuliotis and Bhatt, 1982).

2.4.3. Plant Age at Time of Inoculation

Effect of nematode infection on growth and development of host plant is influenced by plant age at the time of inoculation. Older plants have well developed and differentiated roots, which is not preferred by nematodes to penetrate, thus more roots remain undamaged (Jaffe and Mai, 1979).

Increased nematode density in aged plants is probably due to greater availability of roots and less competition (Fawole and Mai, 1979). The scarce root system of young plants at the time of transplanting may cause concentration of juveniles at root tips, leading to stoppage of root tip growth leading to reduction in volume of root system (Canto-Sánez and Brodie, 1984).

In a study conducted in cucumber by Kayani *et al.* (2017), the *M. incognita* inoculum densities and ages of plants at time of inoculation of nematodes has shown

significant effect on growth parameters. The interaction between these two parameters *i.e.* inoculum density and age of plant on growth parameters were shown to be highly significant.

Reproduction rate of nematodes with inoculum density of 500 J_2 pot^{-1} was recorded to be twice on two week old tomato plants when compared with one week old seedlings. But reproduction of nematodes in seedlings of both one week and two week old seedlings decreased with increase in population density. Three week old plants expressed least reproduction among all. Plant growth stopped after two weeks post inoculation due to reduced root growth (Wallace, 1970).

2.4.4 Nematode Inoculation Density

Di Vito *et al.* (1991) investigated the pathogenicity of *M. incognita* Race 1 and confirmed the destructive effect of nematode on tomato plants. The tolerance limit of resistant and susceptible tomato cultivars was 0.55 juveniles cc $soil^{-1}$. The minimum relative yields were 0 and 0.7 for susceptible and resistant cultivars at Pi ≥32 eggs cc $soil^{-1}$ respectively.

Chandra *et al.* (2010) conducted pot culture experiments under greenhouse conditions to study the effect of population density on pathogenic potential of *M. incognita* on various cucurbits. Different densities of inoculum ranging from 10, 100, 1000 second stage juveniles $plant^{-1}$ were inoculated at 15 days seedling stage and found the presence of an inverse relation between population density, population growth and number of galls.

Kankam and Adomako (2014) investigated the effect of inoculum densities of root knot nematodes (0, 500, 1000, 2000 freshly hatched second stage juveniles (J_2) kg $soil^{-1}$ pot^{-1}) in tomato cv. Pectomec. Increased inoculum level resulted in corresponding increase in number of galls and population build up and damage was most severe at 2000 J_2 kg $soil^{-1}$.

Kayani *et al.* (2017) reported the effects of southern root knot nematode population densities and age of plant on growth and yield parameters of cucumber. Effects of five initial population densities (500, 1000, 2000, 4000 and 8000 freshly hatched second stage juveniles) were studied. All these inoculum densities exhibited a positive correlation with percent reductions of growth and yield.

2.4.5 Nematode Population in Soil

Singh *et al*. (2018) conducted a survey to determine the status of phytoparasitic nematodes associated with various vegetable crops (tomato, french bean, cucurbits, crucifers and potato) under polyhouse conditions. Of all the nematodes studied, *M. incognita* was predominant with a population range of 37-1200 200 cc soil^{-1}, followed by *Helicotylenchus dihystera* (28-832 200cc soil^{-1}), *Pratylenchus coffeae* (20-360 200cc soil^{-1}) and *Mesocriconema xenoplax* (30-260 200cc soil^{-1}).

Patil *et al.* (2017) conducted a survey of polyhouses in different districts of Haryana during 2015-16 to determine incidence of important plant parasitic nematodes on vegetable crops. *M. incognita* was found to be the major plant parasitic nematode with 63.15 per cent frequency of distribution and population density range of 30-10000 J_2 200cc soil^{-1}.

2.4.6 Genetics of Virulence in Root Knot Nematodes

Few root knot nematodes reproduce by facultative meiotic parthenogenesis a mechanism by which an embryo produces from female gamete without genetic contribution from male gamete without the usual process of meiosis (Rashed *et al.,* 2017). Due to this significant volatility within and between root knot nematode populations for host range and also shift to virulence or avirulence, which is less in parthenogenic populations of root knot nematodes (Roberts and Thomason, 1986; Molinari and Miacola, 1997; Ogallo and McClure, 1996). After repeated cultivation of resistant varieties, avirulent populations of nematodes turned virulent (Triantaphyllou, 1987; Jarquin-Barberena *et al*., 1991; Verdejo-Lucas *et al*., 2009).

2.5 SCREENING FOR RESISTANCE

Coyne and Ross (2014) reported galling damage assessment using a rapid visual indication of root infection to root knot nematodes and a relative indication of resistance ranging from no galling damage, slight galling damage, mild galling damage, heavy galling and severe galling damage.

Sasser and Taylor (1978) reported scoring for resistance based on galls and mature egg masses. Categorisation of root systems as no galls or egg masses = 0; 1-2 galls or egg masses = 1; 3-10 galls or egg masses = 2; 11-30 galls or egg masses = 3; 31-100 galls or egg masses = 4; more than 100 galls or egg masses = 5.

Hussey and Janssen (2002) suggested scoring based on percentage of the root system with galls, where 0 = no galling; 1 = trace infection with a few small galls; 2 = ≤ 25% roots galled; 3 = 26 to 50%; 4 = 51 to 75%; and 5 = >75% roots galled.

Classification of resistance may indicate the relative success or failure of a plant pest's survival, development and reproduction on plant species or may describe qualitative and quantitative terms of damage caused to host plant. Susceptible cultivars are usually used as controls for measuring resistance. A host plant can be more or less resistant but not immune, an immune plant is a non-host. The degree of reaction by host which is less than immunity is resistance while more than immunity is impossible.

The following scale to classify degrees of resistance was used by Painter (1951). These terms indicate classes used by most workers in plant resistance at field level without indicating the mechanisms involved.

Immunity: The cultivar that one specific plant parasite will never consume or injure under any known condition.

High resistance: Shown by cultivars that possess qualities which result in small damage by specific plant parasite under given conditions.

Low resistance: Indicates qualities that cause a cultivar to express less damage by a specific plant parasite over the average of crop considered.

Susceptibility: Cultivar shows average or above average damage by a specific plant parasite.

High susceptibility: Cultivar shows more than average damage due to specific plant parasite infestation.

Begum *et al*. (2014) evaluated thirteen different brinjal cultivars using a root knot index ranging from 0 to 4 (0 = no galls/immune, 1 = 1-2 galls/resistant, 2 = 3-10 galls/moderately resistant, 3 = 11-30 galls/susceptible and 4 = above 31 gall/highly susceptible). No genotype was recorded to be immune or resistant while nine genotypes are highly susceptible, three genotypes are susceptible and one genotype was moderately resistant.

Thirty one tomato genotypes were screened for their reaction to root knot nematode by Mounika (2018) using root knot index ranging from 0 to 5 (0 = no galls/immune, 1 = 1-2 galls/highly resistant, 2 = 3-10 galls/resistant, 3 = 11-30 galls/moderately resistant, 4 = 31 – 100 gall/ susceptible and 5 = >100 gall/ highly susceptible). No genotype was found to be resistant, Hisar Lalit was moderately resistant, EC – 631364, EC – 620395 were susceptible and remaining twenty eight genotypes were highly susceptible.

Nisha and Sheela (2015) screened two improved varieties of coleus (Sree Dhara and Nidhi), five lines from CTCRI (Line-74, 64, 79, 76 and 71) and two accessions from Vellanikkara (TC-9 and M-131) for relative tolerance to *M. incognita*. The variety Sree Dhara showed significant superiority over the rest of varieties/lines/accessions in reducing the nematode population (larvae, females, egg masses and eggs egg mass^{-1}). Sree Dhara recorded minimum root-knot index of 1.00 and ranked first in yield.

Ankitha (2019) screened thirty Chinese potato (*Solenostemon rotundifolius* (Poir) J. K. Morton) genotypes against root knot nematode resistance using modified method of Heald *et al.* (1989) for scoring. Fifteen genotypes were found to be resistant of which the genotypes Kenichira local, Suphala, CP 8 and Edayur local were found to be having high yield with nematode tolerance. Genotype Pattambi local recorded highest mean values for root knot count , number of larvae, number of females and number of egg masses 5 g root^{-1}.

Nayak (2019) screened thirty three tomato genotypes and found that final nematode population in root and soil was highest in highly susceptible varieties like Utkala Kumari (1987) and Pusa Ruby (1925.67) and least in resistant cultivars Bani Local (326) and Rajsunakhala local (500).

2.6 LACK OF RESISTANCE ON ALL TYPES OF NEMATODES

Mi gene is effective against three major tropical and sub-tropical root knot nematodes (*M. arenaria, M. incognita* and *M. javanica*), but the resistance is absent against *M. enterolobii* and *M. hapla* (Brito *et al*., 2007). Similarly *M. mayaguensis* overcomes the resistance of tomato and pepper (*Capsicum annum*. L), which contained *Mi-1, N* and *Tabasco* genes (Kiewnick *et al.*, 2009).

2.7 RESISTANCE BREAKDOWN

Presence of natural resistance genes serves as the major line of defense against root knot nematodes. However, plants turning insensitive to *Mi* gene is noticed for which the reasons are yet unknown. The probable source of resistance breakage may be due to temperature, changes in nematodes and changes in resistant cultivars. Resistant cultivars when subjected to monoculture systems serves as a source of breaking resistance rapidly (Philis and Vakis, 1977).

All the present resistant cultivars possess the same source of resistance, namely the *Mi*-1 gene. Although resistant cultivars fight against nematode infestation,

enhancing the effectiveness of resistant strains is highly preferred (El-Sappah *et al.*, 2019).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The experiment entitled “Phenotyping of tomato germplasm for root knot nematode resistance” was conducted in the Department of Plant breeding and Genetics, College of Agriculture, Vellayani during 2018-2020. The study was conducted as two experiments. The first experiment was collection, identification and multiplication of *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood culture for artificial inoculation and the second experiment was screening of tomato germplasm (37 genotypes including released varieties of KAU) for root knot nematode resistance.

The details of materials and methods followed during the course of work are mentioned below.

Experiment I: Collection, Identification and Multiplication of *M. incognita* Culture for Artificial Inoculation

3.1 COLLECTION OF ROOT KNOT NEMATODES

Root knot nematode, *M. incognita* infested root and soil samples were collected from tomato plants at Department of Nematology, College of Agriculture, Vellayani. Soil sample of around 250 g was collected from rhizosphere area along with galled roots in polythene covers and labelled carefully.

3.2 EXTRACTION OF NEMATODES FOR IDENTIFICATION

3.2.1 Extraction of Nematodes from Roots

3.2.1.1 Extraction of Mature Females from Roots

The roots collected were washed thoroughly, cut into small pieces and stained in lactophenol-acid fuchsin (Daykin and Hussey, 1985) and left overnight in clear lactophenol for destaining. The mature females were teased out from galls and kept in

lactophenol.

3.2.1.2 Extraction of Males and Second Stage Juveniles from Roots

The males and second stage juveniles were extracted from roots using modified Baermann's funnel technique (Christie and Perry, 1951). The roots were washed in tap water, chopped into small pieces and placed on a double layered tissue paper lined on a wire gauze. The wire gauze was placed on a Petri dish filled with water up to the level that it just touched the bottom of gauze and roots. Second stage juveniles and males moved through the filter paper and got collected in the water present in Petri dish.

Second stage juveniles were also extracted by picking the egg masses from roots into a Petri dish containing water. The hatched out juveniles were then killed and fixed.

3.2.2 Extraction of Nematodes from Soil

Nematodes were extracted from soil samples using Cobb's decanting and sieving method which is followed by Baermann's funnel technique (Cort *et al*., 1922). Thus the nematodes extracted are identified under stereo microscope followed by perineal pattern observation.

3.3 MULTIPLICATION OF ROOT KNOT NEMATODE CULTURE

The identified populations were maintained under shade net conditions at Department of Plant Breeding and Genetics by inoculating the population to fifteen healthy tomato plants, which were planted earlier in earthen pots containing sterilized soil.

Experiment II: Screening of Tomato Germplasm (37 Genotypes Including Released Varieties of KAU) for Root Knot Nematode Resistance

3.4 EXPERIMENTAL MATERIAL

3.4.1 Germplasm

A total collection of 37 genotypes enlisted in Table 1 were collected from ICAR – National Bureau of Plant Genetic Resources, Regional Station, Hyderabad and other institutes in India for evaluation.

3.4.2 Location of Experiment

The experiment was conducted in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani during October and November months of 2019 under shade net conditions.

3.4.3 Experimental Details

The experiment with thirty seven genotypes was laid out in Completely Randomized Design (CRD) with three replications.

3.4.4 Cultivation Details

3.4.4.1 Nursery

Seeds of each genotype were sown in portrays and maintained well under automated polyhouse. The seeds germinated within a week and were transplanted to main field after 28 days from sowing

Table 1. List of tomato genotypes used in the study

Genotype No.	Name of the genotype	Source
T1	Shakthi	KAU
T2	Akshaya	KAU
T3	Anagha	KAU
T4	Vellayani Vijai	KAU
T5	Manulakshmi	KAU
T6	Manuprabha	KAU
T7	Kashi Vishesh	IIVR, Varanasi
T8	Palam Pride	CSKHPKV, Palampur
T9	Arka Meghali	IIHR, Bengaluru
T10	Pusa Rohini	IARI, New Delhi
T11	*Solanum hirsutum*	NBPGR, Regional Station, Hyderabad
T12	IIHR – 2200	IIHR, Bengaluru
T13	IIHR – 2868	IIHR, Bengaluru
T14	IC – 45	NBPGR, Regional Station, Hyderabad
T15	EC – 164563	NBPGR, Regional Station, Hyderabad
T16	EC – 249574	NBPGR, Regional Station, Hyderabad
T17	EC – 249508	NBPGR, Regional Station, Hyderabad
T18	EC – 631368	NBPGR, Regional Station, Hyderabad
T19	EC – 165700	NBPGR, Regional Station, Hyderabad
T20	EC – 549819	NBPGR, Regional Station, Hyderabad
T21	EC – 164670	NBPGR, Regional Station, Hyderabad

T22	EC – 631364	NBPGR, Regional Station, Hyderabad
T23	EC – 620388	NBPGR, Regional Station, Hyderabad
T24	EC – 620395	NBPGR, Regional Station, Hyderabad
T25	EC – 620417	NBPGR, Regional Station, Hyderabad
T26	EC – 620422	NBPGR, Regional Station, Hyderabad
T27	EC – 145057	NBPGR, Regional Station, Hyderabad
T28	EC – 631359	NBPGR, Regional Station, Hyderabad
T29	EC – 620406	NBPGR, Regional Station, Hyderabad
T30	EC – 160855	NBPGR, Regional Station, Hyderabad
T31	EC – 620394	NBPGR, Regional Station, Hyderabad
T32	EC – 620431	NBPGR, Regional Station, Hyderabad
T33	EC – 620373	NBPGR, Regional Station, Hyderabad
T34	EC – 620401	NBPGR, Regional Station, Hyderabad
T35	PNR – 7	PAU, Ludhiana
T36	Hisar Lalit	CCSHAU, Hisar
T37	PKM – 1	TNAU, Coimbatore

3.4.4.2 Transplanting

Large polythene grow bags (40 x 24 x 24 cm dimensions) were filled with sterile sand mixture (Red soil, Sand and FYM in ratio 2:2:1, respectively) and kept under shade net. Healthy, uniformly grown seedlings were selected from protrays and are transplanted in grow bags.

3.4.4.3 Inoculation of Nematodes

M. incognita egg masses were collected from tomato plants maintained for nematode multiplication. The egg masses were handpicked using forceps and transferred to Petri plates of 5 cm diameter containing sterile distilled water.

Two days after hatching, the juveniles produced were transferred to a beaker. The population of juveniles in beaker and artificially inoculated in soil on transplanted tomato seedlings near the root zone at 15 days after transplanting @ $2J_2$ g of $soil^{-1}$.

3.4.4.4 Uprooting of Tomato Plants

The inoculated plants were uprooted carefully at 45 days after inoculation. To ensure ease in uprooting mild irrigation was given a day before uprooting of plants. The grow bags were pressed slightly to loosen the soil. Upon uprooting the plants were shaken slightly to remove clods of soil adhered to roots. The uprooted roots were further washed individually under tap water to remove adhering soil particles.

3.5 OBSERVATIONS RECORDED

The following observations were recorded from thirty seven genotypes with three replications (one plant per every replication).

3.5.1 Nematode Population in Soil (200 cc)

200 cc soil samples were collected from rhizosphere of tomato plants for nematode population estimation at the time of uprooting. Nematodes were extracted from each soil sample using Cobb's sieving and decanting technique (Cobb, 1918) and Baermann's method (Schindler, 1961). The nematodes thus extracted were counted using a stereo microscope.

3.5.2 Number of Larvae in 5 g Root

Five gram washed root sample of each genotype under every replication were taken and cut into bits of 2 – 3 cm length and placed above tissue paper supported by wire guage placed on Petri plate filled with distilled water. After 24 hours the nematode suspension was collected, pooled and counted using a stereo zoom microscope.

3.5.3 Root Knot Count (5 g root)

The number of galls 5 g root^{-1} were counted individually for each genotype. Root knot index for genotypes under evaluation was performed using modified method of Heald *et al.* (1989) as detailed in table 2.

3.5.4 Number of Females (5 g Root)

Five gram washed root sample of each genotype under every replication were taken and cut into small bits of 2 – 3 cm. The cut pieces were stained by differential staining method using acid fuschin-lactophenol mixture. Lactophenol solution was prepared by mixing liquid phenol (500 ml), lactic acid (500 ml), glycerine (100 ml) and distilled water (500 ml). Stock solution of acid fuchsin was prepared by dissolving 3.5 g acid fuschin in 250 ml of acetic acid and 750 ml of distilled water. Working solution of the stain was prepared by adding 1 ml of stock solution of stain into 100 ml of lactophenol solution. The stain was boiled in a beaker on a hot plate. The infected roots of each genotype were immersed in boiling stain for one minute, rinsed under tap water

Table 2. Method of scoring resistance as given by Heald *et al*., 1989

Number of galls / root knots plant^{-1}	Root knot index	Reaction
0	0	Highly Resistant
1-25	1	Resistant
26-50	2	Moderately Resistant
51-75	3	Moderately Susceptible
76-100	4	Susceptible
>100	5	Highly Susceptible

and finally destained in lactophenol solution until the maximum contrast between nematodes and the root tissue was observed. The processed roots were pressed between glass slides, teased with a clean needle and observed under microscope to count number of females present.

3.5.5 Number of Egg Masses (5 g Root)

Five gram washed root sample of each genotype under every replication were taken and cut into small bits of 2 – 3 cm. The cut pieces were then stained in acid fuschin-lactophenol solution and observed under microscope to count the number of egg masses.

3.5.6 Average Number of Eggs in Egg Mass

Sterile egg mass were collected from the infected root portion and kept between the glass slides, crushed thoroughly, stained and examined under microscope to count the number of eggs.

3.5.7 Reproduction Factor

The reproduction factor was measured using initial and final nematode population in soil contained in grow bags. The reproduction factor was thus achieved using the formula;

$$\text{Reproduction factor (Rf)} = \frac{\text{Final nematode population (Pf)}}{\text{Initial nematode population (Pi)}}$$

3.5.8 Fresh Weight of Shoot

Fresh shoot weight of each plant was measured using electronic balance.

3.5.9 Fresh Weight of Root

Fresh root weight of each plant was measured using electronic balance upon careful and thorough washing of roots.

3.6 STATISTICAL ANALYSIS

The data obtained was analysed using analysis of variance (ANOVA) technique in OPSTAT software and were compared using Duncan's Multiple Range Test (DMRT) at 5% level of probability.

Plate 1: Tomato seedlings in nursery at 3 weeks after sowing

Plate 2: Tomato plants at 15 days after transplanting

Plate 3: Tomato plants at 30 days after transplanting

Plate 4: Field view of tomato plants under screening

Plate 5: Plant yellowing after infection

Plate 6: Initiation of gall formation

Plate 7: Tomato plants at uprooting stage

Plate 8: Galls at uprooting stage of tomato

RESULTS

4. RESULTS

The present investigation entitled “Phenotyping of tomato germplasm for root knot nematode resistance” was undertaken with the objective of screening tomato germplasm including released varieties of KAU and wild tomato species for root knot nematode resistance artificially. The data generated in this study was statistically analyzed and presented in this chapter.

4.1 COLLECTION, IDENTIFICATION AND MULTIPLICATION OF *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood CULTURE FOR ARTIFICIAL INOCULATION

Root knot nematode infected tomato plants and soil samples were collected from Department of Nematology, College of Agriculture, Vellayani and morphological identification of juveniles, females and egg masses was performed.

The juveniles were collected from root and soil samples and observed under stereomicroscope. The *M. incognita* females collected from root samples were pear shaped when identified under stereomicroscope upon staining. The neck of females were curved to sickle shaped directed at an angle to the body. Egg masses were collected from the surface of root knots using forceps, stained and observed under stereomicroscope. Each egg mass comprised of 200 – 250 eggs present in a gelatinous matrix. The J_2 are inoculated on tomato plants grown in sterile media for pure source of inoculum.

4.2 SCREENING OF TOMATO GERMPLASM FOR ROOT KNOT NEMATODE RESISTANCE

Second stage juveniles of *M. incognita* obtained from pure culture were inoculated at the rate of 2 J_2 g $soil^{-1}$ at 15 days after planting of tomato seedlings in grow bags.

4.2.2 Nematode Population Characteristics in Root

Analysis of variance was performed for all the characters studied and the results showed significant variation among all the genotypes screened for all the characters studied under pot culture experiment.

Data on reaction of different accessions of tomato germplasm to root knot nematode, *M. incognita* are presented in the table 3a.

4.2.2.1 Number of Larvae in 5 g Root

There was statistically significant variation in the number of larvae in 5 g root among all the genotypes and the C.D value was 2.539. Number of larvae were higher in genotype EC – 549819 (401.67) and it was significantly different from other treatments (Fig.1). The genotype PNR-7 (71.33) recorded lesser number of larvae 5 g root^{-1} and was statistically on par with Hisar Lalit (84), EC – 631359 (108.67), EC – 620373 (111.3), Manuprabha (113), and Akshaya (114.33).

4.2.2.2 Root Knot Count (5 g Root)

Number of root knots 5 g root^{-1} varied from 356 to 13.2 and exhibited significant variation with C.D value of 1.662. Genotype Arka Meghali recorded lesser root knots (13.2) 5 g root^{-1} and was statistically on par with IIHR – 2200 (14.9), EC – 620401 (16.6), EC – 145057 (21.7), EC – 160855 (22.3), PNR – 7 (23.1), Hisar Lalit (24.6), IC – 45 (24.7), Vellayani Vijai (25.2) and Anagha (25.6) (Fig.2). Genotype EC – 165700 recorded higher number of root knots 5 g root^{-1} with 356 and was significantly different from all other genotypes.

4.2.2.3 Number of Females in 5 g Root

Number of females in 5 g root varied from 34.33 to 245.67 and exhibited significant variation with C.D value of 2.572. Number of females 5 g root^{-1} was found

to be lower in genotype EC – 164563 (34.33) and was statistically on par with EC – 249574 (51.67), Kashi Vishesh (51.67), Vellayani Vijai (52.67) and Shakthi (62) (Fig.3). The genotype EC – 165700 (245.67) recorded higher number of females 5 g^{-1} root and was statistically on par with EC – 164670 (189.67), EC – 620422 (184.67), EC – 620394 (176.33) and EC – 549819 (173.67).

4.2.2.4 Number of Egg Masses in 5 g Root

Number of egg masses in 5 g root ranged from 18.67 to 318.33 and exhibited significant variation with C.D value of 2.593. The genotype PNR – 7 (18.67) recorded the lower count of females 5 g $root^{-1}$ and was statistically on par with Vellayani Vijai (22) and Hisar Lalit (25.33). Number of females 5 g $root^{-1}$ was recorded to be higher in EC – 145057 (318.33) and was statistically on par with IC – 45 (311.67), EC – 164563 (265.33), Akshaya (252.67) and EC – 249574 (236.67) (Fig.4).

4.2.2.5 Average number of eggs in egg mass

Average number of eggs egg $mass^{-1}$ varied from 100.67 to 350.00 and exhibited significant variation with C.D value of 1.721. Genotype EC – 620394 (100.67) was recorded lower number of eggs egg $mass^{-1}$ and was found to be statistically on par with EC – 620431 (119.67), EC – 549819 (125) and EC – 620417 (131.67) (Fig.5). Higheer number of eggs egg $mass^{-1}$ was observed in EC – 620388 (350) and was significantly different from others.

Table 3a. Response of different tomato genotypes on population characteristics of *M. incognita*

Sl. No	Genotypes	Number of Larvae (5 g Root)	Root Knot Count (5 g Root)	Number of Females (5 g Root)	Number of Egg Masses (5 g Root)	Average Number of Eggs in Egg Mass
1.	T1	141.33 (11.87)	35.87 (6.06)	62.67 (7.82)	147.00 (12.13)	214.67 (14.66)
2.	T2	114.33 (10.68)	66.10 (8.17)	113.67 (10.63)	252.67 (15.92)	147.00 (12.12)
3.	T3	168.67 (12.99)	25.60 (5.12)	95.67 (9.73)	138.67 (11.80)	219.67 (14.84)
4.	T4	122.67 (11.05)	25.20 (5.09)	52.67 (7.03)	22.00 (4.74)	212.67 (14.60)
5.	T5	200.33 (14.14)	96.13 (9.83)	107.67 (10.35)	47.67 (6.91)	197.67 (14.07)
6.	T6	113.00 (10.63)	46.97 (6.9)	154.67 (12.43)	152.67 (12.38)	154.67 (12.44)
7.	T7	186.67 (13.67)	72.50 (8.56)	51.67 (6.90)	90.67 (9.52)	174.00 (13.12)
8.	T8	198.00 (14.06)	62.07 (7.9)	70.33 (8.31)	183.67 (13.56)	249.33 (15.80)
9.	T9	251.00 (15.83)	13.26 (3.72)	99.00 (9.90)	94.67 (9.70)	240.67 (15.53)
10.	T10	206.00 (14.36)	48.39 (6.98)	141.33 (11.88)	83.00 (7.56)	190.67 (13.81)
11.	T11	221.00 (14.87)	56.47 (7.54)	112.67 (10.62)	177.67 (13.34)	211.00 (14.53)
12.	T12	187.67 (13.69)	14.93 (3.98)	77.00 (8.76)	89.00 (9.38)	232.67 (15.27)
13.	T13	127.00 (11.23)	67.39 (8.2)	88.67 (9.41)	95.67 (9.77)	229.00 (15.15)
14.	T14	152.00 (13.93)	24.77 (4.97)	161.67 (12.72)	311.67 (17.67)	172.00 (13.13)
15.	T15	127.00 (12.57)	141.97 (11.93)	34.33 (5.56)	265.33 (16.31)	195.67 (14.00)
16.	T16	194.67 (12.67)	125.93 (11.25)	51.67 (7.00)	236.67 (15.40)	183.67 (13.57)
17.	T17	158.67 (15.62)	86.03 (9.31)	118.00 (10.86)	138.33 (11.79)	264.33 (16.27)
18.	T18	161.00 (20.05)	65.97 (8.15)	164.67 (12.84)	96.33 (9.79)	216.00 (14.71)
19.	T19	243.67	355.93	245.67	215.67	144.00

		(15.06)	(18.88)	(15.69)	(14.70)	(12.01)
20.	T20	401.67 (15.99)	34.33 (5.865)	173.67 (13.19)	79.67 (8.90)	125.00 (11.17)
21.	T21	226.67 (14.51)	62.43 (7.93)	189.67 (13.78)	116.00 (10.77)	196.00 (14.01)
22.	T22	255.67 (12.41)	145.47 (12.08)	116.33 (10.74)	132.00 (11.47)	247.67 (15.75)
23.	T23	210.67 (13.66)	121.60 (11.04)	162.67 (12.73)	143.67 (11.99)	350.00 (18.73)
24.	T24	154.00 (12.84)	34.74 (5.91)	153.67 (12.40)	76.67 (8.69)	251.00 (15.86)
25.	T25	186.33 (16.64)	66.83 (8.19)	143.67 (11.99)	227.00 (15.08)	131.67 (11.46)
26.	T26	165.00 (10.40)	75.27 (8.69)	184.67 (13.59)	113.00 (10.63)	168.67 (13.00)
27.	T27	276.67 (11.31)	21.70 (4.69)	161.67 (12.72)	318.33 (17.86)	147.67 (12.16)
28.	T28	108.67 (12.73)	149.33 (12.21)	88.67 (9.38)	144.67 (12.04)	253.67 (15.94)
29.	T29	128.33 (15.99)	49.97 (7.09)	113.67 (10.64)	191.67 (13.86)	260.67 (16.16)
30.	T30	177.00 (14.91)	22.30 (4.75)	123.67 (11.11)	55.67 (7.39)	236.33 (15.39)
31.	T31	255.33 (10.53)	44.78 (6.70)	176.33 (13.28)	151.00 (12.29)	100.67 (10.02)
32.	T32	222.00 (13.19)	73.37 (8.53)	145.67 (12.07)	191.67 (13.85)	119.67 (10.93)
33.	T33	111.33 (8.34)	106.34 (10.3)	151.67 (12.32)	111.67 (10.54)	154.67 (12.45)
34.	T34	174.00 (9.10)	16.66 (4.08)	90.67 (9.49)	74.67 (8.61)	262.67 (16.23)
35.	T35	71.33 (13.55)	23.10 (4.79)	83.00 (9.04)	18.67 (4.25)	200.67 (14.18)
36.	T36	84.00 (11.23)	24.67 (4.96)	74.67 (8.60)	25.33 (5.03)	226.67 (15.07)
37.	T37	183.67 (13.93)	71.50 (8.43)	165.00 (12.84)	158.33 (12.59)	256.00 (16.02)
	SE(m)	0.899	0.589	0.911	0.918	0.61
	C.D (0.05)	2.539	1.662	2.572	2.593	1.721

4.2.3 Nematode Population in Soil (200 cc)

Data pertaining to nematode population in 200 cc soil was presented in table 3b and Fig.6. Nematode population in soil ranged from 224.33 to 868.00 and exhibited significant variation with C.D value of 6.258. Genotype IIHR – 2868 (224.33) recorded lower number of nematodes 200cc soil^{-1} sample and was statistically on par with Arka Meghali (326.67), EC – 620395 (334.67), EC – 631359 (339) and *Solanum hirsutum* (448.67). Genotype EC – 165700 (868) reported higher number of nematodes 200cc soil^{-1} and was statistically on par with PKM – 1 (779), Vellayani Vijai (742.67), EC – 620401 (736), EC – 620406 (714), EC – 164563 (706), EC – 160855 (685), EC – 249508 (661), Anagha (658.67), EC – 549819 (642), Manulakshmi (624.67), EC – 620373 (613), Shakthi (612.33), Pusa Rohini (607), EC – 145057 (603.67), EC – 620388 (603), Akshaya (589), Hisar Lalit (584), Kashi Vishesh (581), IC – 45 (577), EC – 249574 (571.67), Manuprabha (566) and EC – 631368 (552.67).

4.2.4 Reproduction factor

Data pertaining to reproduction factor is presented in table 3b and fig.7. Reproduction factor among the genotypes ranged from 4.340 to 1.122 and exhibited significant variation with C.D value of 1.443. Genotype IIHR – 2868 (1.122) reported lower reproduction factor and was found to be statistically on par with genotypes Arka Meghali (1.633), EC – 620395 (1.673), EC – 631359 (1.695), *Solanum hirsutum* (2.243), EC – 620422 (2.410), EC – 620417 (2.410), EC – 164670 (2.488), PNR – 7 (2.515) and IIHR – 2200 (2.520). Higher reproduction factor was found in genotype EC – 165700 (4.340) and was statistically on par with PKM – 1 (3.895), Vellayani Vijai (3.713), EC – 620401 (3.680), EC – 620406 (3.570), EC – 164563 (3.530), EC – 160855 (3.425), EC – 249508 (3.018), Anagha (3.293), EC – 549819 (3.210), Manulakshmi (3.123), EC – 620373 (3.065), Shakthi (3.062), T Pusa Rohini (3.035), EC – 145057 (3.018), EC – 620388 (3.015), Akshaya (2.945), EC – 620431 (2.920), Hisar Lalit (2.920) and Kashi Vishesh

(2.905)

Table 3b: Response of different tomato genotypes on population of *M. incognita* in soil and reproduction factor

Sl. No.	Genotypes	Nematodes in soil (200 cc)	Initial Nematode Population (Pi)	Final Nematode Population (Pf)	Reproduction Factor (Rf)
1.	T1	612.33 (24.6)	2000	6123.33	3.06
2.	T2	589.00 (24.1)	2000	5890	2.95
3.	T3	658.67 (25.5)	2000	6586.67	3.29
4.	T4	742.67 (24.1)	2000	7426.67	3.71
5.	T5	624.67 (24.8)	2000	6246.67	3.12
6.	T6	566.00 (23.6)	2000	5660	2.83
7.	T7	581.00 (23.9)	2000	5810	2.91
8.	T8	533.00 (22.9)	2000	5330	2.67
9.	T9	326.67 (17.6)	2000	3266.67	1.63
10.	T10	607.00 (24.5)	2000	6070	3.04
11.	T11	448.67 (21.2)	2000	4486.67	2.24
12.	T12	504.00 (22.2)	2000	5040	2.52

13.	T13	224.33 (15.0)	2000	2243.33	1.12
14.	T14	577.00 (23.9)	2000	5770	2.89
15.	T15	706.00 (26.4)	2000	7060	3.53
16.	T16	571.67 (23.7)	2000	5716.67	2.86
17.	T17	661.00 (25.6)	2000	6610	3.31
18.	T18	552.67 (23.3)	2000	5526.67	2.76
19.	T19	868.00 (29.4)	2000	8680	4.34
20.	T20	642.00 (25.2)	2000	6420	3.21
21.	T21	497.67 (22.1)	2000	4976.67	2.49
22.	T22	514.00 (22.4)	2000	5140	2.57
23.	T23	603.00 (24.4)	2000	6030	3.02
24.	T24	334.67 (17.7)	2000	3346.67	1.67
25.	T25	482.00 (21.7)	2000	4820	2.41
26.	T26	482.00 (21.7)	2000	4820	2.41
27.	T27	603.67 (24.4)	2000	6036.67	3.02

28.	T28	339.00 (18.0)	2000	3390	1.70
29.	T29	714.00 (26.6)	2000	7140	3.57
30.	T30	685.00 (26.0)	2000	6850	3.43
31.	T31	523.00 (22.7)	2000	5230	2.62
32.	T32	584.00 (24.0)	2000	5840	2.92
33.	T33	613.00 (24.6)	2000	6130	3.07
34.	T34	736.00 (27.0)	2000	7360	3.68
35.	T35	503.00 (22.2)	2000	5030	2.52
36.	T36	584.00 (24.0)	2000	5840	2.92
37.	T37	779.00 (27.8)	2000	7790	3.90
	SE(m)	2.216			0.511
	C.D (0.05)	6.258			1.443

4.2.5 Fresh Weight of Root and Shoot

4.2.5.1 Fresh Weight of Root (g)

Data pertaining to fresh weights of root and shoot are enlisted in table 3c and fig.8. Fresh weight of root ranged from 10 g to 101 g and exhibited significant variation with C.D value of 11.08. Genotype EC – 145057 recorded higher root weight with 101 g and was found to be significantly different from all other genotypes. Genotype EC – 631364 recorded lower root weight with 10 g and was found to be statistically on par with EC – 249574 (20 g), EC – 631368 (20 g), EC – 160855 (18 g), EC – 164670 (18 g), EC – 164563 (17 g), EC – 620422 (16 g), EC – 165700 (15 g), Manulakshmi (14 g), EC – 249508 (14 g), Vellayani Vijai (12 g), EC – 620388 (12 g), Kashi Vishesh (10 g) and EC – 620373 (10 g).

4.2.5.2 Fresh Weight of Shoot (g)

Fresh weight of shoot ranged from 22 g to 251 g and exhibited significant variation with C.D value of 20.83. Genotype EC – 620406 recorded higher shoot weight with 251 g and was statistically on par with EC – 145057 (244 g). Lower shoot weight was recorded in EC – 631364 with 22 g and was statistically on par with EC – 620388 (41 g).

Table 3c: Fresh weights of root and shoot in tomato genotypes

Sl. No.	Genotypes	Root Weight (g)	Shoot Weight (g)
1.	T1	36.00	100.00
2.	T2	31.67	146.00
3.	T3	35.67	51.67
4.	T4	11.67	49.67
5.	T5	13.67	114.00
6.	T6	32.00	162.00
7.	T7	9.67	79.67
8.	T8	31.67	212.00
9.	T9	42.00	85.67
10.	T10	26.00	117.67
11.	T11	32.00	204.67
12.	T12	35.00	197.67
13.	T13	22.00	175.67
14.	T14	87.67	151.67
15.	T15	17.67	83.67
16.	T16	19.67	93.67
17.	T17	14.33	114.00
18.	T18	20.00	153.67
19.	T19	5.67	45.67
20.	T20	32.00	177.67
21.	T21	18.00	72.00

22.	T22	10.00	22.00
23.	T23	12.33	41.67
24.	T24	29.67	131.67
25.	T25	24.00	80.00
26.	T26	16.00	148.00
27.	T27	101.67	244.67
28.	T28	7.33	51.67
29.	T29	44.00	251.67
30.	T30	17.67	133.67
31.	T31	24.67	99.67
32.	T32	25.67	107.67
33.	T33	10.00	88.00
34.	T34	65.67	183.67
35.	T35	26.00	114.67
36.	T36	23.00	163.00
37.	T37	33.67	187.67
	SE(m)	3.927	7.377
	C.D (0.05)	11.08	20.83

Table 4: Analysis of Variance for 9 characters of 37 tomato genotypes in the study

Sl. No.	Characters	Mean sum of squares	
		Genotypes	Error
1.	Number of larvae in 5 g root	15.91	2.43
2.	Root Knot Count in 5 g root	27.74	1.04
3.	Number of females in 5 g root	22.047	2.48
4.	Number of egg masses in 5 g root	15.17	2.45
5.	Number of eggs in egg mass	10.217	1.12
6.	Nematode population in 200cc soil	26.13	1.77
7.	Reproduction factor	1.28	0.78
8.	Fresh root weight	1143.36	46.25
9.	Fresh shoot weight	10,154.54	163.25

Significant at 5% LOS

4.2.6 Varietal Reaction to Root Knot Nematode Resistance Screening

Varietal scoring to root knot nematode, *M. incognita* resistance was performed using the modified method of Heald *et al.* (1989) and indicated in the table 5.

Among the 37 genotypes screened for resistance, no genotype was found to be highly resistant, resistant and moderately resistant. Genotype T4 (Vellayani Vijai) was found to be moderately susceptible and genotype T30 (EC-160855) was susceptible. All other genotypes (T1, T2, T3, T5, T6, T7, T8, T9, T10, T11, T12, T13, T14, T15, T16, T17, T18, T19, T20, T21, T22, T23, T24, T25, T26, T27, T28, T29, T31, T32, T33, T34, T35, T36 and T37) were found to be highly susceptible.

Table 5: Tomato varietal reaction to root knot nematode, *M. incognita*

Sl. No.	Genotypes	Number of Galls	Root Knot Index	Reaction
1.	T1	258.67	5	Highly Susceptible
2.	T2	419.00	5	Highly Susceptible
3.	T3	181.67	5	Highly Susceptible
4.	T4	59.00	3	Moderately Susceptible
5.	T5	263.00	5	Highly Susceptible
6.	T6	301.00	5	Highly Susceptible
7.	T7	140.33	5	Highly Susceptible
8.	T8	393.33	5	Highly Susceptible
9.	T9	111.67	5	Highly Susceptible
10.	T10	251.67	5	Highly Susceptible
11.	T11	361.67	5	Highly Susceptible
12.	T12	105.00	5	Highly Susceptible
13.	T13	296.67	5	Highly Susceptible
14.	T14	434.67	5	Highly Susceptible
15.	T15	502.00	5	Highly Susceptible
16.	T16	495.67	5	Highly Susceptible
17.	T17	246.67	5	Highly Susceptible
18.	T18	264.00	5	Highly Susceptible
19.	T19	403.67	5	Highly Susceptible
20.	T20	220.00	5	Highly Susceptible
21.	T21	225.00	5	Highly Susceptible
22.	T22	291.00	5	Highly Susceptible
23.	T23	300.00	5	Highly Susceptible
24.	T24	206.33	5	Highly Susceptible
25.	T25	321.00	5	Highly Susceptible
26.	T26	241.00	5	Highly Susceptible
27.	T27	442.00	5	Highly Susceptible

28.	T28	219.00	5	Highly Susceptible
29.	T29	440.33	5	Highly Susceptible
30.	T30	79.00	4	Susceptible
31.	T31	221.00	5	Highly Susceptible
32.	T32	376.67	5	Highly Susceptible
33.	T33	212.67	5	Highly Susceptible
34.	T34	219.00	5	Highly Susceptible
35.	T35	120.33	5	Highly Susceptible
36.	T36	113.67	5	Highly Susceptible
37.	T37	481.67	5	Highly Susceptible

Plate 9. Juveniles of *M. incognita*

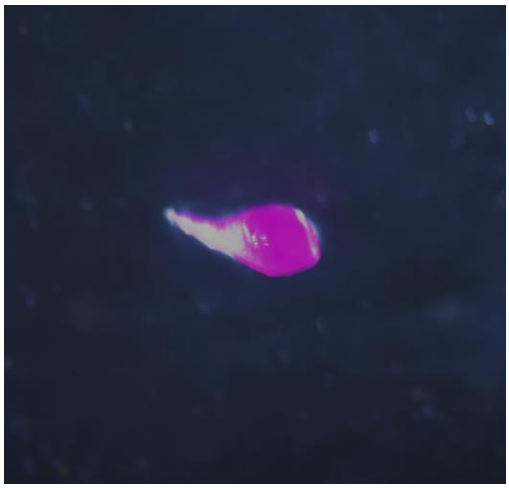


Plate 10. Female of *M. incognita*

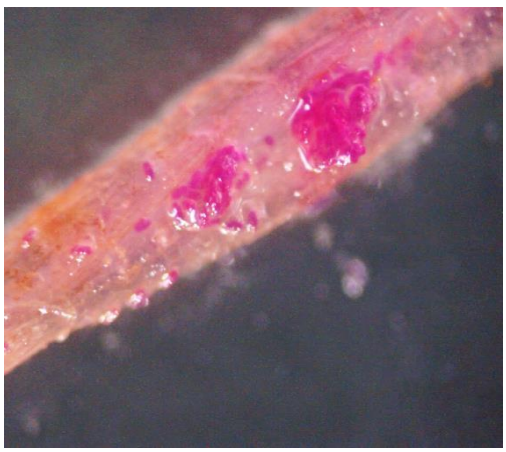
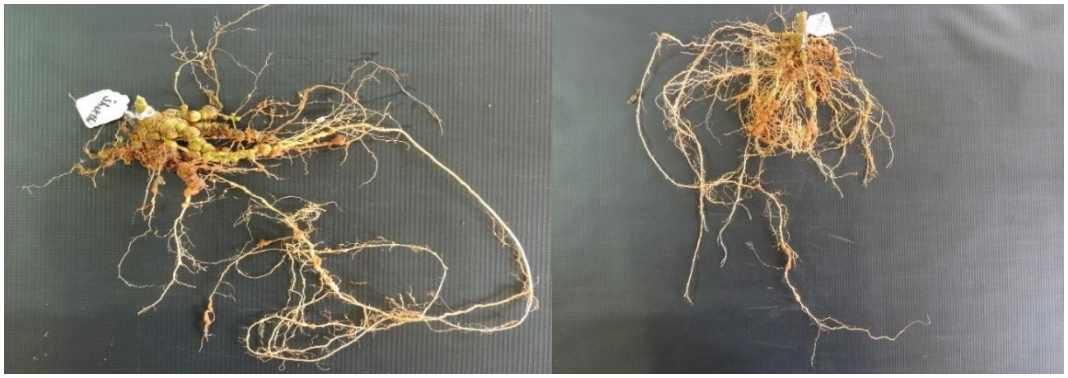


Plate 11. Egg Masses of *M. incognita*

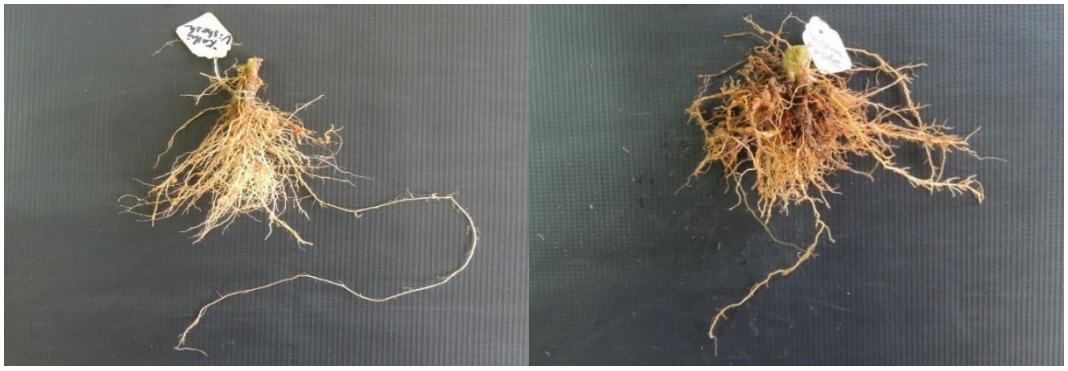


Shakthi **Akshaya**

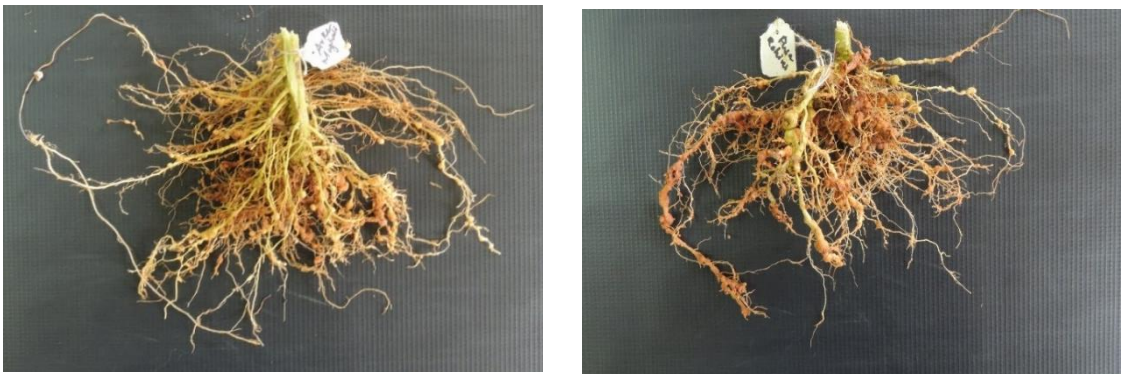
Anagha **Vellayani Vijai**

Manulakshmi **Manuprabha**

Plate 12. Root knot formation on tomato germplasm



Kashi Vishesh **Palam Pride**



Arka Meghali **Pusa Rohini**

Solanum hirsutum **IIHR 2200**

Plate 12 (continued). Root knot formation on tomato germplasm

IIHR 2868

IC – 45

EC – 164563

EC – 249574

EC – 249508

EC – 631368

Plate 12 (continued). Root knot formation on tomato germplasm

EC – 165700

EC - 549819

EC – 164670

EC – 631364

EC – 620388

EC – 620395

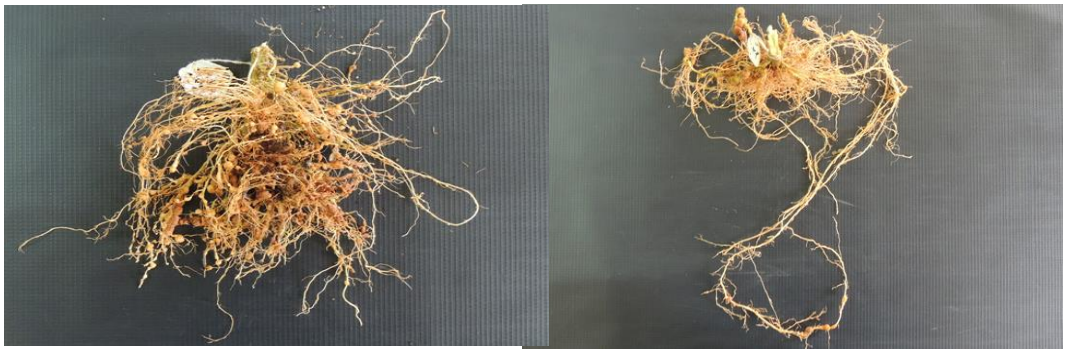
Plate 12 (continued). Root knot formation on tomato germplasm

EC - 620417 **EC - 620422**

EC – 145057 **EC – 631359**

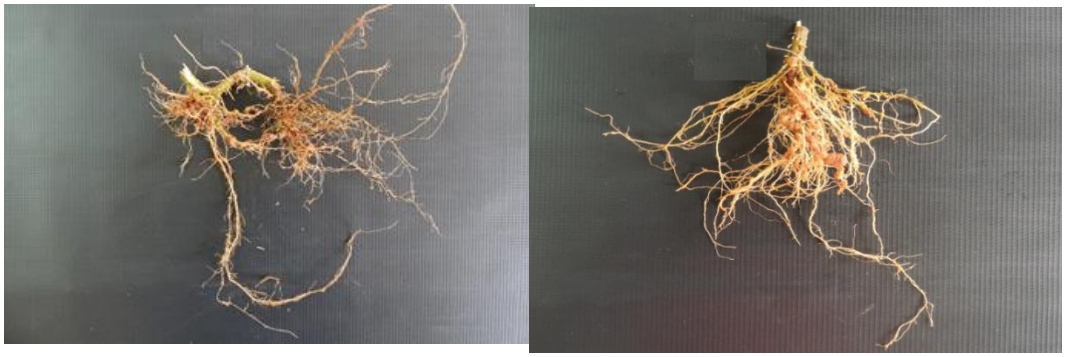
EC – 620406 **EC – 160855**

Plate 12 (continued). Root knot formation on tomato germplasm



EC – 620394 **EC – 620431**

EC – 620373 **EC – 620401**



PNR – 7 **Hisar Lalit**

Plate 12 (continued). Root knot formation on tomato germplasm

PKM – 1

Plate 12 (continued). Root knot formation on tomato germplasm

DISCUSSION

5. DISCUSSION

Tomato is an important vegetable crop all over the world. Global tomato production faces potential risk due to *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood. Host plant resistance provides effective and economical method of managing nematodes in both low and high value cropping systems. Tomato varieties released by Kerala Agricultural University were not evaluated for root knot nematode resistance and thus an attempt has been made here through screening of these germplasm for root knot nematode resistance including other genotypes collected from different universities and institutions in India.

The present study was conducted as two experiments. The first experiment comprised of collection, identification and multiplication of *M. incognita* culture and the second experiment screening tomato germplasm for root knot nematode resistance in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani.

In the current investigation, thirty seven genotypes of tomato were screened for nematode resistance under polyhouse conditions.

5.1 Screening for Resistance to *M. incognita*

Root Knot Nematode, *M. incognita* is a serious pathogen attacking all Solanaceous vegetables especially tomato. Root knot nematode attack leads to production of conspicuous galls on root system that leads to poor development of tomato plants leading to higher economic damage due to poor fruit quality as well as reduced number of harvests. Identification of varieties with resistance to nematode infestation is necessary for eco-friendly management of this pest.

The study was performed to evaluate the reaction of thirty seven tomato genotypes against root knot nematode, *M. incognita*. Each pot was artificially

inoculated with 2000 second stage juveniles of *M. incognita.* Comparative reaction of the genotypes screened were evaluated in terms of nematode characteristics in root and soil.

Reduction in growth parameters and nematode infestations were found to be proportional to inoculum density and *M. incognita* was found to be pathogenic to tomato at all inoculum levels and damage was most severe at 2000 J_2 kg $soil^{-1}$ (Kanakam and Adomako, 2014).

5.1.1. Nematode Popuation Characteristics in Root

Regarding the root-knot count, number of larvae, females, egg masses and egg egg $mass^{-1}$ all the varieties found to be highly susceptible to *M. incognita* infestation.

Genotype PNR – 7 recorded lowest number of larvae and egg masses in 5 g root. EC – 165700 recorded highest root knot count and number of females 5 g $root^{-1}$. This result is in accordance with the findings observed in tomato cultivars screened for root knot nematode resistance by Begum *et al.* (2014)

According to Cousins and Walker (1998) root knot nematode eggs developed poorly on root knot nematode resistant tomato genotypes compared to susceptible ones. This is in accordance with the current investigation where, genotype PNR – 7 recorded lower number of egg masses 5 g $root^{-1}$ and genotypes Hisar Lalit and Vellayani Vijai are on par with it.

Also quantity of eggs reflects the number of nematodes that reached reproductive maturity and therefore provide a measure of resistance. Karssen and Moens (2006) reported that highly susceptible plants allowed juveniles to enter the roots, reached maturity stage and produce eggs while resistant cultivars does not allow reproduction of nematodes.

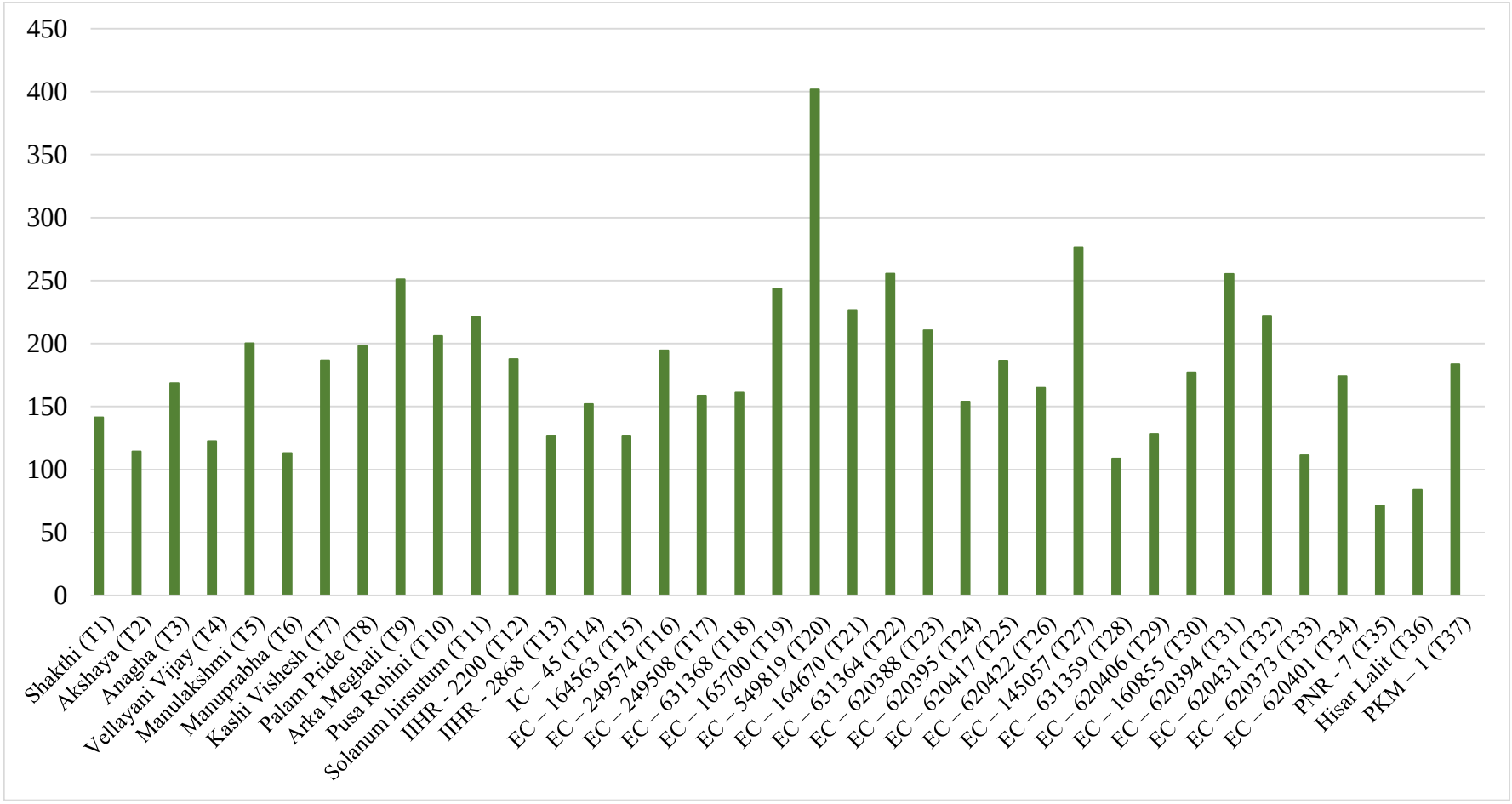


Fig.1: Number of larvae in 5 g root of tomato germplasm

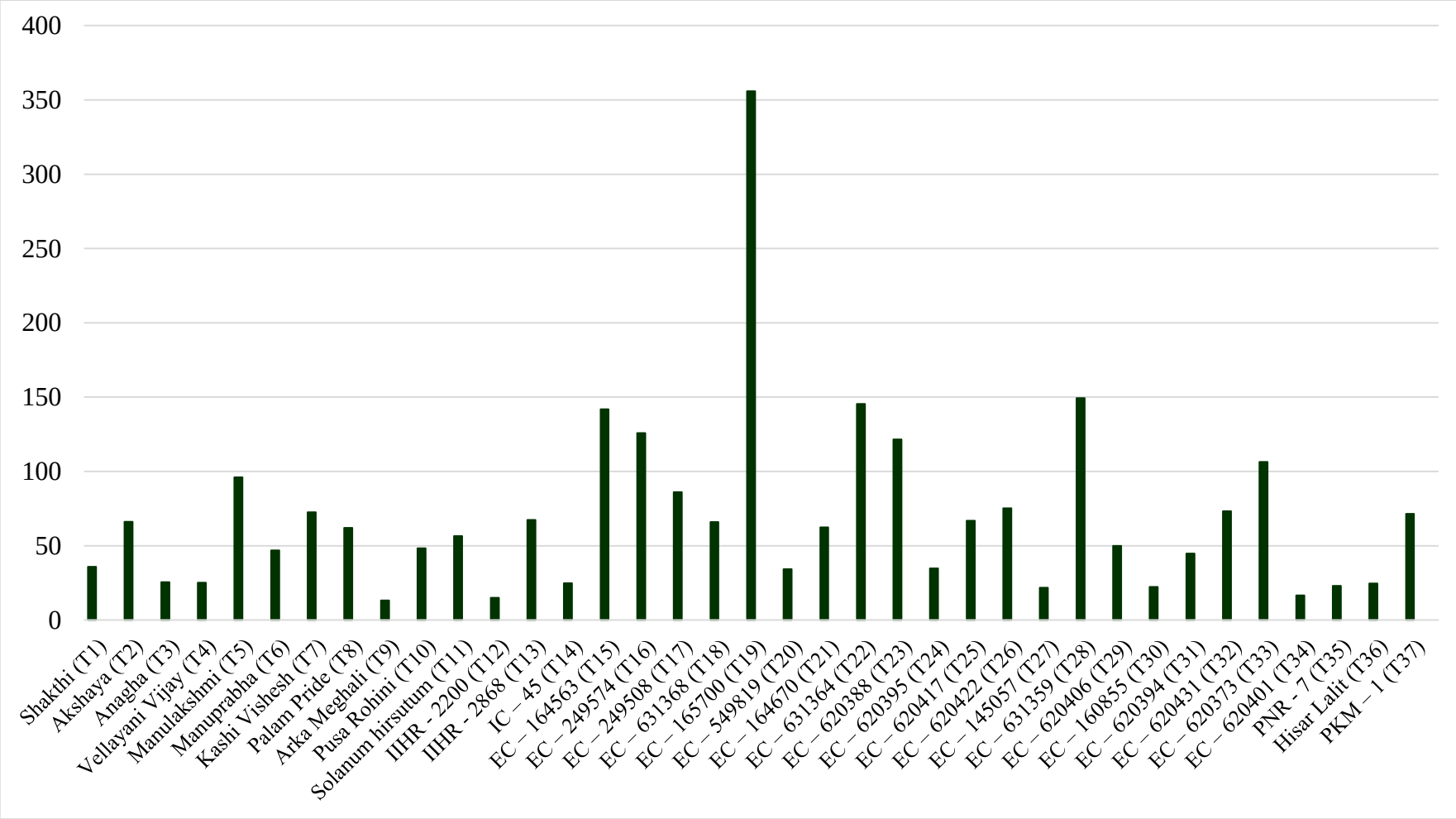


Fig.2: Root knot count in 5 g root of tomato germplasm

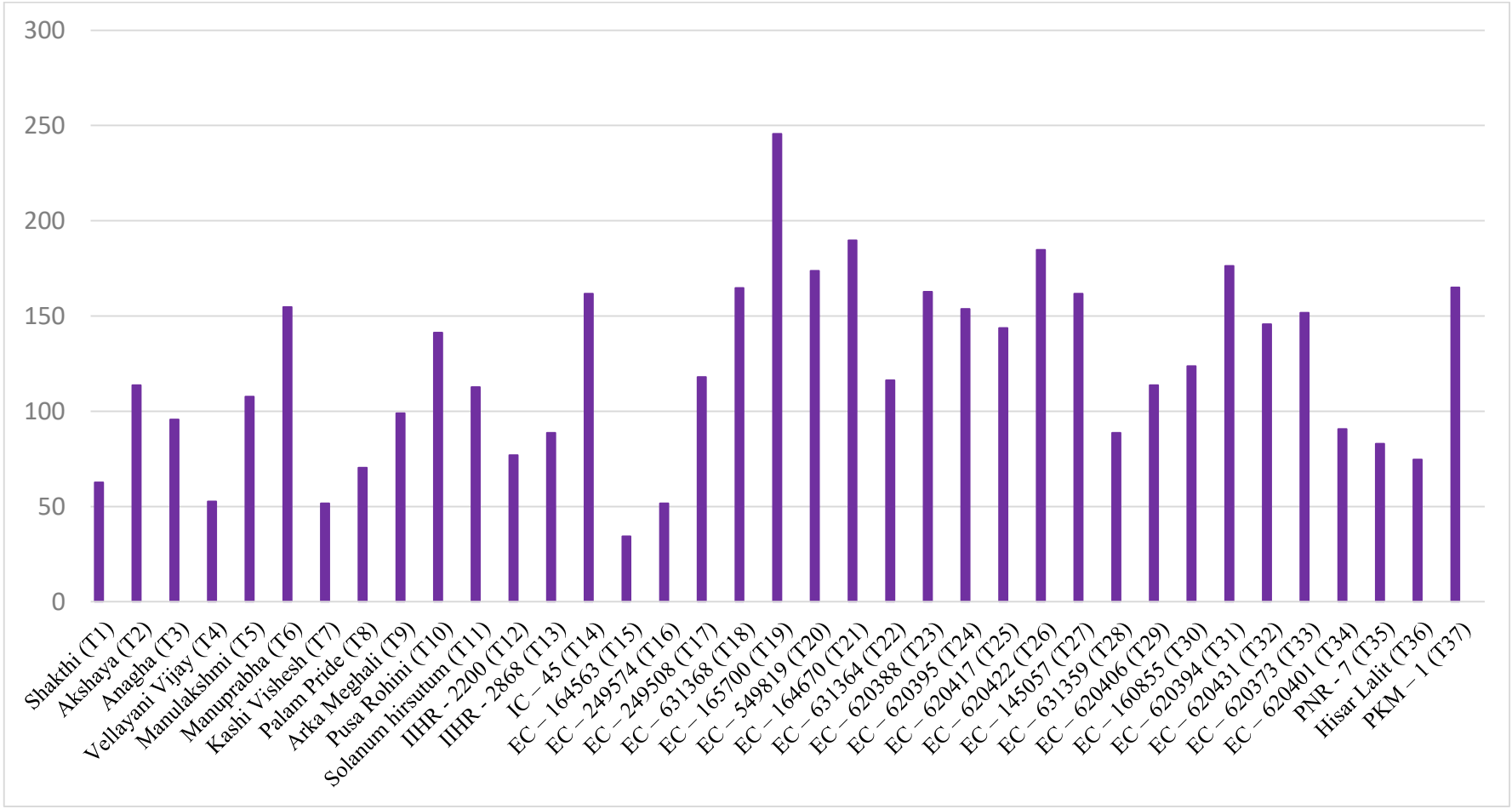


Fig.3: Number of females in 5 g root of tomato germplasm

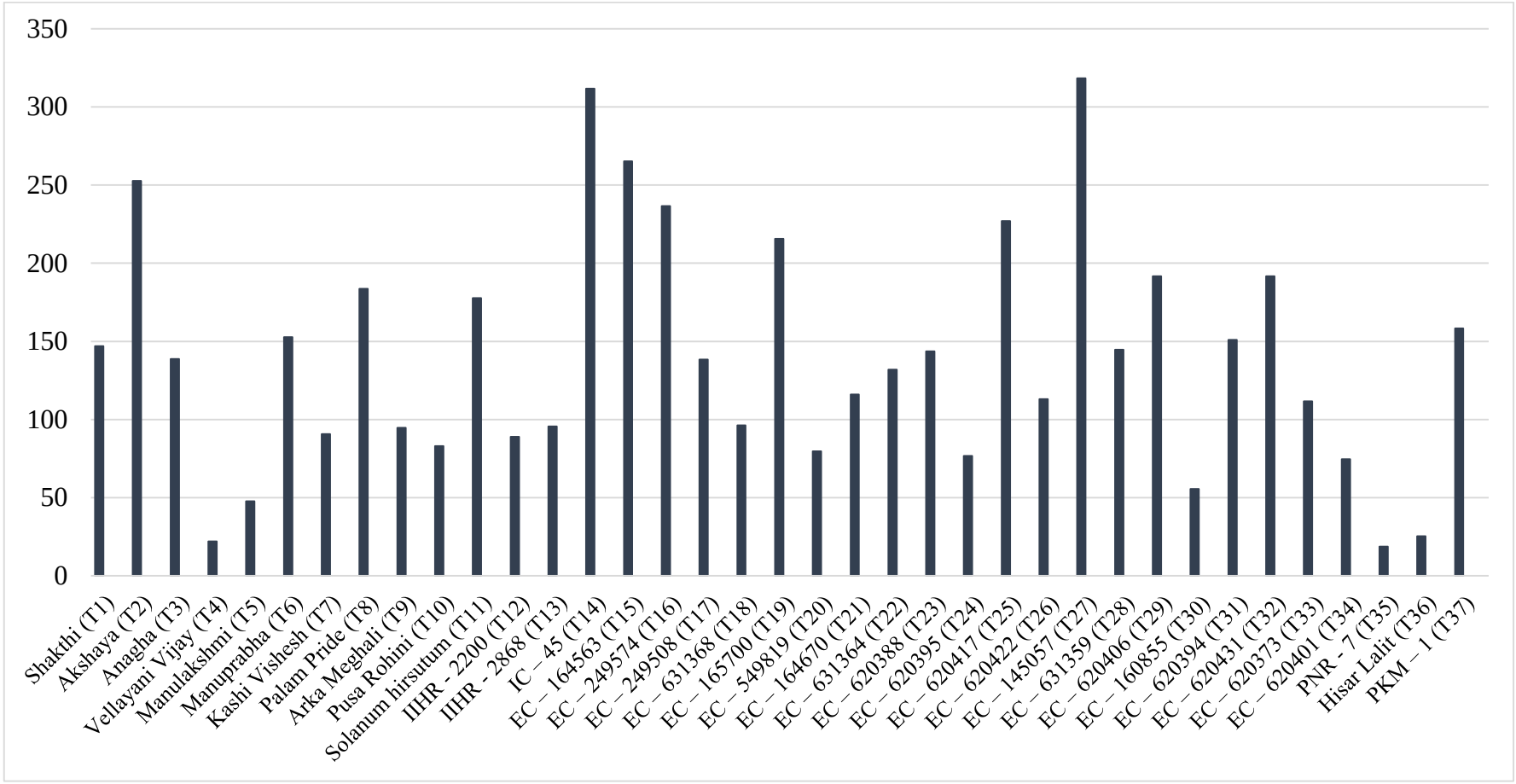


Fig.4: Number of egg masses in 5 g root of tomato germplasm

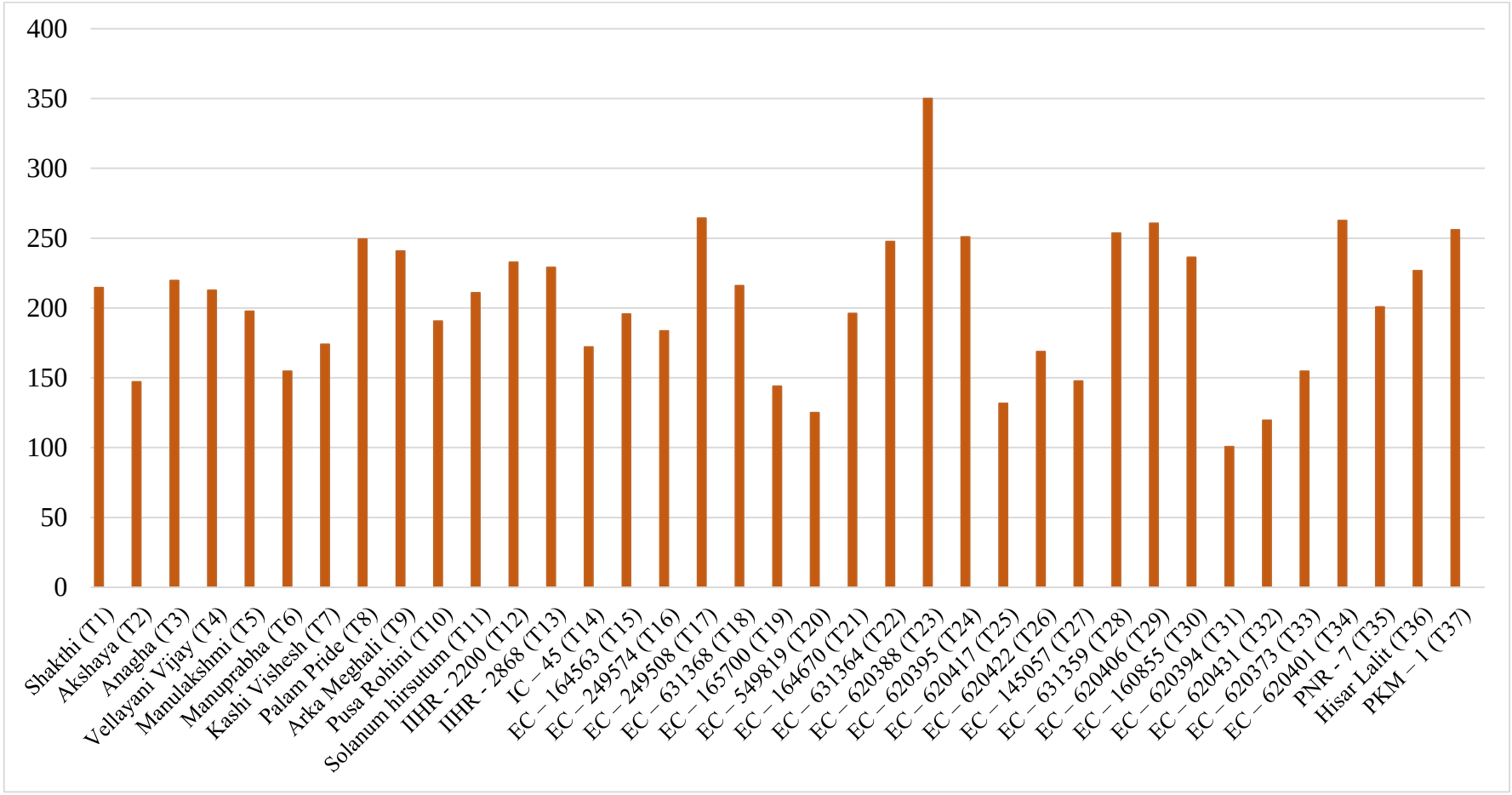


Fig.5: Number of eggs in egg mass of tomato germplasm

5.1.2 Nematode Population in 200 cc soil

Minimum nematode population in 200 cc soil was observed in the genotype IIHR – 2868, while EC – 165700 recorded highest number of nematodes in 200 cc soil. Seenivasan and Devarajan (2008) reported an increase in *M. incognita* nematode population at the time of uprooting the plants under screening.

The result obtained is in accordance with the work done by Danso (2010) where the tomato genotype H24 which recorded lower number of nematodes in 200cc soil with 67 nematodes. But the same H24 genotype did not record lower number of nematodes in root (92 J_2 g of root^{-1}).

EC – 165700 which recorded highest nematodes 200 cc soil^{-1} also recorded higher root knot count and number of females in 5 g root. This result is supported by the findings of Swetha *et al.* (2019), where the tomato genotype IC249503 was reported with higher number of juveniles 200 cc soil^{-1} (3052.80 J_2 200cc soil^{-1}).

5.1.3 Reproduction Factor

IIHR – 2868 recorded lower reproduction factor (1.122), while EC – 165700 recorded higheer reproduction factor (4.340) which also recorded higher root knot count and number of females in 5 g root. This result is in accordance with the data obtained from experiment conducted by Karjeh *et al*. (2005), where the tomato cultivar Betterboy indicated high root gall index (4.73) and high reproduction factor (3.73) when screened for root knot nematode resistance.

Host plants having varying degrees of susceptibility, allows juveniles to enter the roots, reach maturity and produce many eggs, while resistant cultivars do not allow nematode reproduction (Taylor and Sasser, 1978; Karssen and Moens, 2006).

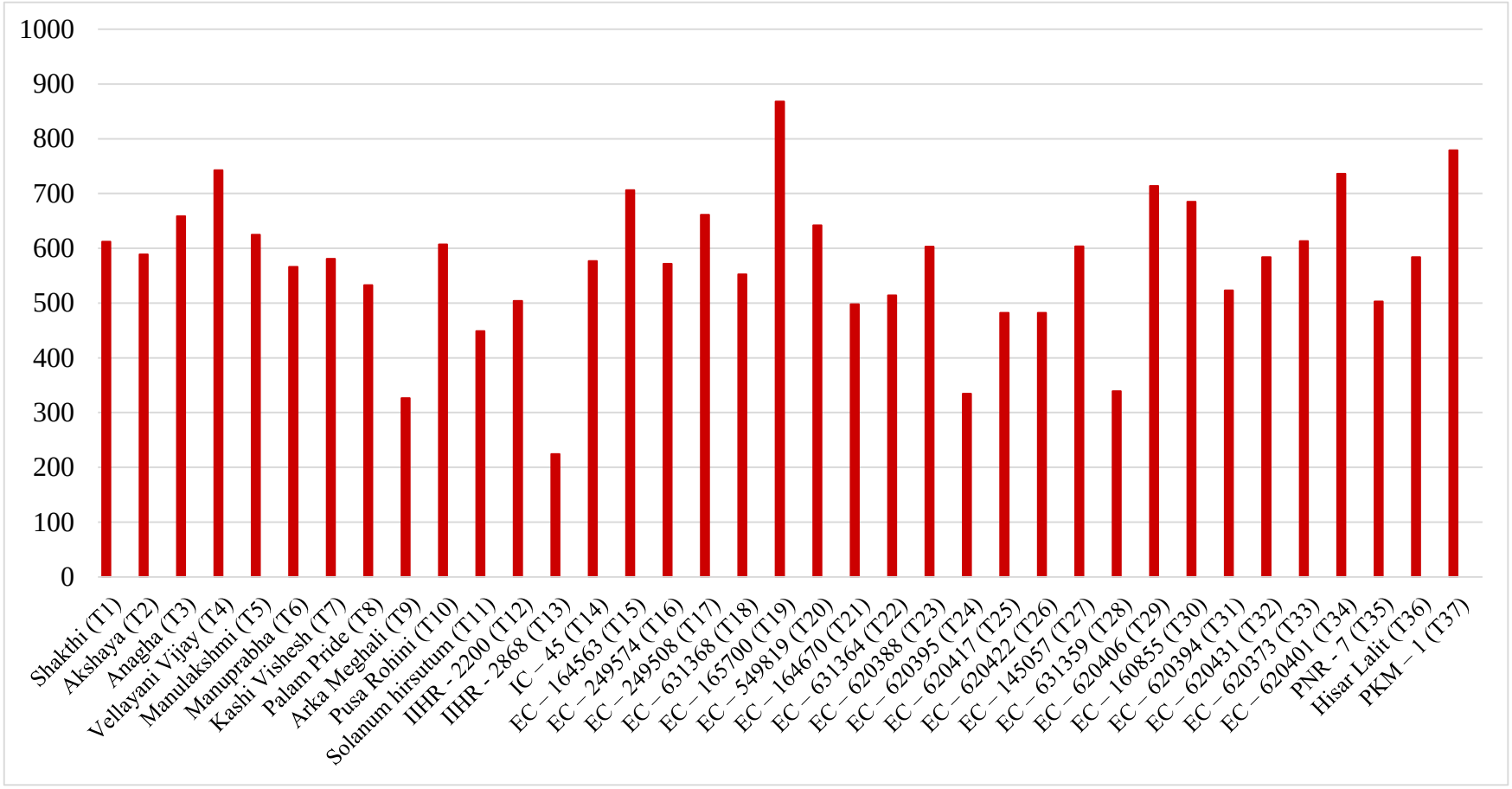


Fig. 6: Nematode population in 200 cc soil

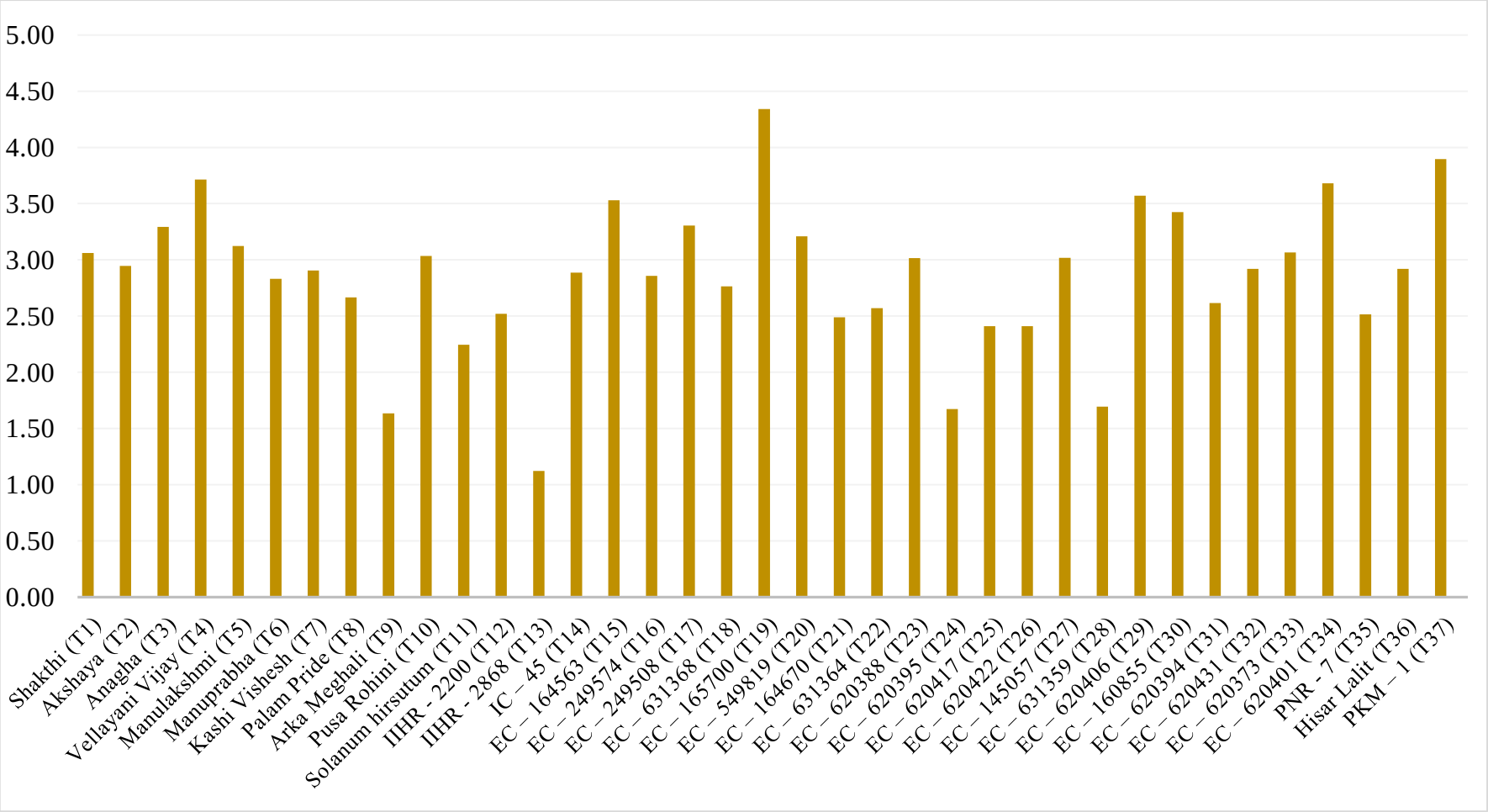


Fig. 7: Nematode reproduction factor in tomato germplasm

Plant's resistance and susceptibility to *M. incognita* reflects nematode ability to reproduce (Cook and Evans, 1987). Hirunsalee *et al*. (1995) mentioned that reproduction and galling of nematodes on plant root were favoured in tolerant and resistant cultivars but inhibited on resistant genotypes.

5.1.4 Fresh Root and Shoot Weight

The induction of galls and giant cells in the stellar region by *M. incognita* disrupts xylem tissues and retards the absorption and upward movement of water and nutrients which ultimately leads to reduction of shoot weight and increase in root weight due to development of galls. EC – 620406 recorded higher shoot weight. EC – 145057 recorded higher root weight. EC – 631364 recorded lower shoot and root weight. Stunted growth in tomato plants heavily infested with root knot nematodes was observed by Siddique and Alam (1985).

Roberts *et al*. (1995) observed increase in root weight of susceptible cultivars due to nematode infestation. The specialized feeding cells in roots and alter root - shoot balance by redirecting photosynthates produced in leaves to supply the demand of nematodes in roots (Hunt *et al*., 2005).

El-Sherif *et al.* (2007) reported an increase in root weight for the most susceptible cultivar compared to resistant cultivar upon root knot nematode infestation. This is due to root knots functioning as metabolite sinks as nutrients produced from leaves are distributed to root galls and bodies of nematodes.

5.1.5 Varietal Reaction to Root Knot Nematode, *M. incognita*

Among thirty seven genotypes screened no genotype was found to be highly resistant, resistant or moderately resistant. The KAU released variety Vellayani Vijai was moderately susceptible, EC – 160885 was susceptible and all other genotypes are highly susceptible.

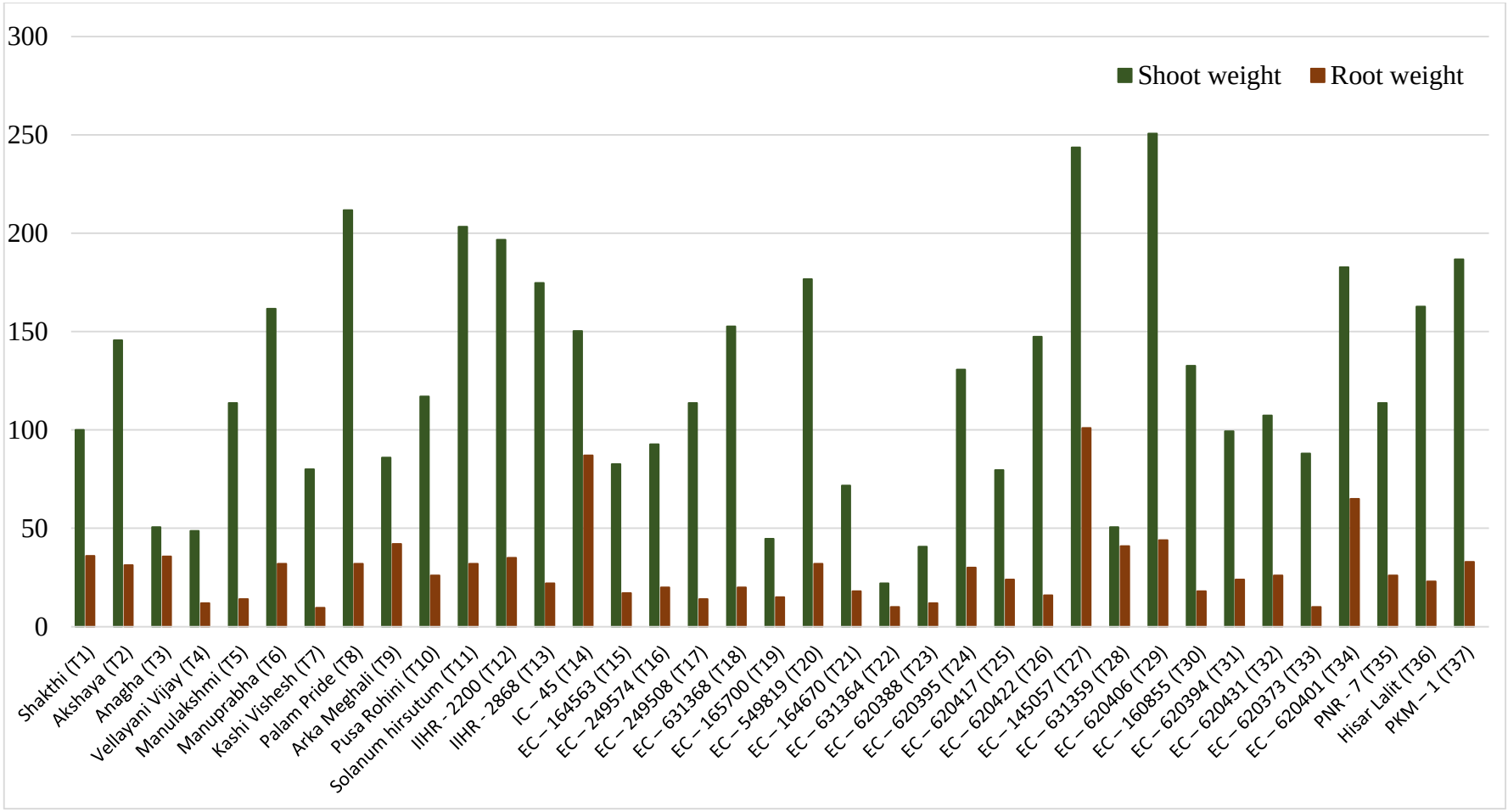


Fig.8: Fresh weight of root and shoot of tomato germplasm

The vigour of a plant influences resistance to root knot nematodes (Kher, 1996). Vellayani Vijai is determinate and short statured with specific advantage of high yield (37.26 t / ha) coupled with tolerance to bacterial wilt, high temperature and partial yield. It adapts well to southern districts of Kerala.

The moderately susceptible Vellayani Vijai showed resistance potential by exhibiting lower root knots in root system and a significant record of low number of root knots, number of females and egg masses in 5 g root. Vellayani Vijai recorded a root weight of 11.67 g which was on par with the lower root weight recorded in EC – 631364 (10 g).

Maximum mean temperature inside the polyhouse under current investigation was recorded to be 35.5ºC and a minimum mean temperature was recorded to be 25.4ºC. Most studies reported a complete loss of resistance at higher temperature above 32ºC (Dropkin, 1969; Williamson, 1998). El-Sappah *et al*. (2019) reported that resistance breakdown as a major problem at higher temperatures >28ºC. The nematode becomes active when the soil temperature is 18 - 32ºC. The temperature factor is highly essential since the natural resistance only works when temperature is below 27ºC. Other studies have shown that there is active resistance at soil temperatures >34ºC, which is possibly due to heat stable resistance (Verdejo-Lucas *et al.*, 2009; Abdul-Baki *et al.*, 1996). So, the possible reason for loss of resistance in resistant cultivars like PNR – 7 and Hisar Lalit under current study may be due to higher temperature.

No genotype was found to be resistant to root knot nematode in the current study. This result was supported by the findings of Nihal *et al.* (2019) where fifty one genotypes of tomato were screened against root knot nematode, *M. incognita* (Kofoid and White, 1919) Chitwood race 2 resistance and no genotype was found to be highly resistant in the work.

Sujatha *et al.* (2017) conducted a similar screening work in forty tomato genotypes for root knot nematode, *M. incognita* resistance and found Hisar Lalit, PNR – 7 and IIHR – 2868 as resistant. This result is not supported for the current study since both Hisar Lalit and PNR – 7 were found to be highly susceptible. This could possibly due to progressive increase in virulence of the nematode upon prolonged selection and temperature that prevailed during screening *i.e*. 35.5ºC, where resistance is broken.

Hisar Lalit and PNR – 7 are the resistant cultivars used in the present study but both of them resulted in lack of resistance. Kaloshian *et al.* 1996 reported a similar conclusion where, the resistant cultivars were reported to be susceptible to *M. incognita* populations probably due to repeated planting of resistant cultivars since the resistance is conferred by a single gene *Mi* in most commercial cultivars. Soil temperatures above 28ºC coupled with mono cropping may also lead to resistance breaking more rapidly.

The nematodes used in the current study tend to show resistance breaking in resistant cultivars. This is supported by the findings of Tzortzakakis *et al*. (2016) where six resistance breaking genotypes of root knot nematodes (four *M. javanica* and two *M. incognita*) in tomato fields were observed from Crete region of Greece apart from already existing thirteen resistance breaking genotypes of root knot nematodes in the country Greece. The six nematode populations reproduced on resistant genotypes to a level, where there was no significant difference to that of their reproduction on susceptible genotypes.

Differences in reaction of genotypes against root knot nematode could be due to interaction between nematodes and secondary plant metabolites or defense enzymes produced. Afifah *et al*. (2019) reported upregulation of metabolites like sugar transporters, starch synthases, myoinositol phosphate oxygenase, sucrose UDP-glucose dehydrogenase (UGD) and ascorbic acid in resistant cultivars in comparison to susceptible cultivars.

The identified superior genotype Vellayani Vijai which was moderately susceptible for root knot nematodes can be evaluated for resistance to nematode infestation under field conditions and evaluate the yield loss due to infestation.

SUMMARY

6. SUMMARY

The present study on "Phenotyping of tomato germplasm for root knot nematode resistance" was carried out in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani during 2018-2020. Thirty seven tomato genotypes including released varieties of KAU were screened for root knot nematode resistance.

The study comprised of two experiments. In the first experiment; collection, identification and multiplication of *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood culture for artificial inoculation was performed upon collecting root knot nematode infested root and soil samples from tomato plants at Department of Nematology, College of Agriculture, Vellayani. Root knot nematode females, egg masses and juveniles were extracted from the samples and identified under stereomicroscope. The collected soil samples are then inoculated to healthy seedlings for multiplication and maintained as pure source of inoculum.

The second experiment was performed by screening tomato germplasm for root knot nematode resistance. Thirty seven tomato genotypes (including released varieties of KAU) were evaluated in Completely Randomized Design with three replications for root knot nematode resistance. The seedlings were transplanted to grow bags comprising sterile sand mixture and raised under polyhouse conditions. Fifteen days after transplanting the seedlings, hatched juveniles were inoculated @ 2000 juveniles plant^{-1}. The inoculated plants were carefully monitored and uprooted at forty five days after inoculation.

Observations were recorded for number of larvae in 5 g root, root-knot count in 5 g root, number of females in 5 g root, number of egg masses in 5 g root, average number of eggs egg mass^{-1} and nematode population in 200 cc soil. Reproduction factor was calculated to assess the reproductive potential. Weight of root and shoot under plant were recorded. Analysis of variance was found to be significant for all

the parameters observed in the study.

PNR – 7 recorded lower number of larvae (71 in 5 g root) and was on par with Hisar Lalit (84), EC – 631359 (109), EC – 620373 (111), Manuprabha (113) and Akshaya (114). IIHR – 2200 recorded lower number of root knots 5 g root^{-1} (14.9) and was on par with EC – 620401 (16.6), EC – 145057 (21.7), EC – 160855 (22.3), PNR – 7 (23.1), Hisar Lalit (24.6), IC – 45 (24.7), Vellayani Vijai (25.2) and Anagha (25.6). EC – 164563 reported lower number of females 5 g root^{-1} (34) and was on par with Kashi Vishesh (52), EC – 249574 (52), Vellayani Vijai (53) and Shakthi (63). PNR – 7 recorded lower number of egg masses 5 g root^{-1} (19) and was on par with Hisar Lalit (25) and Vellayani Vijai (22). EC – 620394 reported lower number of eggs egg mass^{-1} (101) and was on par with EC – 620431 (120), EC – 549819 (125) and EC – 620417 (132).

IIHR – 2868 recorded lower and EC – 165700 recorded higher for number of nematodes in 200 cc soil as well as reproduction factor.

Fresh root and shoot weights of all plants under screening were recorded after uprooting. EC – 145057 reported higher root weight (101 g) and was significantly different from other treatments. EC – 620406 reported higher shoot weight (251 g) and was on par with EC – 145057 (244 g).

Root knot indexing was done using the method given by Heald *et al.* (1989). The genotypes were categorized on a root knot index scale of 0-5 using total root knots in root system (0 – highly resistant, 1 – resistant, 2 – moderately resistant, 3 – moderately susceptible, 4 – susceptible and 5 – highly susceptible). No genotype among the thirty seven genotypes screened was observed under highly resistant, resistant or moderately resistant categories, while Vellayani Vijai was under moderately susceptible category, EC – 160855 under susceptible category and all other genotypes under highly susceptible category.

The study revealed the lack of resistance in all the genotypes. The temperature inside polyhouse being above 28ºC was found to be most probable reason for break of resistance even in nematode resistant checks. Vellayani Vijai and EC – 160855 genotypes can be forwarded further for estimation of fruit yields under nematode infected fields. Genotyping can also be performed to check for the presence of gene *Mi* conferring resistance to *M. incognita*.

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ABSTRACT

The present study entitled “Phenotyping of tomato germplasm for root knot nematode resistance” was carried out in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani during 2018-2020, with the objective to screen tomato germplasm including released varieties of KAU for root knot nematode resistance through artificial screening.

The study comprised of two experiments. In the first experiment, collection, identification and multiplication of *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood culture for artificial inoculation was performed. Root knot nematode infested root and soil samples were collected from tomato plants in the Department of Nematology, College of Agriculture, Vellayani. Root knot nematode females, egg masses and juveniles were extracted from the samples and identified. The collected soil samples upon identification are then inoculated to healthy seedlings for multiplication and maintained as pure source of inoculum.

The second experiment was screening of tomato germplasm for root knot nematode resistance using thirty seven tomato genotypes (including released varieties of KAU) which were evaluated in Completely Randomized Design with three replications. Fifteen days after transplanting the seedlings, hatched juveniles were inoculated @ 2000 juveniles plant^{-1}. Observations were recorded for number of larvae in 5 g root, root-knot count in 5 g root, number of females in 5 g root, number of egg masses in 5 g root, average number of eggs in egg mass and nematode population in 200 cc soil. Reproductive potential was assessed by calculating reproduction factor. Weight of root and shoot were recorded. Analysis of variance was found to be significant for all the parameters observed.

PNR – 7 recorded lower for number of larvae and egg masses in 5 g root. EC – 165700 recorded higher for root knot count and number of females 5 g root^{-1}.

IIHR – 2868 recorded lower and EC – 165700 recorded higher for number of nematodes in 200 cc soil as well as reproduction factor.

Root knot indexing was done using the method given by Heald *et al.* (1989). The genotypes were categorized on a root knot index scale of 0-5 using total root knots in root system (0 – highly resistant, 1 – resistant, 2 – moderately resistant, 3 – moderately susceptible, 4 – susceptible and 5 – highly susceptible).

The study revealed the lack of resistance in all the genotypes. No genotype was found to be highly resistant, resistant or moderately resistant. Vellayani Vijai was found to be moderately susceptible with a root knot index of 3 and EC – 160855 was susceptible with a root knot index value of 4, while all other genotypes in the study were highly susceptible. Vellayani Vijai can be forwarded further for fruit yields under nematode infected fields. Genotyping can also be performed to check for the presence of gene *Mi* conferring resistance to *M. incognita.*