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Biodiversity of Root-Knot Nematodes (*Meloidogyne* spp.) associated with Tomato and Characterisation and Screening of Tomato Genotypes against *Meloidogyne* spp. from Ethiopia

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Dedication

I dedicate this PhD thesis to my beloved wife ‘Nej’ (Nejat Hassen Yimer) for her constant support, understanding and unconditional love and to all my family. I love you all dearly.

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List of Abbreviations

AF - Adult Females

AMF - Arbuscular Mycorrhizal Fungi

ANOVA - Analysis of Variance

AVRDC - Asian Vegetable Research and Development Centre

CI - Confidence Interval

COR - Crude Odds Ratio

DD - Degree-days

EIAR - Ethiopian Institute of Agricultural Research

EMI – Egg mass Index

EPPO - European and Mediterranean Plant Protection Organization

EST - Esterase

HPR - Host-Plant Resistance

HU - Haramaya University

ILVO - Institute for Agricultural and Fisheries Research

J1 – First-stage juveniles

J2 - Second-Stage Juveniles

J3 – Third-stage Juveniles

J4 – Fourth-stage juveniles

LSD - Least Significant Difference

MARC - Melkassa Agricultural Research Centre

MDH - Malate Dehydrogenase

MIG - *Meloidogyne incognita* Group

PCR - Polymerase Chain Reaction

PPN - Plant-Parasitic Nematodes

RCBD - Randomized Complete Block Design

RGI - Root-Gall Index

RKN - Root-Knot Nematodes

SARC - Sirinka Agricultural Research Centre

SCAR - Sequence Characterized Amplified Region

SJ - Swollen Juveniles

SSF - Small-Scale Farmers

VJ - Vermiform Juveniles

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Chapter 1: General Introduction

1.1.Tomato Production in the World

Tomato (*Solanum lycopersicum*) belongs to the Solanaceae family, which contains many important food crops such as potato, pepper and eggplant (Inga *et al*., 2006). The crop has a diploid chromosome number (2n=24) (Naika *et al*., 2005). It originated in the Andes, South America. The cultivated tomato was first taken to Europe by Spanish conquistadors in the 16th century and then introduced into southern and eastern Asia, Africa, the Middle East and distributed throughout the world (Purseglove, 1968; Naika *et al*., 2005). The crop has developed into a great number of cultivated types suitable to different environments, methods of production, and food uses (Guillaume & Mathilde, 2012). Today tomato is considered as one of the most widely grown vegetable crops and constitutes a major agricultural industry (Foolad, 2007). It is undoubtedly the most popular garden crop too. World tomato production in 2013 was about 164.5 million tons from an estimated 4.7 million hectares. The top 5 tomato producing countries in the same year were China, India, USA, Turkey and Egypt in descending order (FAOSTAT, 2014).

The tomato fruit contains abundant and well balanced nutritional components which includes minerals (calcium, iron, phosphorus), vitamins (vitamin A, B, C), protein (essential amino acids), sugars, dietary fibers (pectin) and citric acid (Sekyewa, 2006). Tomatoes are major sources of lycopene, a dietary carotenoid found in high concentrations in processed tomato products (Di Mascio *et al*., 1998). This compound is an antioxidant known to combat cancer, heart diseases and premature aging (Wener, 2000). Fresh and processed tomatoes such as concentrates, puree and paste are increasingly in demand in the world where they form an essential part of the human diet (Guillaume & Mathilde, 2012).

1.2. Tomato Production in Ethiopia

It is believed that cultivated tomato was introduced into Ethiopian agriculture between 1935 and 1940 (Gemechis *et al*., 2012). No local cultivars of tomato have evolved or been developed and hence all varieties grown are introduced (Yayeh, 1989). However, right after the establishment of the Ethiopian Institute of Agricultural Research (EIAR) in 1966, tomato was recognized as a commodity crop (Gemechis *et al*., 2012). Its versatility in fresh or processed form has played a major role in its rapid and widespread adoption as an important food commodity (Bezabeh *et al*., 2014).

The first record of commercial tomato cultivation dates back from 1980 with a production area of 80 ha (Lemma, 2006) in the upper Awash by Merti Agroindustry for both domestic as well as export markets. By 1993, its total area increased to 833 ha and later on the cultivation spread towards other parts of the country. Since 1994 up to 2014, tomato acreage increased to 7,257.45 ha to produce 393,730.22 tons of tomatoes (FAOSTAT, 2014).

Tomatoes constitute a major farming activity in Ethiopia due to the favourable ecological conditions, importance in daily diet and proximity for export marketing to the Republic of Djibouti and Middle East countries (Tadele & Mengistu, 2000). It is one of the most important regional export crops in the country (Joosten *et al*., 2011). Tomato is an important cash vegetable crop produced by both commercial and small-scale farmers (SSF) in Ethiopia (Mekete *et al*., 2003). Recently, due to expansion of state farms and private investments, commercial production of tomato both in terms of area and production is on the rise.

Tomato is grown in many parts of Ethiopia: on large-scale basis under irrigation at Merti, Upper Awash Agro Industry Enterprise (UAAIE) and for fresh consumption in Melkasa, Koka, Meki, Zeway, Wondo-Genet, Guder, Bako, Wollo, Haragahe, Shawa, Jimma and Wallaga (Shimeles, 2000; Gemechis *et al*., 2012) and in areas where sufficient irrigation supplies are present. SSF are taking the lion share of the tomato production in the country. The smallholders are interested in tomato production more than in any other vegetables due to its multiple harvest potential and high profit per unit area (Bezabeh *et al*., 2014).

Since 1969, more than 300 tomato varieties were tested for their agronomic qualities and disease resistance potentials. Most of the varieties tested showed susceptibility to late blight (*Phytophthora infestans*), powdery mildew (*Oidium neolycopersici*) and mosaic virus (Lemma, 2002) but no information was reported about their growth and yield when grown in soil infested with root-knot nematode (RKN) populations. Since the inception of its commercialization SSF have grown tomato to satisfy their livelihood needs. However, tomato growers have often been challenged by inconsistent tomato production and significant yield loss due to pests and diseases (Mandefro & Mekete, 2002). Different biotic and abiotic factors may be attributed for lower tomato production in Ethiopia. RKN are among the most damaging threats for tomato production due to their wide distribution and pathogenicity in tropical and sub-tropical climates coupled with their wide host range (Coyne *et al*., 2009). Despite several reports on the damage of RKN on tomato crop in Ethiopia there were no

management practices used by the SSF of Ethiopia. The average yield of tomato has always been low (8 tons/ha) compared with yield in the neighbouring country Kenya (21 ton/ha) and the average yields of 54, 42, 35, 20 and 35 tons/ha in America, Europe, Asia, Africa and the entire world (FAO, 2013) respectively.

1.3. Tomato and Food Security

In the 21st century, one of the most pressing issues for humanity is global food security (Sundström *et al*., 2014). That was why the first of the UN's eight millennium development goals is to reduce the proportion of the world's population that suffers from hunger by half between 1990 and 2015 (UN, 2015). Agricultural production is critical for achieving global food security (Nayyar & Dreier, 2012). More than half of the global population growth between 2015 and 2050 is expected to occur in Africa. Africa has the highest rate of population growth among major areas, growing at a pace of 2.55% annually in 2010-2015. Consequently, of the additional 2.4 billion people projected to be added to the global population between 2015 and 2050, 1.3 billion will be added in Africa. The population of Ethiopia is also projected to be 188 million by 2050 (UN Report, 2015), which means double from the current population (90million). It is clear that as the population grows, the land available for agricultural purposes will continue to decrease (Lemma, 2015). Boosting agricultural production in the face of a growing population is one of the major challenges of Sub-Saharan Africa (Gilbert *et al*., 2014). To meet the escalating demand for food owing to the growth of global population, a vertical increase in crop production is the only viable means in the decades to come. Agricultural intensification with the use of agricultural inputs and improved technologies such as high yielding and disease resistant crop varieties are highly needed (Pender *et al*., 2006). However, intensification of agriculture has its own drawbacks: the appearance of emerging diseases and the occurrence of virulent populations will be the most important threat of crop production (Tilman, 1999).

In many countries, rapid urbanization is accompanied by increasing urban poverty, food insecurity and malnutrition (Lemma, 2015) and Ethiopia is not an exception. Improving SSF tomato production capacity would contribute to enhancing food security and alleviating poverty (Gemechis *et al*., 2012). Tomato production perfectly suits to urban agriculture and cultivation in small areas with water sources (such as gardens) and highly contributes to the economy of Ethiopia. The production of staple crop is small-scale in Ethiopian agriculture and is impacted by a number of factors, among which crop diseases are the most critical

(Abebe *et al*., 2015). Reducing the damaging effect of *Meloidogyne* spp. is one strategy that satisfies the needs of farmers, agricultural extension agents, decision makers, researchers and private and commercial tomato producers. Developing ecologically sustainable disease management strategies such as resistant cultivars will help in preserving the environment, avoiding toxic residues (Starr & Mercer, 2009) and further improve public health and livelihood through diet diversification and high financial return of tomato.

1.4. Plant-Parasitic Nematodes on Tomato in Ethiopia

In Ethiopia, only a few and sporadic surveys on plant-parasitic nematodes (PPN) have been conducted and the communication of findings to policy makers and stakeholders has always been poor (Abebe *et al*., 2015). The Earliest report of *Meloidogyne* species identification work was by Whitehead (1968) who was a nematologist based in East Africa Agriculture and Forestry Research Organization based in Kikuyu, Kenya. Survey and identification research conducted by O'Bannon (1975) reported nematode species belonging to the genera *Helicotylenchus*, *Heterodera*, *Meloidogyne* and *Pratylenchus* associated with vegetable crops. Out of 53 crops (including tomato) sampled, RKN were found on 40 (O'Bannon, 1975). Following this finding, limited survey and identification effort was made and focused on different crops across the country (Abebe *et al*., 2015). The presence of RKN from Ethiopia mainly in vegetables growing areas has been documented (Stewart & Daganachew, 1967; O'Bannon, 1975; Tadele & Mengistu, 2000; Mandefro & Dagne, 2000; Mekete *et al*., 2003; Seid *et al*., 2015b). A survey was conducted to characterize the abundance and distribution of *Meloidogyne* species from different vegetable crops (tomato, pepper, onion, snap bean, cabbage, beetroot, carrot and potato) in Ethiopia (Mandefro & Mekete, 2002). The authors reported a 62% incidence of RKN on these vegetables. Pepper and tomato were found seriously infected with *Meloidogyne* species and the authors recommended an immediate research towards their management. More than a decade has already passed since these recommendations were forwarded and the productivity of tomato is still declining (personal communication with tomato growers and MARC-vegetable breeding unit).

1.4.1. *Meloidogyne* species (Root-Knot Nematodes)

RKN are obligate biotrophic pathogens that invade plant roots and establish prolonged and intimate relationships with their host (Niu *et al*., 2016). RKN are distributed worldwide (Jones *et al*., 2013). As of October 2016, there were 101 described species in the genus *Meloidogyne* and 22 of these were reported from Africa (Onkendi *et al*., 2014). Their vernacular name comes from the galls (root knots) induced by these nematodes on the roots

of their host plant (Fig 1.1). It is generally accepted that four major species, i.e., *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949; *M. javanica* (Treub, 1885) Chitwood, 1949; *M. arenaria* (Neal, 1889) Chitwood, 1949 and *M. hapla* (Chitwood, 1949), as well as a few emerging species such as *M. enterolobii* (Yang & Eisenback, 1983) and *M. chitwoodi* (Golden, O'Bannon Santo & Finley, 1980*)*, cause damage on a vast majority of crops (Moens *et al*., 2009). In Africa, *M. incognita*, *M. javanica* and *M. arenaria* are considered the most dominant species, reported in 37, 36 and 26 different countries across the continent, respectively (De Waele & Elsen, 2007). These three species have collectively been reported to cause damage in many economically important crops including tomato (Onkendi *et al*., 2014; Seid *et al*., 2015). Due to the fact that these three major species are widespread it could have led to bias against accurate identification of the emerging species. Several of the species considered as major species might have been inaccurately identified (Onkendi *et al*., 2014). The problem is even worse when one considers that the majority of these *Meloidogyne* species have been identified based solely on morphology before the advent of molecular tools. The occurrence of RKN species mixture is common which makes the problem even worse. For instance, both *M. enterolobii* and *M. paranaensis* for many years have been misidentified as *M. incognita* (Carneiro *et al*., 1996). It is possible that the potential impact of new and emerging species has been overlooked. The recent adoption of molecular diagnostic tools in many laboratories globally will most likely lead to an increase in the number of species due to the discovery of cryptic species, less misidentifications and new species identification (Onkendi *et al*., 2014). Accurate identification of *Meloidogyne* spp. inflicting a yield loss in any crop and in any given area is a prerequisite to devise a sound, sustainable and eco-friendly management approach. Information on the biodiversity, distribution, prevalence, economic importance and management of PPN, including RKN, in Ethiopia is limited. In Ethiopia, RKN, have largely been identified on the basis of female perennial pattern and second-stage juveniles (J2) morphometric and in few cases using cytological and biochemical methods (Mandefro & Dagne, 2000; Mekete *et al*., 2003). From these studies, *M. incognita*, *M. ethiopica* and *M. javanica* were reported on tomato in Ethiopia. There is little information available in the literature about Ethiopian RKN population identification using a combination of DNA-based and isozyme electrophoresis.

Figure 1.1. Typical damage symptoms caused by *Meloidogyne* spp. on roots of tomato (*Solanum lycopersicum*) A) Jittu farm-Ethiopia B) *M. chitwoodi* on tomato from a pot test

1.4.2. Life Cycle of Root-Knot Nematodes

A typical life cycle of the RKN (*Meloidogyne* spp.) is presented in Fig 1.2. The mature females lay eggs into gelatinous masses composed of a glycoprotein matrix produced by their rectal glands. This keeps the eggs together and protects them against environmental extremes and predation (Moens *et al*., 2009). The egg masses are usually found on the surface of galled roots or embedded within the gall tissue and can contain up to 1000 eggs (Jones *et al*., 2013). Within the egg, embryogenesis proceeds to the first-stage juvenile (J1), which moults to J2. J2 hatch from the egg and in general, hatching is dependent solely on suitable temperature and moisture conditions, with no stimulus from host plants being required (Karssen *et al*., 2013; Moens *et al*., 2009; Jones *et al*., 2013). The emerged J2 are attracted to the roots of host plants by exudates emanating from the plant root and invasion of the roots takes place usually behind the root tip (Karssen *et al*., 2013). Juveniles (J2) then move through the root to initiate and develop a permanent feeding site, which consists of several giant cells. This feeding site serves as the only nutrient sink for the developing J2. The nematode growth and reproduction entirely depend on this established feeding site (Castagnone-Sereno *et al*., 2013). Under favourable conditions, the J2 moults to the third-stage juvenile (J3) after about

14 days, then to the fourth-stage juvenile (J4), and finally to the adult stage (Moens *et al*., 2009). The J3 and J4 do not feed. The adult females continue to feed and enlarge to become round to pear shaped. There is a tremendous variation exhibited in reproductive strategies of *Meloidogyne* species that ranges from amphimixis to obligatory mitotic parthenogenesis (Chitwood & Perry, 2009). Most species are parthenogenetic and males are only formed under adverse conditions. RKN have unbalanced sex ratios (Jones *et al*., 2013; Castagnone-sereno *et al*., 2013). The life cycle of RKN takes three to six weeks to complete, depending on the species, the host plant and environmental conditions (Castagnone-sereno *et al*., 2013). This short life cycle enables RKN populations to survive well in the presence of a suitable host (Shurtleff & Averre, 2000) and their populations build up to a maximum usually as crops reach maturity. RKN have several generations in one cropping season (Karssen *et al*., 2013) and more generations could be produced in tropical conditions due to higher soil temperatures compared to temperate conditions. Many *Meloidogyne* species have a broad host range. The potential host range of *Meloidogyne* species encompasses from 3000 to 5500 plant species (Trudgill & Blok, 2001; Abad *et al*., 2003).

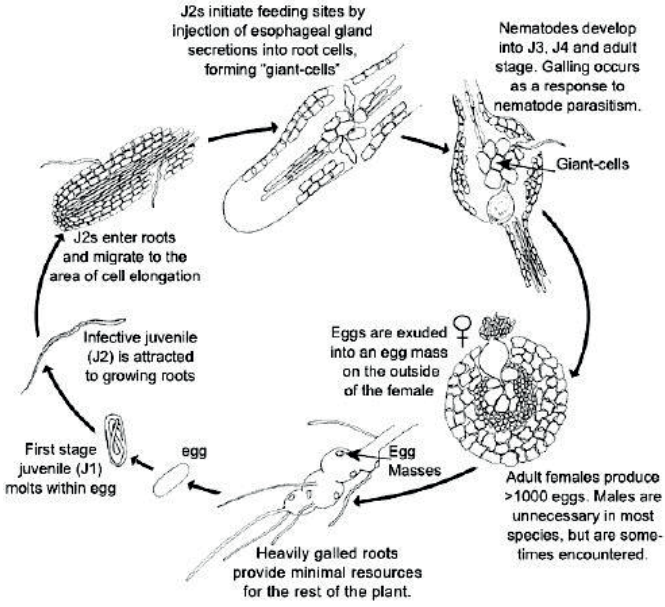

Figure 1.2. Life cycle of RKN (*Meloidogyne* spp.) Diagram Courtesy of V. Brewster (Mitowski & Abawi, 2011)

1.4.3. Economic Importance of Root-Knot Nematodes

In a recently conducted survey, researchers working on PPN ranked RKN first in the list of the top ten PPN based on scientific and economic importance (Jones *et al*., 2013). RKN (*Meloidogyne* spp.) are economically important pests of a wide range of vegetables throughout the world (Castagnone-Sereno, 2006). They are considered to be the most destructive and difficult pest to control in tropical and subtropical countries (Simpson & Starr, 2001). Moreover, their involvement in many disease complexes together with their ability to break down plant resistance made them a severe pest of vegetables (Luc *et al*., 2005). Species of the RKN genus *Meloidogyne* are estimated to cause a global loss of US$ 157 billion (Abad *et al*., 2008). With a 30-100% reported crop loss within Africa alone they are undermining the continent's agriculture (Murungi *et al*., 2014). The RKNs alone result in 60% yield loss of vegetable crops worldwide (Mateille *et al*., 2008). In Western Anatolia (Turkey) *Meloidogyne* spp. caused up to 80% yield losses in processing tomato-growing areas (Kaşkavalci, 2007). However, much higher percentages have been documented on tomato (see Chapter 2 of this thesis) in different regions, depending on species, population density, frequency of infestations and cultivars (Seid *et al*., 2015a).

1.4.4. Management of Root-Knot Nematodes

Owing to their polyphagous nature, management of RKN is a difficult task. Different RKN management options such as chemical, regulatory, biological, cultural, resistance and physical have been tried worldwide (see chapter 2). Host-plant resistance (HPR) has been given more attention over the other approaches as a major goal for pest management since it provides an effective, eco-friendly, sustainable and economical method for managing nematodes in both high and low value cropping systems (Starr *et al*, 2001). Moreover, it is a significant component of a solution to many nematode problems especially in tropical agriculture for the SSF and when used integrated with cultural management methods and traditionally grown crops (Luc *et al*., 2005). In developing countries with many SSF such as Ethiopia, there is a high demand to identify sources of resistance in tomato cultivars for seed multiplication or breeding against RKN (Cook & Starr, 2006). However, so far, no effort has been made to identify local sources of resistance from commercial tomato varieties and breeding lines against RKN. The variety *Roma VFN* has widely been grown in Ethiopia and is known to be resistant for some species of RKN. However, this variety became out of production due to its low agronomic qualities. Currently, in Ethiopia, several commercial farmers and some SSF are growing hybrid (for agronomic traits) varieties obtained from

various sources such as Israel, South Africa, The Netherlands, and Denmark (Gemechis *et al*., 2012). However, the vast majority of SSF are also growing tomatoes from local retailers who retail both the hybrid and locally released varieties.

1.5. Objectives

The overall objectives of this research were to identify RKN problems from major tomato growing areas in Ethiopia and to develop management strategies that are easily adopted by resource poor farmers of Ethiopia. These objectives were further translated into the following specific objectives:

i. To characterize the biodiversity of *Meloidogyne* species present in major tomato growing areas of Ethiopia with DNA-based and isozyme techniques.
ii. To screen locally available tomato cultivars and breeding lines for resistance against local aggressive *Meloidogyne* species.
iii. To check the heat stability of potentially resistant tomato breeding lines under elevated soil temperatures.
iv. To investigate the reaction of tolerant tomato cultivars and breeding lines on *Meloidogyne* spp. infested fields.
v. To determine damage threshold and tolerance limit of selected tomato genotypes against two *M. incognita* populations.
vi. To study the relationship between *Pi* and *Pf* of two *M. incognita* populations on selected tomato cultivars and breeding line.

1.6. Thesis Outline

This thesis contains eight chapters.

Chapter 1. Gives a **general introduction** of this research work. It introduces the context and significance of the study. It answers the question why tomato and RKN were selected for investigation in Ethiopia.

Chapter 2. **Tomato (*Solanum lycopersicum* L.) and root-knot nematodes (*Meloidogyne* spp.)- a century-old battle.** This chapter describes a century-old battle between tomato and RKN. It highlights the history of the battle and the review of works done on the topic in this time span. It focuses more on RKN yield loss potential in view of tomato cultivars with *Mi* gene.

Chapter 3. "**Biodiversity of root-knot nematodes (*Meloidogyne* spp.) from major tomato growing areas of Ethiopia"** presents the biodiversity of RKN on major tomato growing areas mainly Rift Valley, Upper Awash and East Hararghe of Ethiopia. *Meloidogyne* species

were identified using DNA-based and isozyme techniques. It documents the distribution of RKN across these sampled areas in Ethiopia.

Chapter 4. "**Resistance screening of breeding lines and commercial tomato cultivars for *Meloidogyne incognita* and *M. javanica* populations from Ethiopia"** presents the aggressiveness of *M. incognita* and *M. javanica* populations collected from major tomato-growing areas of Ethiopia. It shows the results of resistance screening of 33 tomato cultivars and breeding lines against four local aggressive populations (two from each) of *M. incognita* and *M. javanica* in growth chamber conditions. The experiment was completed with the investigation of resistance mechanisms of selected tomato cultivars or breeding lines based on the screening results against the populations tested.

Chapter 5. "**Heat stability of resistance in selected tomato breeding lines against *Meloidogyne incognita* and *M. javanica* populations under elevated soil temperatures**" demonstrates the results of the experiments on the heat stability test of selected tomato cultivars in laboratory conditions (in a water bath) with different soil temperatures (28, 32 and 36°C) and exposure time (24 hr and 48 hr).

Chapter 6. "**Tolerance and resistance of selected breeding lines and commercial tomato cultivars in *Meloidogyne* spp. infested fields in Eastern Ethiopia**" shows the tolerance of selected tomato cultivars and breeding lines after growing them in two RKN infested hotspot field conditions in Ethiopia.

Chapter 7. "**Damage potential of *Meloidogyne incognita* populations on selected tomato genotypes in Ethiopia"** presents the tolerance limit and minimum yield of selected tomato cultivars and a breeding line and the damage threshold on 'Babile' and 'Jittu' of *M. incognita* populations performed in bigger pot experiments on the open field at Tony farm, Dire Dawa, Ethiopia.

Chapter 8. Presents **the general discussion and future prospects of this thesis**. It integrates the main findings of all the scientific chapters (3-7). It compares these findings in view of other related works performed elsewhere, and will point out some future prospects. It highlights the new findings of this study.

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Chapter 2: Tomato (*Solanum lycopersicum*) and root-knot nematodes (*Meloidogyne* spp.) – a century-old battle

Modified from:

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Abstract

The encounter between *Meloidogyne* species and tomato is many centuries old. *Meloidogyne* species are known to cause high levels of economic loss worldwide in a multitude of agricultural crops, including tomato. This review was initiated to provide an overview of the damage potential of *Meloidogyne* spp. on cultivars of tomato (*Solanum lycopersicum*), and to compile the different studies done on the management of *Meloidogyne* spp. on tomato with particular emphasis on the *Mi* resistance gene. Numerous studies have been conducted to assess the damage potential of RKN on various tomato cultivars; its yield loss potential ranges from 25 to 100%. A range of management options from using synthetic nematicides to soilless cultures have been tried and are available for managing *Meloidogyne* spp. Resistant commercial cultivars and rootstocks carrying the *Mi* gene have been used successfully to manage *Meloidogyne incognita*, *M. javanica* and *M. arenaria*. However, virulent populations have been detected. Relying on a single RKN management strategy is an out-dated concept and different management options should be used in an integrated management context by considering the whole system of disease management. In future management of *Meloidogyne* species, care must be taken in directly extrapolating the tolerance limit determined elsewhere, since it is affected by many factors such as the type of initial inoculum and physiological races of *Meloidogyne* spp., environmental conditions, types of cultivars and experimental approaches used.

Keywords – damage potential, durability, management, *Mi* gene, nematode control, pest management.

2.1. Introduction

Tomato is a popular vegetable crop worldwide. Africa and Asia account for more than 80% of the global tomato production area, with about 70% of world output (FAO, 2012). It is ranked first in the world for vegetables and accounts for 14% of world vegetable production (US$ 1.6 billion market value) (FAO, 2010). Apart from being an important food crop, tomato is an acknowledged model species for evolutionary studies and research on fruit development and metabolite accumulation (Guillaume & Mathilde, 2012). However, there are many pests and diseases damaging both the quality and quantity of tomato production. PPN are one of them. They represent an important constraint on the delivery of global food security. Damage caused by PPN has been estimated at US$ 80 billion per year (Nicol *et al.*, 2011). This is likely to be a significant underestimate of the actual figure as many growers in developing nations are unaware of the existence of PPN (Jones *et al.*, 2013). One of the major obstacles to the production of adequate supplies of food in many developing nations is damage caused by *Meloidogyne* spp. (Sasser, 1980).

It is generally documented that the four major species (*M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla*) as well as few emerging species such as *M. enterolobii* and *M. chitwoodi*, cause damage on a vast majority of crops (Moens *et al.*, 2009). *Meloidogyne* species cause high levels of economic loss in a multitude of agricultural crops worldwide with dramatic yield losses being reported on vegetables in tropical and sub-tropical agriculture (Sikora & Fernandez, 2005). They are impacting both the quality and quantity of marketable yields. Next to direct losses due to nematode attacks, many indirect losses through loss of irrigation water and fertilisers can occur since damaged roots do not utilise water and fertiliser as efficiently as healthy roots (Mai, 1985). In addition, RKN interact with other plant pathogens, resulting in increased damage caused by other diseases, affecting world food supplies (Sasser, 1980). Numerous studies have been conducted to determine the damage potential of *Meloidogyne* species on several vegetable crops including tomato, and different management strategies have been proposed. With the phase-out of methyl bromide, in particular, the problem of *Meloidogyne* spp. on tomato gained new interest. However, these studies were not compiled and presented in a way to help different stakeholders. Thus, the objectives of this review were: *i*) to provide an overview of the damage potential of *Meloidogyne* spp. on tomato cultivars; and *ii*) to compile different studies on the management of *Meloidogyne* spp. on tomato with particular emphasis on the *Mi*-resistant gene.

2.2. Root-Knot Nematodes on Tomato

Tomato is often referred to as a universal host for *Meloidogyne* species. However, from the 101 described species in the genus, tomato is a non-host for several of them. Most likely the term 'universal host' comes from the fact that the economically most important species reproduce well on tomato. This was also shown in the North Carolina Differential Host Range test. Reports on *Meloidogyne* spp. infecting tomato plants date back to the end of the 19th century. In the botanical garden of Pavia (Italy), tomato plants showed severe galling on the root system and after investigation this was ascribed to *Heterodera radicicola* (Cavara, 1895), a former name for RKN. During the same period, similar symptoms were observed on tomatoes in a vegetable garden in the Sahara (Cavara, 1895). In 1889, 'exceptionally knotty' tomato roots were found near an agricultural experiment station in Auburn, Alabama (USA). Similar symptoms were observed on the roots of various plants. In a bulletin following these observations, *Heterodera radicicola* was identified as being the cause of the symptoms (Atkinson, 1889). Since then, many reports of RKN on tomato have become available and at present we know that several species can cause severe damage to the crop.

2.3. Damage and Yield Losses of Tomato due to *Meloidogyne* Species

RKN can cause severe damage to the roots of tomato. Symptoms are more prevalent with tropical species compared to temperate RKN (Fig 1.1). Tomato cultivars have different degrees of susceptibility towards different *Meloidogyne* spp. Damage and yield loss studies conducted so far have shown a considerable difference in degree of susceptibility among tomato cultivars. Moreover, different populations of the same species of *Meloidogyne* even exhibit different degrees of pathogenicity on a specific tomato cultivar. A tomato cultivar that is absolutely susceptible to one population may be moderately resistant to another population of the same species. Several studies report the damage potential of different *Meloidogyne* spp. on different tomato cultivars under pot, microplot and field experiment conditions throughout the world. Experiments were done in different conditions and localities with different experimental approaches, making it difficult to extrapolate the results. Many factors affect the results. These include: *i*) differences in laboratory extraction techniques and efficiency; *ii*) variations in soil type and environmental conditions that may affect nematode population development; *iii*) differing levels of resistance or tolerance among crops or crop varieties to be tested and cropping systems; *iv*) nematode species and population levels or inoculum densities; *v*) inoculum types and inoculation techniques used and vi) RKN species

mixtures (Greco & Di Vito, 2009; Nyczepir & Thomas, 2009). A frequently cited average yield loss due to *Meloidogyne* spp. is 10% (Koenning *et al.*, 1999). Nevertheless, much higher percentages have been documented (Table 2.1) in different regions, depending on population level, genus, frequency of infestations and crop species. Yield losses of 22-30% have been reported on tomato due to *M. incognita* (Sasser & Carter, 1985). In Western Anatolia (Turkey) *Meloidogyne* spp. caused up to 80% yield losses in processing tomato-growing areas (Kaskavalci, 2007). In north-eastern Spain, an initial population density in soil of 4750 juveniles 250 cm^{-3} of *M. javanica* caused a 61% yield reduction in tomato cropped in summer plastic houses (Verdejo-Lucas *et al.*, 1994).

2.4. Management Options

Control refers to specific acts designed to reduce the number of nematodes, while management has the objective of minimising economic losses and considers the whole system of care and treatment of crop pests (Hooper & Evans, 1993). Different management options that are commonly used for PPN are applicable for *Meloidogyne* spp. on tomato as presented below.

2.4.1. Prevention

2.4.1.1. Quarantine

Quarantine strategies are considered a preventive and not a curative approach in stopping the introduction and/or increased dissemination of economically important nematodes into a new site (Nyczepir & Thomas, 2009). With the aim of reducing the adverse impact of *Meloidogyne* spp. on agricultural crops, phytosanitary measures have major importance especially for resource poor farmers (Coyne *et al.*, 2009). To avoid introduction of *Meloidogyne* spp. into a field, awareness and regulation are required (Wesemael *et al.*, 2011). New nematode species to a country are often first intercepted by quarantine and inspection services, which assist in preventing the unintended spread of species to new areas (Coyne *et al.*, 2009). The four major RKN species, *M. arenaria*, *M. incognita*, *M. javanica* and *M. hapla*, are generally not regulated because these species of economic concern are distributed globally. The temperate RKN, *M. chitwoodi* and *M. fallax* Karssen, 1996, are quarantine organisms in the EU (EC Directive 2000/29/EC) and the (sub) tropical *M. enterolobii* is placed on the EPPO A2 list as recommended for regulation as a quarantine pest (EPPO, 2014). The latter is known to be highly aggressive and able to break the *Mi* resistance in

tomato and N and *Tabasco* resistance in pepper (Fargette *et al.*, 1994; Brito *et al.*, 2007; Kiewnick *et al.*, 2009).

2.4.1.2. Sanitation

Sanitation is important to prevent new infestations (introduction into a production site) and to avoid secondary infestations (spreading within the production site). Human activities, such as the transport of infested planting material, soil, plant debris and irrigation water, can provide transfer channels between contaminated and healthy areas and easily spread *Meloidogyne* spp. (Collange *et al.*, 2011). For tomato production in protected cultures and in the open field, introduction with planting material poses a risk. Transplants are mostly provided in growth media that are free of pests and diseases, and should be obtained from reliable nurseries and, if possible, certified nematode-free plants should be used. At the farm level, cleaning all agricultural machinery and tools can avoid transporting PPN with the soil. Irrigation water can also be a source of nematode infection or a means to spread it within the field (Hugo & Malan, 2010). Due to environmental concerns and reduced water availability, closed systems (= recycling of irrigation water) are preferable. Proper sanitation of this water is of paramount importance to avoid the spread of pests and diseases. Moens & Hendrickx (1990) showed that *M. incognita* present in drainage water could re-infect tomato plants. Potential and available control measures for PPN in irrigation water are chlorination, electrical discharge, filtration, heat treatment, hydrogen peroxide, ozonation, sedimentation and flocculation and UV radiation. However, each treatment comes with advantages and disadvantages (Hugo & Malan, 2010).

Table 2.1. Damage potential of the major RKN (*Meloidogyne* spp.) on different tomato cultivars

Species	Cultivar	Cultivar info	ET	YLP (%)	TL* (J2 cm^{-3} soil)	Pi*	ST	Reference
M. hapla	Veebrite	NM	Microplots	40	2.6	0, 0.34, 2.39, 7.96, 36.35 J2 cm^{-3} soil	3	Potter & Olthof, 1977
M. hapla	Manapal	NM	Microplots	50	0.04	0, 0.4-1.6, 2-4.4, 8-16 eggs + J2 cm^{-3} soil	2	Barker *et al*., 1976
M. hapla	Campbell 135	Susceptible	Field	NS	_	0, 0.29, 2.58, 22.94 J2 cm^{-3} soil	4	Sayre & Toyana, 1964
M. incognita	Manapal	NM	Microplots	85	0.04	0, 0.4-1.6, 2-4.4, 8-16 eggs + J2 cm^{-3} soil	2	Barker *et al*., 1976
M. incognita	Chico III	Susceptible	Field	100	4	0, 0.125, 0.25, 0.5, 1, 2,.., 256 eggs + J2 cm^{-3} soil	1	Di Vito *et al*., 1981
M. incognita	U.C.105J	Susceptible	Pots	100	4	0, 0.25, 0.5, 1, 2, …, 512 eggs + J2 cm^{-3} soil	1	Di Vito & Ekanayake, 1984
M. incognita	IAS-1	Resistant	Pots	60	4	0, 0.25, 0.5, 1, 2, …, 512 eggs + J2 cm^{-3} soil	1	Di Vito & Ekanayake, 1984
M. incognita	Ventura	Susceptible	Field	100	0.55	0, 0.031, 0.062, 0.125, 0.25..128 eggs + J2 cm^{-3} soil	1	Di Vito *et al*., 1991
M. incognita	DISA N	Resistant	Field	30	0.55	0, 0.031, 0.062, 0.125, 0.25,. 128 eggs +J2 cm^{-3} soil	1	Di Vito *et al*., 1991
M. incognita	Shaft-Falat	NM	Pots	-	0.33	0, 0.25, 0.5, 1, 2, 4 eggs + J2/g soil	2	Hamzehzarghani *et al*., 2012
M. incognita	Pusa Ruby	Susceptible	Pots	25	_	1.3 J2 cm^{-3} soil	_	Ganaie & Khan, 2011
M. javanica	Campbell 135	Susceptible	Field	NS	_	0, 0.29, 2.58, 22.94 J2 cm^{-3} soil	4	Sayre & Toyana, 1964
M. javanica	Ramon	Resistant	Microplots	29	-	10000 eggs cm^{-3} soil	1	Ornat *et al*., 2001
M. javanica	Cobra	Susceptible	Microplots	29	-	10000 eggs cm^{-3} soil	1	Ornat *et al*., 2001
M. javanica	Marglobe	Susceptible	Pots	60	0.28	0, 1, 2, 4, 8, 16 J2 cm^{-3} soil	1	Mekete *et al*., 2003
M. javanica	Shaft-Falat	NM	Pots	-	0.33	0, 0.33, 0.65, 1.3, 2.6, 5.2 eggs + J2 cm^{-3} soil	2	Hamzehzarghani *et al*., 2012
Meloidogyne spp.	Asesewa	NM	Field	95.2	-	Natural infested field	2	Hemeng, 1980
Meloidogyne spp.	St. Peters	Resistant	Field	100	-	Natural infested field	2	Hemeng, 1980

*NM: the information is not mentioned in the original article; ET: experiment type; YLP: yield loss potential; TL: tolerance limit; Pi: initial population density; NS: not significant; ST: soil type: 1 = sandy soil, 2 = sandy loam, 3 = loam, 4 = clay soil. *Original data were recalculated to* cm^3 *assuming an average soil density of 1.3 g* cm^{-3}.

2.4.2. Physical Soil Treatments

2.4.2.1. Steam heat and solarisation

The effectiveness of soil solarisation and steam heat in managing *Meloidogyne* spp. under field and glasshouse conditions is dependent on soil temperature (Nyczepir & Thomas, 2009). A soil temperature considered sufficient to control PPN is 45°C (Sikora & Fernandez, 2005). Lethal effects on eggs and J2 of *M. incognita* have been observed below 45°C when nematodes were exposed to sub-lethal temperatures for a sufficient period of time (Wang & McSorley, 2008). In Florida, solarisation of a fine sandy soil for 3 months (July-September) suppressed *M. incognita* populations in tomato fields, resulting in increased yields (Overman & Jones, 1986). However, solarisation is more suited for annual crops, nurseries and raised beds (McGovern *et al.*, 2002). Disadvantages limiting the use of solarisation for the control of *Meloidogyne* spp. include the non-specificity (McSorley, 1998), the duration of time needed, decreasing efficacy with increasing soil depth below 5 cm and the size of the area to be treated (Nyczepir & Thomas, 2009).

The extensive use of steam heat in glasshouse conditions as a means to manage PPN has been limited in recent years, due to the high cost of heating fuel (Viaene *et al.*, 2013), non-specific effects on non-target (beneficial) microorganisms, possible emission of phytotoxic chemicals into treated soil and change in soil pH (Nyczepir & Thomas, 2009).

2.4.2.2. Flooding

Flooding and bare fallow treatments lowered soil populations of the four major *Meloidogyne* species. Rhoades (1982) reported that flooding reduced the density of *M. incognita* but the optimal duration of flooding depended on air temperature. Alternating drying cycles and flooding appeared to be more effective than prolonged flooding (Noling & Becker, 1994). However, a 3-week flooding period followed by a 5-week drying and a second 3-week flooding period in winter was not successful to control RKN on tomato in Florida (Nelson *et al.*, 2002). Time duration and salinity problems limit the use of flooding in tomato production.

2.4.3. Rotation

In general, a rotation of a minimum 3 years is recommended for tomato to reduce pests and diseases. Due to the wide host range of several important RKN species, rotation options are limited. Rotation with corn and velvet bean reduced *M. incognita* population levels and increased tomato yield in a field in Puerto Rico (Acosta *et al.*, 1991). Rotation with *Mi* gene cultivars does not imply changes in farming systems or market supply (Ornat & Sorribas, 2008) and can be a solution for intensive tomato production.

2.4.4. Organic Amendments

Organic amendments cover several sources and products, including green manures from cover crops or crop residues, industrial or town waste, animal manures, composted or not composted. They are incorporated into the soil or applied on top of the soil as mulches. In general, soil amendments improve the nutrient and water holding capacity of the soil, improve soil fertility and structure, reduce erosion and release specific compounds that may be nematicidal and stimulate microbial activity in the soil (Akhtar & Malik, 2000; Oka, 2010; Thoden *et al.*, 2011). The results of studies on organic amendments to control RKN are not straightforward. Biofumigation with *Brassica juncea* and *Eruca sativa* showed promising results both in increased yield of tomato and reduction of *M. incognita* population in Italy (Colombo *et al.*, 2008). By contrast, Noling & Gilreath (1999) found no reduced levels of *M. incognita* in amended plots compared to an untreated control, and lower tomato yields than in fumigated plots. There are studies that show increased RKN populations after application of the amendment (Thoden *et al.*, 2011). This gave rise to the hypothesis that interactions between several factors may contribute to the results, including the dosage of organic amendment and the number of application years, the chemical characteristics of different products, such as release of nematotoxic compounds, physiological stages of the incorporated plant tissues, compost maturity and decomposition stage of organic matter, C/N ratios of the organic amendment and soil infestation level, and nematode community structures.

2.4.5. Chemical Control

Historically, chemical control has been the most important strategy to reduce *Meloidogyne* populations (Nyczepir & Thomas, 2009). According to Talavera *et al.* (2012), 78.3% of the farm advisors in south-eastern Spain mentioned chemical soil fumigation as the most commonly used management method for RKN, followed by non-fumigant nematicides (59.8%). A combination of nematicides with soil solarisation and grafting on resistant

rootstocks were considered to be the most effective methods of RKN management. Increasing environmental and health concerns resulted in the ban of methyl bromide, and chemical nematicides are being discouraged specifically as a sole management method. However, new generations of less harmful nematicides are becoming available as a result of renewed interest by the crop protection industry (Desaeger, 2014).

2.4.6. Biological Control

Natural enemies are promising for RKN control. Several fungi and bacteria have been identified and classified based on their nematophagous and antagonistic characteristics, respectively. Nematophagous fungi include trappers, endoparasites, egg parasites and toxin producers. The egg-parasitising *Purpureocillium lilacinum* was reported to reduce *M. javanica* and *M. incognita* on tomato crops (Goswami *et al.*, 2006; Kumar *et al.*, 2009) but results have been difficult to reproduce (Hallmann *et al.*, 2009). A single pre-plant application of the fungus *P. lilacinum* strain 251 reduced root galling of *M. incognita* on tomato by 66% and egg mass formation by 74%, and also for *M. hapla* sufficient control was achieved on tomato (Kiewnick & Sikora, 2006). This strain of *P. lilacinum* has been commercialised in several countries. A one-off application of *Pochonia chlamydosporia* was able to slow down the build-up of *M. javanica* for at least 5-7 months in tomato and lettuce rotations in glasshouses (Van Damme *et al.*, 2005). However, Tzortzakakis & Petsas (2003) reported that *P. chlamydosporia* did not show any effect on *M. javanica* on tomato in glasshouse studies in Greece, and also in a double cropping system of lettuce and tomato in Spain *M. javanica* could not be controlled (Verdejo-Lucas *et al.*, 2003). *Aspergillus* spp. and *Trichoderma* spp. have shown potential to reduce populations of *M. incognita* on tomato (Goswami & Mittal, 2004; Goswami & Tiwara, 2007; Affokpon *et al.*, 2011a). When inoculation of arbuscular mycorrhizal fungi (AMF) was done 3 weeks before *M. incognita* inoculation, tomato plants were protected against *M. incognita* and its reproduction reduced (Talavera *et al.*, 2001). In a split-root experiment, Dababat & Sikora (2007) showed that *Fusarium oxysporum* Fo162 induced systemic resistance in tomato against *M. incognita*. In Benin (West Africa), a field application of AMF on a *Meloidogyne*-infested field increased tomato yields by 26% compared to the non-AMF control treatment (Affokpon *et al.*, 2011b). Important for control with AMF is successful root colonisation before nematode attack (Talavera *et al.*, 2001; Hallmann *et al.*, 2009).

The obligate endoparasitic bacteria *Pasteuria penetrans* effectively parasitised *M. incognita*

in rotations that included tomato, eggplant and common beans or cabbage (Amer-Zareen *et al.*, 2004). In *M. incognita* infested microplot, the application of 5×10^{10} spores m^{-2} increased tomato yield by 46% (Talavera *et al.*, 2002). The efficacy of *P. penetrans* depends on soil conditions, temperature and nematode age (Talavera & Mizukubo, 2003). Moreover, its host specificity requires mixtures to enable proper management of mixed *Meloidogyne* infestation (Hallmann *et al.*, 2009). *Streptomyces* spp. are important producers of antibiotics. Avermectins, which are produced by them, were found to have strong nematicidal effects (Hallmann *et al.*, 2009). Bélair *et al.* (2011) showed in a glasshouse bioassay that a combined soil treatment with *Streptomyces* and chitin reduced *M. hapla* populations and galls on tomato. However, the high cost of the soil treatments and variability in the results prevent the use as an alternative control method. Seed treatments proved to be successful to manage *M. incognita*, *M. arenaria* and *M. javanica* on tomato (Cabreira, *et al.*, 2009) and might be more promising compared to soil treatments. However, biological control agents alone rarely provide adequate management and should be integrated with other management methods such as resistant cultivars, crop rotations, trap crops or antagonistic plants, either to promote the establishment of biological control agents or to reduce nematode populations in the soil (Viaene *et al.*, 2013).

2.4.7. Soilless Culture Systems

Soilless culture is a good alternative to soil-based culture particularly in glasshouse vegetable production. The use of soilless culture systems as a management strategy for PPN has long been tried. It is widely practised because it is more practical and cheaper than repeated soil fumigation (Hochmuth & Hochmuth, 2012). However, the development from growing plants in field soil to soilless culture systems has not resulted in the eradication of problems caused by PPN (Hallmann *et al.*, 2005; Ornat & Sorribas, 2008). *Meloidogyne incognita* and *M. arenaria* were found on roses grown in soilless culture in Sicily (D'Errico & Ingenito, 2003). *Meloidogyne hapla* was found in rock wool and coconut-peat cultures of roses in Germany (Hallmann *et al.*, 2004; Ornat & Sorribas, 2008). Almost all commonly used substrates are suitable for nematode infestation (Stapel & Amsing, 2004), and the most common sources of nematode infestation are infested planting material and irrigation water (Hallmann *et al.*, 2005). Control of PPN in soilless culture systems is extremely difficult. Nevertheless, heat treatment of circulation water (Evans, 1991; Runia & Amsing, 2001a, b), ultra violet radiation and filtration (Moens & Hendrickx, 1989; Amsing & Runia, 1995), resistance, plant

growth management, avoidance of nematode infestation, routine monitoring of planting material and recirculation water, and the use of certified planting material can substantially reduce nematode problems (Hallmann *et al.*, 2005). In organic farming, hydroponics and the use of inorganic growing media are not allowed.

2.4.8. Resistant Cultivars

Nematologists use the term resistance and tolerance differently than other plant pathologists. Nematologists assess the resistance of a cultivar by its effect on nematode multiplications whereas other pathologists use disease symptoms to determine resistance (Trudgill, 1986; Starr & Mercer, 2009). In other branches of plant pathology, resistance is defined as the ability of a host plant restricting or hindering pathogen invasion, development, or multiplication while in nematology resistance does not imply non-invasion (Schafer, 1971). A highly resistant plant supports little or no nematode reproduction, whereas a susceptible plant supports abundant reproduction. A plant that supports an intermediate level of reproduction is considered partially or moderately resistant. Tolerance is defined as the ability of a host plant to survive nematode attack and gives satisfactory yields at a level of infection that causes economic loss to other varieties of the same host species (Robinson, 1969). Tolerance is measured by the growth or yield parameters after infection (Starr & Mercer, 2009).

Resistant cultivars are an economical and environmentally safe method for controlling *Meloidogyne* species. They are cultivated with a dual purpose; to reduce nematode population levels and to avoid crop damage by nematodes. Therefore, resistant cultivars also need to be tolerant to *Meloidogyne* species. It is particularly important for organic farming or integrated production since these systems do not allow, or they restrict, the use of chemical control (Ornat & Sorribas, 2008). Resistant cultivars do not require significant changes in farming operations or in market supply (Ornat & Sorribas, 2008).

Resistance against *Meloidogyne* spp. has been reported in many agricultural crops (Cook & Starr, 2006; Starr & Mercer, 2009) but is not often used (Cook, 2004; Wesemael *et al.*, 2011). Tomato is one of the few crops in which *Meloidogyne* resistance has been widely used, and commercial resistant cultivars and rootstocks are available for tomato (Ornat & Sorribas, 2008). Resistance against *M. incognita*, *M. javanica* and *M. arenaria* has been developed in widely used tomato cultivars bearing the *Mi* gene (Ornat *et al.*, 2001). Fruit yields of the susceptible tomato cv. Blitz were higher when grafted on cultivars Beaufort and Hypeel45

tomato rootstocks carrying the *Mi* gene and inoculated with different populations of *M. incognita* (Lopez-Perez *et al.*, 2006). Nematode-resistant tomato rootstocks can be used for grafting desirable tomato scions. However, expression of resistance is affected by different factors such as soil temperature, species and populations of *Meloidogyne*, *Mi* dosage and tomato genetic background (Ornat & Sorribas, 2008). Thus, tomato cultivars should be carefully chosen, particularly when they are followed by nematode-susceptible crop (Lopez-Perez *et al.*, 2006). The use of the *Mi* gene and its limitations are discussed below.

2.4.9. Genetics-Based Management

2.4.9.1. Brief history of Mi *gene from where to where?*

Resistance in tomato to RKN was first observed in the wild species *Lycopersicon* (the genus *Lycopersicon* is now a synonym of *Solanum*) *peruvianum* Mill. P.I.128657 by Bailey (1941). It was later introgressed into the cultivated *S. lycopersicum* (Smith, 1944) and has proved useful in the management of *M. arenaria*, *M. incognita* and *M. javanica* (Roberts, 1992), the aphid (*Macrosiphum euphorbiae*) (Rossi *et al.*, 1998) and *Bemisia tabaci* biotypes Q (Nombela *et al.*, 2001) and B (Jiang *et al.*, 2001). Its resistance against *M. incognita* gave it its name-*Mi* gene (Williamson, 1998). *Mi* cultivars of tomato were introduced in the 1980s and have gained importance ever since. In California, USA, the majority of field-grown processing tomatoes have the *Mi* gene (Cook, 2004; Williamson and Roberts, 2009).

2.4.9.2. Structure and mechanism of action of the Mi *gene*

Although the exact numbers of responsible genes are unknown (Sidhu & Webster, 1975; Roberts *et al.*, 1990), the resistance in tomato cultivars against *Meloidogyne* species is believed to be controlled by a single dominant gene (Gilbert & McGuire, 1956; Roberts & Thomason, 1989; Messeguer *et al.*, 1991). The *Mi* gene was mapped to the short arm of tomato chromosome 6 near the centromere (Kaloshian *et al.*, 1998). It belongs to the NBS-LRR group of genes, which are characteristic of a family of plant proteins, including several that are required for resistance against bacteria, fungi and viruses (Milligan *et al.*, 1998). Two homologues of this gene, *Mi-1.1* and *Mi-1.2*, conferred resistance in an experimental assay (Milligan *et al.*, 1998). The functional *Mi-1.2* gene is referred to as '*Mi*'. *Mi*-mediated resistance triggers a hypersensitive reaction (Dropkin, 1969a) that involves cellular disorganisation, localised host-cell necrosis and restricted nematode development at the infection site near the vascular bundle. The tomato *Mi* resistance gene confers resistance, but not immunity, to *M. arenaria*, *M. incognita* and *M. javanica* (Roberts & Thomason, 1989),

since a few juveniles are able to infect roots, but they develop slowly, resulting in a reproduction rate smaller than on susceptible cultivars (Talavera *et al.*, 2009). The same phenomenon was reported on alfalfa (Griffin & Elgin, 1977) and soybean (Pedrosa *et al.*, 1996). More detailed information about the structure and function of the *Mi* gene is given by Williamson (1998) and Williamson & Roberts (2009).

2.4.9.3. Effectiveness and profitability of the Mi *gene*

The *Mi* gene has been incorporated into many commercially available tomato cultivars (Devran *et al.*, 2010) and is used against RKN in home gardens, tomatoes for the fresh market and processing tomato cultivars (Roberts & Thomason, 1989). For almost 70 years it has been the only source of resistance in all commercial tomato cultivars and it has been effective for RKN management, especially when used in combination with other management techniques such as rotation and sanitation (Roberts, 1992). In successive field trials, the resistant cultivars PSR 8991994 and Sanibel greatly suppressed root galling and *M. javanica* populations; fruit weight, number of fruit and weight per fruit as compared with the susceptible cv. *Colonial* increased significantly (Rich & Olson, 1999). Cultivars *Monika* (*Mi*-resistant) and *Durinta* (susceptible) were cropped for three consecutive seasons in non-fumigated soil and soil fumigated with methyl bromide at 75 g m^{-2} and at a cost of € 2.44 m^{-2} to determine the effectiveness and profitability of the *Mi* gene. Growth of cv. *Monika* increased profits by € 30 000 ha^{-1} compared with cv. *Durinta* in non-fumigated soil (Sorribas *et al.*, 2005a). The resistant cv. *Monika* increased yield with 5.6, 4.4 and 4.7 kg m^{-2} after one, two and three consecutive crops, respectively, compared with the susceptible cv. *Durinta* in nematode infested soil. The use of tomatoes with the *Mi* resistance gene was economically justified based on its cost efficacy (Sorribas *et al.*, 2005b).

A cropping cycle with *Mi* tomato genotypes reduced initial population density for the next crop, and the effect was similar to the use of nematicides on a susceptible crop (Tzortzakakis *et al.*, 2000; Talavera *et al.*, 2009). Maleita *et al.* (2011) reported that cv. *Rapit* can be used to control the three most common *Meloidogyne* spp. and inhibit the increase of *M. hispanica* populations. Four crop rotations including the *Mi*- resistant tomato cv. *Monika* and the susceptible cv. *Durinta* were assessed for three consecutive cropping seasons in three unheated plastic houses located in different parts of Spain. The *Mi*-resistant cv. *Monika* suppressed *M. javanica*, *M. arenaria* and *M. incognita* populations by more than 90%

compared with the susceptible cv. *Durinta*. Substantial yield increase (+2.6 kg m^{-2} in the rotation including at least one resistant tomato cultivar and +6.1 kg m^{-2} when the resistant cultivar was cropped for 2 consecutive years) was only achieved when initial nematode populations were high and with suitable agroclimatic conditions for the resistant tomato cultivar (Talavera *et al.*, 2009). After growing a tomato cultivar with *Mi* in *M. javanica*-infested fields, yield losses of the succeeding cucurbit crop were significantly reduced (Ornat *et al.*, 1997) and yield was similar to two treatments with fenamiphos on susceptible tomato (Tzortzakakis *et al.*, 2000).

2.4.9.4. Limitations of the Mi *gene*

Despite its effectiveness and profitability, the resistance conferred by the *Mi* gene has some critical limitations. Planting a resistant crop for several consecutive years can increase the risk of selection of virulent nematode populations. This has been reported for the *Mi* gene in Morocco after 3-8 years (Eddaoudi *et al.*, 1997), in Florida, USA, after five consecutive resistant tomato crops (Noling, 2000), and in Spain after three cropping cycles of resistant tomato rootstocks (Verdejo-Lucas *et al.*, 2009). Meher *et al.* (2009) showed that continuous growing of 13 resistant tomato cultivars during 10 years resulted in a 6.6% higher infection by *M. incognita* compared with a susceptible control. The presence of naturally occurring resistance-breaking populations has also been reported (Roberts, 1992; Ornat *et al.*, 2001; Maleita *et al.*, 2011). In Spain, 48% of 29 field populations of *Meloidogyne* spp. were found virulent against the *Mi* gene (Verdejo- Lucas *et al.*, 2012). It was unclear if the presence of virulent populations was due to selection pressure by repeated cultivation of resistant tomato cultivars. Virulent *Meloidogyne* spp. have been found in most tomato-growing areas (Castagnone-Sereno, 2006). Resistance mediated by *Mi* is broad with its effect on the tropical species *M. arenaria*, *M. incognita* and *M. javanica*. However, it is not effective against the aggressive *M. enterolobii* (Kiewnick *et al.*, 2009) and the temperate *M. hapla* and *M. chitwoodi* (Brown *et al.*, 1997; Liu and Williamson, 2006), all species that are known to infect tomato. Another constraint for the *Mi* gene is the irreversible breakage of resistance at high soil temperatures (>28°C) (Dropkin, 1969b; Haroon *et al.*, 1993; Talavera *et al.*, 2009). Mutation(s) in the *Mi* gene or a gene required in the *Mi*-mediated resistance pathway (Lopez-Perez *et al.*, 2006) and failed transcription due to DNA methylation (Liharska, 1998) can hamper the efficacy. The expression of the *Mi* gene is also affected by gene dosage, depending on whether the resistant cultivars are heterozygous (*Mimi*) or homozygous (*MiMi*)

as shown by Tzortzakakis *et al.* (1998). These authors found much greater reproduction of partially virulent populations of *M. javanica* on heterozygous compared to homozygous tomato genotypes. Despite these constraints *Mi*-resistant cultivars remain important for management of *Meloidogyne* spp. on tomato.

2.4.10. Integrated Nematode Management

The primary aims of integrated nematode management are to improve crop yield using a combination of management options, thereby targeting key nematode species such as *Meloidogyne* species (Nyczepir & Thomas, 2009) and consideration of the ecosystem (Barzman *et al*., 2015). The decision as to which management options will be part of the integrated nematode management strategy is governed by many factors such as *Meloidogyne* species present, perennial *vs* annual crops, economics, technology and societal considerations (Nyczepir & Thomas, 2009). To develop an effective integrated nematode management strategy, knowledge is needed on plant damage or crop loss caused by resident *Meloidogyne* species on the crop(s) that will be produced, population densities and population dynamics of RKN populations with and without the use of control measures, and the economic consequences associated with different control methods (McSorley & Phillips, 1993). In integrated nematode management strategies there are interactions within a soil system, among management options, and among microorganisms. According to Collange *et al.* (2011), there are at least four main processes for controlling *Meloidogyne* species using an integrated approach: killing nematodes in the soil with thermal or chemical agents, breaking the nematode biological cycle to limit or delay reproduction sequences, enhancing the competitions from other microorganisms in the soil to reduce nematode populations by predation, trophic competition, or parasitism, and limiting dissemination from a contaminated to an uncontaminated area.

2.5. Future Considerations

Given the withdrawal of effective nematicides, alternative management strategies for *Meloidogyne* spp. in tomato production are needed. Prevention, physical management methods, organic amendments, biological control, resistant cultivars and an integrated nematode management have proved to be effective but have their limitations. Innovations are limited and take time to be accepted and implemented. The most promising results have been achieved with the successful implementation of the *Mi* gene in commercial cultivars. In total, nine resistance genes (*Mi 1-9*) are now known in tomato. In six of them heat stability was

reported (Ammiraju *et al.*, 2003; Jablonska *et al.*, 2007; Wu *et al.*, 2009; Wang *et al.*, 2013) but these genes are not yet cloned or available in commercial cultivars. Pyramiding genes might be the key to overcome the problem of heat stability successfully. Techniques to cool soil temperature to below the critical 28°C through daily watering and the use of white plastic mulch (reflects the incoming solar radiation) until the canopy covers the soil proved to be successful (Rich & Olson, 1999) but seem impractical.

The development of rootstocks containing a heat-stable gene should be a priority in order to control *Meloidogyne* spp. in tomatoes grown at high soil temperatures. The *Mi* resistance gene should also be used in an integrated management context to preserve its durability and prevent the selection of virulent populations of *Meloidogyne* due to variability in isolate reproduction, resistant genotypes, and environmental conditions. The use of tomato genotypes with the *Mi* gene can be optimised in a rotation sequence of a cropping system.

It is advisable to evaluate the pathogenicity of local *Meloidogyne* populations associated with different environmental characteristics before growing the *Mi*-resistant tomato. Farmers can grow a few tomato plants in soil collected from their field to assess the presence of *Meloidogyne* and its aggressiveness under local circumstances. Response to temperature regimes or other abiotic factors, and system compatibility, including undesirable associations with other pests, diseases or agronomic traits, should also be assessed. The damage potential of RKN on tomato crops depends on many factors, such as initial population density, aggressiveness, environmental conditions, cultivar and experimental approach. Thus, the tolerance limit should be determined locally and care must be taken in extrapolating the tolerance limit determined elsewhere. An estimate of the tolerance level can be made by diluting infested soil with sterilised soil and growing tomato plants in a series of nematode densities. However, more precise evaluation will require the aid of a specialist and specialised equipment. All methods of control likely to be used in developing countries should be adaptable to the small-scale farmer with minimum financial resources. Awareness and support are necessary to allow sustainable tomato production both in intensive as subsistence agriculture.

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Chapter 3: Biodiversity of Root-Knot Nematodes (*Meloidogyne* spp.) from Major Tomato Growing Areas of Ethiopia

Awol SEID, Chemeda FININSA, Tesfamariam MEKETE, Toon JANSSEN, Wim M. L. WESEMAEL and Wilfrida DECRAEMER (2016). Biodiversity of root-knot nematodes (*Meloidogyne* spp.) from major tomato growing areas of Ethiopia. In preparation for submission to *Journal of Nematology*.

Abstract

RKN are among the biotic factors that limit tomato production worldwide. The objectives of this study were to assess the distribution and identify RKN (*Meloidogyne* spp.) and associated problems. A total of 212-rhizosphere soil and 123 root samples were sampled from 40 localities in major tomato growing areas of Ethiopia during 2012/13 growing season. A total of 646 respondents participated in the questionnaire survey to assess knowledge and practice of farmers and factors associated with RKN damage on tomato. Out of the 212 composite soil samples collected, 100 samples (47.2%) were found infested by various *Meloidogyne* species eight weeks after the start of the bioassay test. Out of the 123 root samples collected, 80 of them (65%) had root galls. The highest prevalence (100%) of RKN was found on samples collected from Adami Tullu, Babile (Erer and Gende Sudan), Erer Gota, Hurso, Jittu, Tikur Wuha, Tepo Choronke, Zeway and Koka. The highest incidence (100%) of the RKN was found from Adami Tullu, Jara Weyo, Babile (Erer and Gende Sudan), Erer Gota, Hurso, Jittu, Tikur Wuha, Tony farm, Tepo Choronke, Zeway and Koka locations based on direct observation of galls on collected root samples. Out of the 646 respondents, 280 (43.3%) of them reported to have RKN damage symptoms when shown the symptoms of RKN while 366 (56.7%) of them did not report damage. The highest significant effect on the dependent variable RKN damage on tomato roots comes from the previous crop, soil texture, awareness about RKN and source of irrigation water used. The presence of *Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla* on tomato was confirmed using a combination of SCAR primers and biochemical identification tools. The two tropical species, *M. incognita* and *M. javanica,* were identified as the most prevalent species. Both species were also co-infesting tomato plants. *Meloidogyne hapla* was detected for the first time in an open tomato production farmer's field at 'Zeway' location with 1620 m.a.s.l. elevation. The occurrence of these *Meloidogyne* species alone, or in mixed populations from samples collected, clearly shows that RKN are widespread in major tomato growing areas of Ethiopia. In the near future, the economic importance of *M. arenaria* and *M. hapla* on tomato production in Ethiopian agriculture should be investigated.

Key words: Damage, Distribution, Diversity, *Meloidogyne hapla***,** Prevalence, Tomato

3.1. Introduction

Tomato is a widely consumed and popular vegetable crop worldwide (Naz *et al*., 2012). It provides a rich source of micronutrients necessary for a well-balanced human diet (Rice *et al*. 1987) and can be used as a food security and poverty-alleviating crop (Gemechis *et al*., 2012). Tomato is consumed in different ways and a co-staple food in some parts of Ethiopia (Gemechis *et al*., 2012). It is an important cash and profitable vegetable crop produced by both small-scale and commercial farmers in Ethiopia (Lemma *et al*., 1994). Ethiopia is endowed with favourable climatic conditions for the production of vegetables including tomato. Recently, many foreign and domestic investors are being engaged in large-scale tomato production. However, the average yield of tomato in Ethiopia has always been low, 8 tons/ ha compared with the average yields of 54, 42, 35, 20 and 35 tons/ha in America, Europe, Asia, Africa and the entire world respectively (FAO, 2013). Farmers get lower yield mainly due to diseases and pests (Mandefro & Mekete, 2002; Balem, 2008). In East Africa, including Ethiopia, the knowledge of farmers about the presence and management of PPN is very limited (Maina *et al*., 2010; Mwesige *et al*., 2016). Moreover, studies that assess farmer's knowledge and factors associated with RKN damage are lacking. Tackling the problem of PPN on agricultural production thus is highly dependent on increasing farmer's awareness about PPN problems (Coyne, 2009).

RKN are among the biotic factors that limit tomato production worldwide. Tropical conditions are more conducive for the RKN populations' rapid build-up. Moreover, the limited availability of skilled manpower and suitable infrastructure makes the impact of RKN more severe in tropical developing countries (De Waele & Elsen, 2007). Whitehead (1968) and O'Bannon (1975) were among the first to document the occurrence of *Meloidogyne* spp. in Ethiopia. Since then different attempts have been made to study the distribution of the RKN genus (Mandefro & Mekete, 2002). However, there is still a lot unknown about the presence and distribution of this genus in Ethiopia. The study of its biodiversity in major tomato growing areas of Ethiopia is principal. Identification of *Meloidogyne* spp. inflicting a yield loss in any crop and in any given area is a prerequisite to devise a sound, sustainable and eco-friendly management approach. *Meloidogyne* spp. identification based only on classical approaches using morphology, morphometrics, and the North Carolina differential host race test is to some extent inaccurate, unreliable and laborious (Hartmann & Sasser, 1985; Hunt & Handoo, 2009; Janssen *et al*., 2016). Though it depends on the age of the female nematode (egg-laying young female) used isozyme profiles can provide accurate,

routine diagnostic test for RKN (Esbenshade & Triantaphyllou, 1987; Carneiro *et al*., 2000). DNA-based methods can be used for various stages of nematode development, discriminate individual species from mixed populations, and supported by DNA voucher specimens that have been stored for several years (Moleleki & Onkendi, 2013). Recently, Janssen *et al*. (2016) developed a reliable identification method for RKN from clade I using mitochondrial haplotypes with Nad5 gene fragment that contains the largest number of variable positions. RKN in Ethiopia have largely been identified on the basis of morphology (female perennial pattern and J2 morphometrics) and in few cases using cytological and biochemical methods (Mandefro & Dagne, 2000; Mekete *et al*., 2003). There is little information available in the literature about identification of Ethiopian RKN populations using molecular tools (Meressa *et al*., 2015). The objectives of the present study were: 1) to study the distribution of RKN in major tomato growing regions of Ethiopia 2) to characterize RKN populations using DNA and isozyme tools from major tomato growing areas of Ethiopia and 3) to assess knowledge and practice of farmers and factors associated with RKN damage on tomato.

3.2. Materials and Methods

3.2.1. Survey, Sampling and Soil bioassay

A total of 40 localities (Table 3.1 & Fig 3.1) in major tomato growing areas mainly from Rift Valley, Upper Awash and Eastern Hararghe were sampled during the 2012/13 growing season to assess and identify the distribution of RKN (*Meloidogyne* spp.) and possible associated problems. The proportions of samples in each major tomato growing areas were based on the agro-ecological zones of Ethiopia. This zonation was made based on the elevations and length of growing period, which includes the rain fall, potential evapo-transpiration and soil moisture storage properties. In total, 212 rhizosphere soil samples were collected from all localities. A total of 123 root samples were also collected from most fields visited and kept separately from the soil samples. Less number of roots was sampled because some farmers were not willing to provide root samples. Every root sample corresponds with a soil sample. Ten to fifteen plants per 1/2 ha were collected in a zigzag fashion. Approximately, 1.5 kg of composite soil samples and 200 g feeder root samples were collected for each sample to a depth of 0-25 cm (Barker, 1985). The soil samples were placed in clean polythene bags and date, specific locations, tomato cultivar, previous crop and condition of the soil were noted on their labels. The soil and root samples were transported from the sampling areas to Haramaya University (HU) laboratory using insulated containers. The corer, hand shovels and footwear were cleaned after sampling each farm to avoid cross

contamination of soil samples and spread of PPN between farms. The samples were collected from a range of farms: from established commercial greenhouse tomatoes production to gardens (Table 3.1). The stages of the tomato crop during sampling ranged from seedlings in the nursery to senescing tomato plants in the field. The samples were taken from farms when the soil was not too wet or too dry as these extreme conditions make it difficult to collect and prepare samples for analysis (Shurtleff and Averre, 2000).

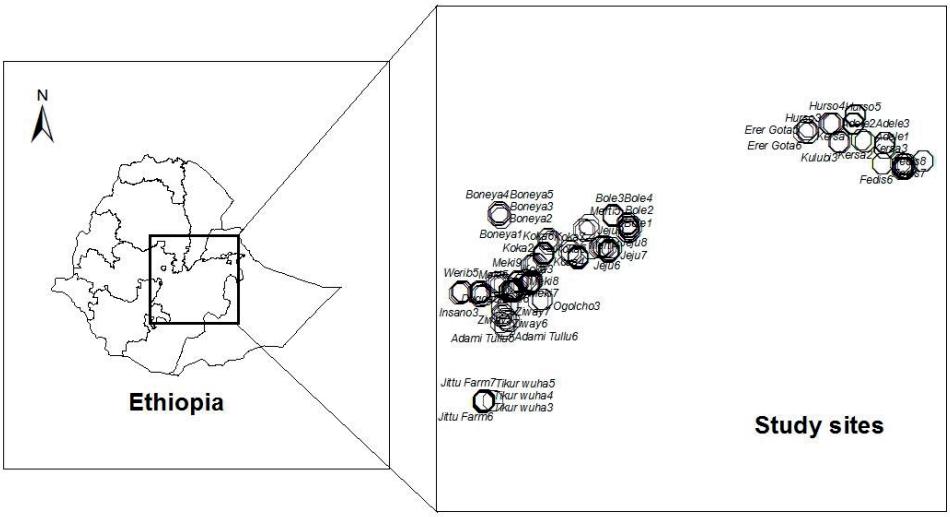

Figure 3.1. Map showing the sampled localities of major tomato growing areas of Ethiopia.

The population density from the original soil samples was not computed for two main reasons. Firstly, due to sampling, it took several days to collect the samples and 'older' samples might have already more dead nematodes. Secondly, not only absolute numbers matter but also the infectivity potential after growing a suitable host. As a result, soil samples collected from each field were tested for the presence of RKN with a bioassay test using tomato as an indicator plant. A three-week-old *Meloidogyne* spp. susceptible tomato cv. *Moneymaker* was grown in the collected soil samples. After eight weeks, the roots of the tomato plant were examined to determine the root gall index (RGI) and egg mass index (EMI). For the RGI and EMI, 0 to 5 rating index scale was used where 0 = no egg masses/galls; 1 = 1-2 egg masses/galls; 2 = 3-10 egg masses/galls; 3 = 11-30 egg masses/galls; 4 = 31-100 egg masses/galls and 5 = >100 egg masses/galls (Taylor & Sasser, 1978). Then from each pot, 100 cm^3 soil and 10 g root were collected to analyse RKN population densities.

3.2.2. Extraction of Nematodes from Soil and Root

Soil was thoroughly mixed and homogenized after the bioassay test and 100cm^3 soil sub-sample was used for nematode extraction using a modified Baermann funnel technique (Hooper *et al*., 2005). The recovered nematodes were counted using a stereomicroscope and the densities expressed as the number of nematodes per 100 cm^3 of soil. Ten gram galled roots of the tomato plant from each pot were washed gently with tap water and cut into smaller pieces (1 to 2 cm long) and agitated for 1 min in 1% NaOCl. The suspension was passed through 75 and 25 µm nested sieves. Eggs and J2 collected on the 25µm sieve were washed several times with tap water, re-suspended and their density determined using a stereomicroscope (Hussey and Barker, 1973).

3.2.3. Root-Knot Nematode Infestation Study

The RGI and EMI were determined from both root samples directly collected during the survey and those obtained after eight weeks of soil bioassay test. For the bioassay test, an indicator susceptible tomato cultivar *Moneymaker* was planted in each collected soil sample. After eight weeks, the roots of this tomato cultivar were uprooted, washed carefully with tap water, blotted dry and gall and eggmass indices determined (Taylor & Sasser, 1978). Ten grams of roots were stained with Phloxin B (15 mg/L) for 15-20 min based on Daykin & Hussey (1985). The stained roots were then rinsed in tap water and egg masses and root galls were counted using a stereomicroscope.

The prevalence (frequency of occurrence expressed as number of samples with RKNs per total number of samples surveyed) and incidence of the disease (number of plants galled per total number of plants sampled) were calculated. Except in a few localities with limited number of egg masses where 1 egg mass was used per plant; from the majority of localities 3 to 5 egg masses per plant were collected for identification. Egg masses were collected from galled roots and a single egg mass was left on Petri dish to get enough J2 for the molecular studies and to start a pure culture.

3.2.4. Molecular and Biochemical Identification of *Meloidogyne* species

3.2.4.1 Molecular identification

The DNA of RKN J2 was extracted based on Holterman *et al*. (2006). One to five J2 were added to 25µl sterile water in a PCR tube. Then, 25µl lysis buffer (0.2M NaCl, 0.2M Tris-HCl (pH 8.0), 1% (v/v) b-mercaptoethanol, and 800µg/ml proteinase-K) was added to the tubes. The samples were then incubated for 1 hour and 30 minutes at 65°C followed by 5 min

incubation at 99°C in a thermo cycler. The presence and quality of the extracted DNA was checked using a universal primer set D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3') and D3B (5'-TCGGAAGGAACCAGCTACTA-3') based on De Ley *et al*. (1999). All DNA extracts giving a positive amplification with the D2A and D3B primer set were further processed using sequence-characterised amplified regions (SCAR) primers for the detection of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* based on Zijlstra *et al*. (2000) (Table 3.2.). For isolates with weak band problems after amplifications using the primers developed by Zijlstra *et al*. (2000), Nad5 gene fragment was used to confirm the identification (Janssen *et al*. 2016). The species-specific primers (SSP) sets developed by Wishart *et al*. (2002) were applied for the detection of *M. hapla, M. chitwoodi*, *M. fallax* and *M. enterolobii*. The PCR products of the D2D3 segment of 28S rDNA region was purified and sent for sequencing at a sequence facility (Macrogen Europe, the Netherlands). The products were sequenced bi-directionally. The sequences were analysed using MEGA6 Software and aligned using the default parameters of clustal X (Tamura *et al*., 2013). The evolutionary history was inferred using the Maximum Likelihood method based on the Tamura 3-parameter model. Initial tree (s) for the heuristic search were obtained automatically by applying Neighbour-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. Evolutionary analyses were conducted in MEGA6. For the 28S rRNA region phylogenetic tree *Pratylenchus penetrans* was used as an out-group while for the Nad5 mitochondrial region *M. enterolobii* was used as an out-group.

3.2.4.2. Biochemical identification

Biochemical identification (EST and MDH) was employed for the weak samples amplified using SCAR primers. These *Meloidogyne* populations were first propagated on the susceptible tomato cultivar '*Marmande*' in the greenhouse. Infected tomato roots were placed in 0.9% NaCl solution and young egg-laying females were isolated under a stereomicroscope and placed in 0.9% NaCl solution till identification. The females were transferred from the NaCl solution to reagent-grade water on ice for a few minutes. After this desalting step eight sample wells were each filled with one female and 0.6µl-extraction buffer (20% sucrose, 2% Triton X-100, 0.01% Bromophenol Blue) (Esbenshade & Triantaphyllou, 1985). The females were then macerated using a small glass rod and 0.3µl of these samples were loaded onto each of two eight wells sample applicators. Both sample applicators were inserted into the cathode slot, one into the left, and the other into the right applicator arm of the PhastSystem

(Pharmacia, Sweden). *Meloidogyne javanica* was used as a reference. Electrophoresis was carried out with PhastGel gradient gels (8-25) in a discontinuous buffer system (Karssen *et al*., 1995). After electrophoresis the gels were stained in a staining box and placed in an incubator at 37 °C. For the EST (esterase) activity the gel was stained for 1hr while for MDH (malate dehydrogenase) activity it was only stained for 5 minutes. The gels were also double stained, first with Est followed by MDH. After staining, the gels were rinsed with distilled water and fixed for 5 min in a 10% acetic acid/10% glycerol/80% distilled water solution. The compounds of the staining solutions used for the activity of EST and MDH were based on Karssen *et al*. (1995).

3.2.5. Farmers Knowledge and Practice towards Plant-Parasitic Nematodes

A cross-sectional study was conducted from January 2013 till March 2013 to assess the knowledge and practice of farmers towards PPN. A total of 646 respondents (small-scale, state-owned and commercial farmers, and extension workers) from the major tomato growing areas of Ethiopia (Rift Valley, Upper Awash and East Hararghe) were included. The survey used closed ended questionnaires. The dependent variable used in the survey was RKN damage symptom (Fig 3.2). The independent variables included previous crop, soil texture, fertilizer used, source of irrigation water, owner of the field, source of seedling, awareness about PPN and pesticide used by farmers. Data were analysed using SPSS16.0 software. The frequency and percentage statistics were used to describe the sample population in relation to relevant variables. The binary logistic regression analysis (odds ratio with corresponding 95% confidence interval) was performed to assess the presence and degree of association between the dependent and independent variables. P-value < 0.05 was considered for statistical significance association.

Figure 3.2. A) Greenhouse tomato production Jittu farm-Ethiopia B) RKN infected tomato roots C) Unhealthy tomato plant infected with RKN at Babile location D) Egg masses stained with Phloxin 'B' during the 2012/13 growing season from Ethiopia.

3.3. Results

3.3.1. Prevalence, Incidence and Density of Root-Knot Nematode

Out of the 212 composite soil samples collected, 100 samples (47.2%) were found infested by various *Meloidogyne* species eight weeks after the start of the bioassay test. Out of the 123 root samples collected, 80 of them (65%) had root galls. There was significant variability in the prevalence and incidence of RKN between sampled localities. The highest prevalence (100%) of RKN was found on samples collected from Adami Tullu, Babile (Erer and Gende Sudan), Erer Gota, Hurso, Jittu, Tikur Wuha, Tepo Choronke, Zeway and Koka. The highest incidence (100%) of RKN was found from Adami Tullu, Jara Weyo, Babile (Erer and Gende Sudan), Erer Gota, Hurso, Jittu, Tikur Wuha, Tony farm, Tepo Choronke, Zeway and Koka locations based on direct observation of galls on collected root samples. RKN were not recovered from the tomato growing areas such as Meki, Jeju and Nura Era. The highest EMI (=5) and RGI (=5) were recorded from 40% of the root samples directly collected from fields

(data not shown) and after the bioassay test (Table 3.1). Five soil samples were found positive after the bioassay test while their respective root sample directly collected from the field was not found infected. The RKN population densities varied among the sampled fields as enumerated after the bioassay test. RKN populations in the soil ranged from 0 (17 locations) to 514 (Jittu farm), with an average of 136 (J2+eggs)/ 100 cm^3 soil, and 0 (17 locations) to 361 (Jittu farm), with an average of 81 (J2 + eggs) in 10 g of roots (Table 3.1).

Table 3.1. Altitude ranges, farm type, prevalence, incidence, root gall index (RGI), egg mass index (EMI), densities after the bioassay test and species of RKN recovered from the 40 tomato growing localities of Ethiopia.

Locality	Altitude (m)	Farm Type [a]	Prevalence [b] %	Incidence [c] %	RGI	EMI	J2+egg/100 cm^3 soil	J2+egg /10g root	*Meloidogyne* species [d]
Adami Tullu	1645-1677	CF, SS	100 (7)	100 (7)	5	5	430	226	Mi (40), Mj (50), Ma (10)
Jara Weyo	1618-1633	SS	33 (3)	100 (1)	1	1	106	32	Mi (66.6), Mj (33.3)
Kintrit	1601-1606	SS	50(2)	50 (2)	2	2	79	64	Mi (50), Mj (50)
Babile Erer	1318-1652	GR, SS	100(7)	100 (5)	5	5	440	243	Mi (40), Mj (60)
Babile Gende Sudan	1311-1363	GR, CF, SS	100(7)	100 (4)	5	5	479	256	Mi (30), Mj (70)
Bati Lijamo	1833-1849	SS	50(4)	50 (2)	3	2	187	102	Mi (50), Mj (25), Ma (25)
Werib	2153-2336	GR, SS	0(5)	0 (2)	0	0	0	0	-------
Insano	1834-1846	SS	40 (5)	67 (3)	2	3	159	44	Mi (20), Mj (40), Ma (40)
Erer Gota	1077-1389	GR, CF, SS	100 (6)	100 (5)	5	5	292	139	Mi (60), Mj (40)
Hurso	1127-1154	SS	100 (5)	100 (3)	5	5	252	139	Mi (80), Mj (20)
Fedis	1898-1909	GR, INT, SS	63 (8)	60 (5)	4	4	121	61	Mi(20), Mj(60), M.spp(20)
Gursum	1700-1711	INT, SS	71 (7)	100 (4)	4	4	177	97	Mi(50), Mj(40), M.spp(10)
Jittu	1702-1705	LSG	100 (7)	100 (7)	5	5	514	361	Mi (70), Mj (20), Ma (10)
Tikur Wuha	1698-1702	CF, SS	100 (5)	75 (4)	5	5	308	185	Mi (60), Mj (40)
Kersa	2139-2147	SS	67 (3)	50 (2)	3	4	152	102	Mi (60), Mj (40)
Kulubi	2258-2460	GR, SS	67 (3)	50 (2)	4	3	72	48	Mi (60), Mj (40)
Shiniele	1035-1063	CF	50 (4)	67 (3)	2	3	138	107	Mi (50), Mj (50)
Tony farm	1188-1203	SF	60(5)	100 (2)	2	2	149	103	Mi (60), Mj (40)
Dugda	1750-1877	SS	0 (5)	0 (0)	0	0	0	0	-------
Tepo Choronke	1637-1668	CF, SS	100 (7)	100 (7)	5	5	313	238	Mi (50), Mj (30), Ma (10), M. spp (10)
Meki	1639-1701	GR, CF, SS	0 (9)	0 (6)	0	0	0	0	-------
Weldia	1706-1786	CF	0 (6)	0 (2)	0	0	0	0	-------
Chele	1697-1705	CF, SS	60 (5)	67 (3)	3	3	84	60	Mi (60), Mj (20), Ma (20)
Girisa	1667-1677	SS	0 (4)	0 (0)	0	0	0	0	-------
Merti	1159-1230	CF, SS	40 (5)	67 (3)	2	3	111	68	Mi (60), Mj (20), Ma (20)
Jeju	1324-1608	CF, SS	0 (10)	0 (5)	0	0	0	0	-------
Zeway	1620-1709	GR, CF, SF, SS	100 (7)	100 (7)	5	5	389	224	Mi(40), Mj(30), Ma(10), Mh(10), M. spp (10)
Ogolcho	1716-1720	CF, SS	0 (3)	0 (2)	0	0	0	0	-------

Koka	1596-1601	GR, CF, SS	100 (7)	100 (7)	5	5	316	219	Mi (60), Mj (30), Ma (10)
Mojo Zuria	1716-1756	GR, SS	0 (4)	0 (2)	0	0	0	0	-------
Welenchiti	1437-1467	SS	0 (4)	0 (0)	0	0	0	0	-------
Bole	1262-1285	CF, SS	0 (4)	0 (0)	0	0	0	0	-------
Tibila	1256-1355	GR, CF, SS	83 (6)	75 (4)	3	4	166	136	Mi (40), Mj (40), Ma (10), M. spp (10)
Nura Era	1100-1126	CF, SS	0 (8)	0 (4)	0	0	0	0	-------
Melkassa	1459-1562	CF, SF, SS	0 (5)	0 (3)	0	0	0	0	-------
Wonji	1549-1632	CF, SS	0 (3)	0 (3)	0	0	0	0	-------
Adele	2041-2042	GR, SS	0 (3)	0 (0)	0	0	0	0	-------
Asarge	2072-2076	GR, SS	0 (4)	0 (0)	0	0	0	0	-------
Kombolcha	2114-2158	GR, SS	0 (5)	0 (2)	0	0	0	0	-------
Boneya	2136-2278	SS	0 (5)	0 (0)	0	0	0	0	-------

[a] Farm Type: CF = commercial farm (>5ha), SS = small scale farmers farm (0.5-5ha), GR = Garden (small areas visited in towns and gardens in rural farmers village too), SF = state farm (owned and managed by the state), INT = intercropped (tomato was intercropped with other solanaceous plants and beans.

[b] Prevalence (%) is the number of fields with RKNs per total number of fields surveyed. Numbers in brackets are the total number of fields visited per locality.

[c] Incidence (%) is number of plants with galls per total number of plants samples determined on samples collected from the field (i.e. before the bioassay test).

[d] *Meloidogyne* species, Mi = *Meloidogyne incognita*; Mj = *Meloidogyne javanica*; Ma = *Meloidogyne arenaria*; Mh= *Meloidogyne hapla* and *M.* spp. = Unidentified *Meloidogyne* species. Numbers beside the species in bracket tells the proportion percentages.

3.3.2. *Meloidogyne* species Present on Tomato

A total of 153 RKN isolates (from locations with positive samples) were studied and identified using molecular and biochemical tools. The presence of *M. incognita*, *M. javanica, M. arenaria* and *M. hapla* on tomato was confirmed using the SCAR primers (Zijlstra *et al*., 2000) and Nad5 gene fragment primers (Janssen *et al*. 2016) (Table 3.3, Fig 3.3-3.7). Out of these 153 isolates, 48.4% were found to be *M. incognita* (74), 41.2% *M. javanica* (63), 6.5% *M. arenaria* (10), 3.3% unidentified *Meloidogyne* spp. (5) and 0.7% *M. hapla* (1). The 5 isolates i.e. Fedis-*FED5*, Gursum-*GUR10*, Tepochoronke-*TEP5*, Tibila-*TIB9* and Zeway-*ZEW8* were unidentified despite trying a combination of molecular and biochemical techniques. *Meloidogyne chitwoodi*, *M. fallax* and *M. enterolobii* were not detected using their respective SSP. The two tropical species, *M. incognita* and *M. javanica* were identified as the most prevalent species. Both species were also found co-infecting tomato plants after the bioassay test.

A study of the 28S large subunit rDNA partial sequences of the isolates (Table 3.3) through BLAST search of GenBank did not give conclusive species identification since 99-100% were similar to *M. incognita, M. javanica*, *M. arenaria*, *M. thailandica* (Handoo, Skantar, Carta & Erbe, 2005) and *M. floridensis* (Handoo, Nyczepir, Esmenjaud, van der Beek, Castagnone-Sereno, Carta, Skantar & Higgins, 2004). From the complete set of the 28S rDNA partial sequences we only encountered a single variable site and a couple of heterozygous positions and three haplotypes. Even this single variable position does not coincide with *M. incognita* or *M. javanica* or *M. arenaria*. A phylogenetic tree was constructed using the three haplotypes including *M. hapla* obtained from the sequence analysis of the 28S rDNA region (Fig 3.8) and Ethiopian RKN isolates identified using Nad5 gene fragment based on Janssen *et al*. 2016 (Fig 3.9).

Table 3.2. Primers used for molecular identification of *Meloidogyne* species from Ethiopia.

Primers Code	Primer Sequence 5'-3'	Specificity	Reference
D2A	ACAAGTACCGTGAGGGAAAGTTG	28S rDNA region	De Ley et al., 1999
D3B	TCGGAAGGAACCAGCTACTA	28S rDNA region	De Ley et al., 1999
Far	TCGGCGATAGAGGTAAATGAC	*M. arenaria* specific SCAR	Zijlstra *et al*., 2000
Rar	TCGGCGATAGACACTACAAACT	*M. arenaria* specific SCAR	Zijlstra *et al*., 2000
Fjav	GGTGCGCGATTGAACTGAGC	*M. javanica* specific SCAR	Zijlstra et al., 2000
Rjav	CAGGCCCTTCAGTGGAACTATAC	*M. javanica* specific SCAR	Zijlstra *et al*., 2000
Finc	CTCTGCCCAATGAGCTGTCC	*M. incognita* specific SCAR	Zijlstra et al., 2000
Rinc	CTCTGCCCTCACATTAGG	*M. incognita* specific SCAR	Zijlstra *et al*., 2000
NAD5F2	TATTTTTTGTTTGAGATATATTAG	NADH dehydrogenase subunit 5	Janssen *et al*., 2016
NAD5R1	CGTGAATCTTGATTTTCCATTTTT	NADH dehydrogenase subunit 5	Janssen *et al*., 2016
Jmv1-5s	GGATGGCGTGCTTTCAAC	5S gene (IGS region)	Wishart et al., 2002
Jmv-hapla	AAAAATCCCCTCGAAAAATCCACC	Between 5S gene and 18S gene	Wishart *et al*., 2002
Jmv-2 (*M. fallax* & *M. chitwoodi*)	TTTCCCCTTATGATGTTTACCC	Between 5S gene and 18S gene	Wishart *et al*., 2002
Jmv tropical	GCKGGTAATTAAGCTGTCA	IGS region for tropical species	Wishart *et al*., 2002

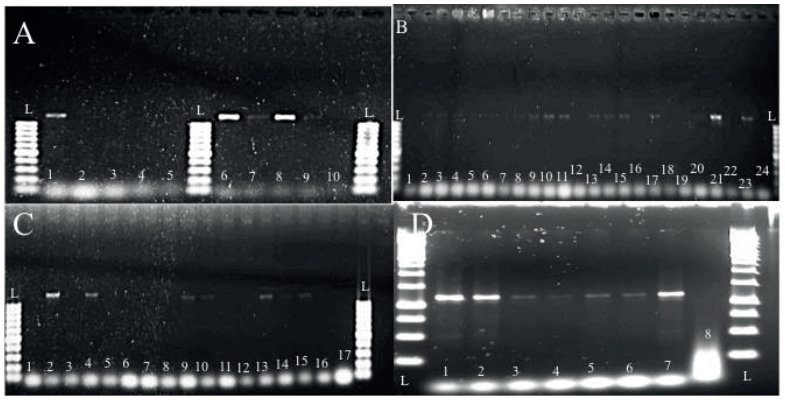

Figure 3.3. Some examples of SCAR amplification patterns obtained using the primer Finc/Rinc (a product length of 1200bp) from infective J2 hatched from a single egg mass. A) Lane1-BGS6, 6-JIT5, 7-JIT9, 8-TEP6, 10-Negative control-water instead of DNA) while Lane 2, 3, 4, 5 & 9 were not amplified. B) Lane 3-ADT3, 4-ADT4, 6-ADT5, 9-BEF1, 10-BEF7, 11-BGS2, 13-ERG2, 14-JIT10, 15-GUR1, 17-TIB6, 21-KER1, 23-positive control (*M. incognita*) and 24-Negative control while on Lane 1, 2, 5, 7, 8, 12,16,18,19,20 amplification was not found. C) Lane 2-CHL1, 4-ALM1, 9-BEF6, 10-BEF8, 13-BGS3, 14-BTL1, 15-ERG5 while Lane 1,3,5,6,7,8, 11, 12 and 16 no product amplified, 17-negative control. D) Lane 1-FED1, 2-GUR 5, 3-GUR6, 4-HUR1, 5-INS2, 6-KOK3, 7-TEP1 & 8-negative control. L in A, B & C is a 100bp ladder (Promega) while the L in D is a 250bp ladder (Promega).

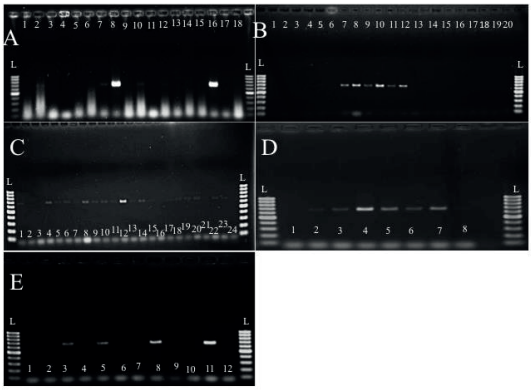

Figure 3.4. Examples of SCAR Amplification patterns obtained using the primer Fjav/Rjav (a product length of 670bp) from infective J2 hatched from a single egg mass, in all PCR pictures L is a 100bp ladder (Promega). A) Lane 8-ZEW6 & 16-JIT7 while samples on Lane 1 to 7, 9-15 & 17 were not amplified, 18-negative control. B) Lane 7-ADT2, 8-BTL2, 9-GUR8, 10-INS1, 11-MRT2, 12-TIB4 while the remaining samples from lane 1 to 6 & 13 to 19 not amplified, 20-negative control. C) Lane1-TNF3, 4-TKW3, 5-TKW5, 6-ADT1, 7-SHN3, 8-BEF3, 9-BEF5, 10-BEF10, 11-ALM2, 12-ADT10, 13-GUR4, 14-CHL4, 18-BGS9, 19-KUL2, 20-TEP3, 22- SHN4, 23-Positive control (*M. javanica*) and 24 is negative control while samples on lane 2, 3, 15, 16, 17 & 21 did not amplify. D) Lane 2-TEP8, 3-TIB2, 4-TIB3, 5-KOK6, 6- KOK7, 7-KOk10 & 8-negative control. E) Lane 3-ADT7, 5-BEF2, 8-BGS4, 11-ERG4 while samples on lane 1, 2, 4, 6,7,9 & 10 were not amplified, 12-negative control.

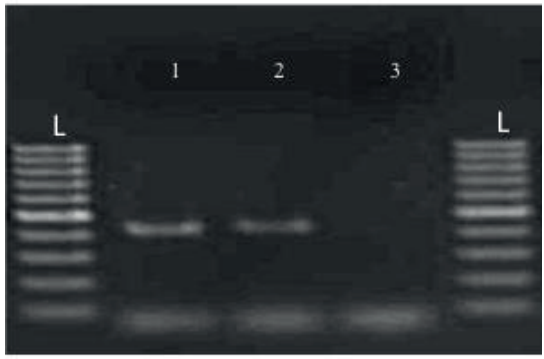

Figure 3.5. An example of SCAR amplification patterns obtained using the primer Fare/Rare (a product length of 420bp) from infective J2 hatched using a single egg mass, in all PCR pictures L is a 100bp ladder (Promega). A) Lane 1-ADT6, lane 2-KOK9, 3-negative control

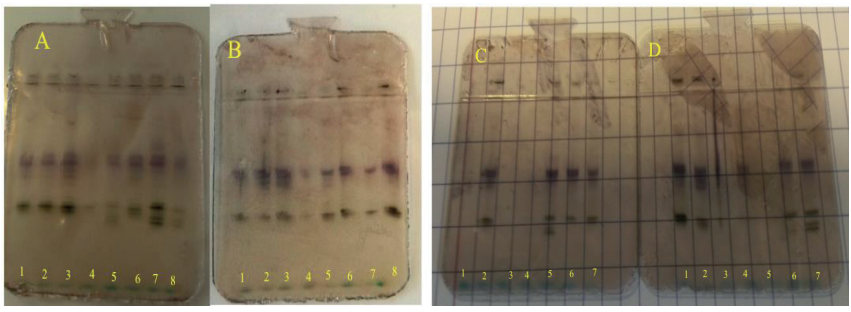

Figure 3.6. Some examples of EST and MDH obtained from *Meloidogyne* species of Ethiopia. A) Lane1-ADT3 (I1), 2-JIT4 (I1), 3-JIT8 (I1), 4-Fed5 (unidentified), 5-*M.javanica* control, 6-KOK5 (I1), 7-BEF9 (J3) & 8- BGS9 (J3); B) Lane 1-JIT9 (I1), 2-TEP9 (I1), 3-TEP10 (I1), 4-BTL1 (I1 too weak), 5-TIB7 (I1), 6-TKW1 (I1), 7-TKW4 (I1) & 8-ZEW3 (I1); C) 1-No female was added, 2-ADT4 (I1), 3-GUR10 (unidentified), 4-TIB8 (unidentified), 5-GUR4 (J3), 6-ADT5 (I1), & 7-CHL5 (I1) and D) Lane1-HUR2 (I1), 2-KER4 (I1), 3-MRT5 (no pattern found), 4-ZEW8 (unidentified), 5-TEP5 (unidentified), 6-GUR3 (J3) & 7-SHN3 (J3). The codes I1 & J3 in the brackets indicate the EST phenotype of *M. incognita* & *M. javanica* respectively

3.3.3. *Meloidogyne hapla*

The identity of *M. hapla* was determined using species-specific primers 5'-3' (GGATGGCGTGCTTTCAAC/AAAAATCCCCTCGAAAAATCCACC) (Wishart *et al*., 2002). A known *M. hapla* population was included as a positive control in the PCR. The PCR product size of 440bp was clearly and reproducibly amplified (Fig 3.7). The 28S large subunit rDNA partial sequence of our *M. hapla* population (deposited in a GenBank

accession number: KU587712) showed 99-100% sequence homology with accessions GQ130139, DQ328685, KP306534, KP306532, KJ755183, KF430798 and KJ645432 (Table 3.4). *Meloidogyne hapla* was detected for the first time in Ethiopia in an open tomato production farmer's field at Zeway location with 1620 m.a.s.l elevation. A soil sample collected from 'Zeway' area was found severely infested by a mixture of *M. incognita, M. javanica, M. arenaria* and *M. hapla* with a mixture of large and numerous small galls observed after the bioassay test.

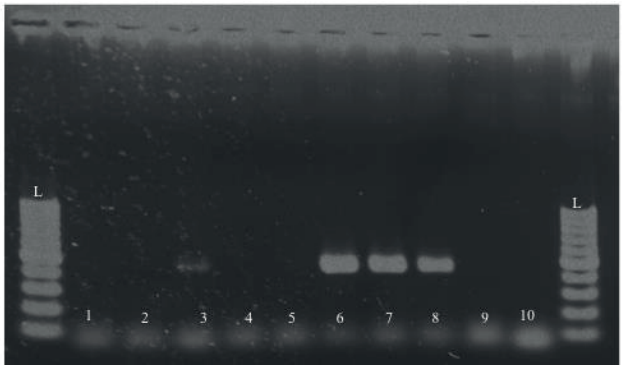

Figure 3.7. Amplification patterns obtained using the primer JMV1-5s/*JMV-hapla* (a product length of 440bp) from infective J2 hatched from a single egg mass, in all PCR pictures 'L' is a 100bp ladder (Promega). A product length of 440bp amplified for *M. hapla* from Zeway Ethiopia Lane-6 & 7-ZEW1 while the sample on Lane 3 & 8 were *M. hapla* positive control. Samples on lane 1-BGS6, 2-ZEW6, 4-BEF6, 5-GUR8, 9-JIT10 and 10-negative control were not amplified.

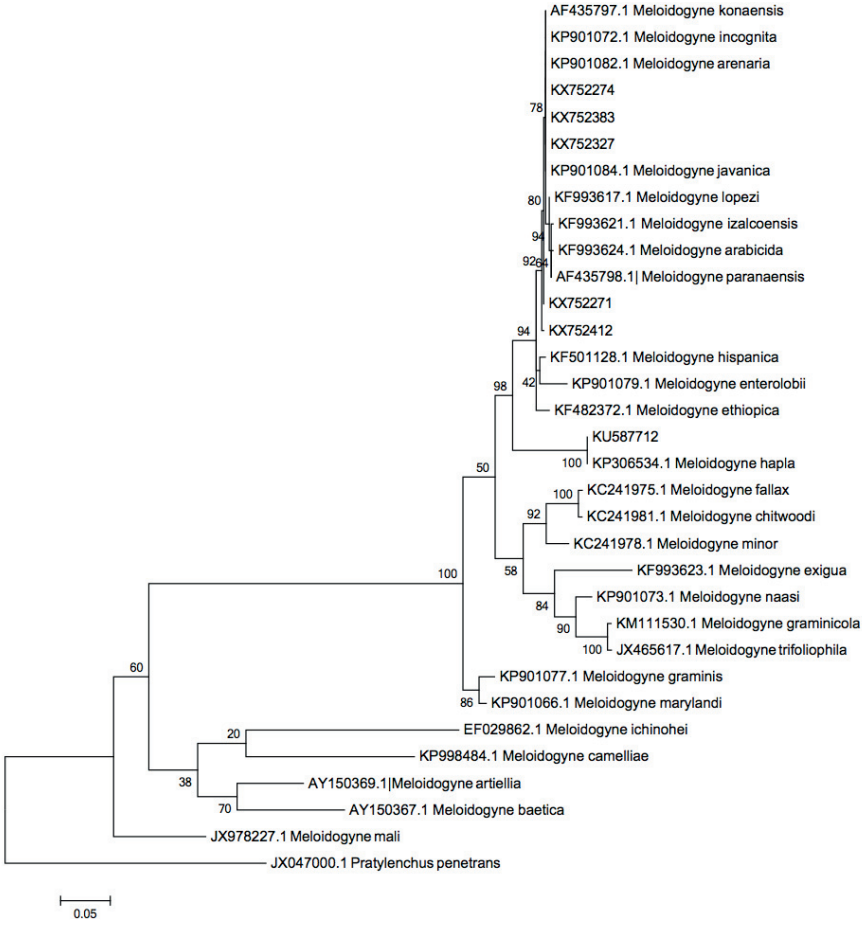

Figure 3.8. Maximum parsimony tree inferred from the 28S rDNA sequences. The accession number of the Ethiopian isolates is KX752274 (Isolate-ERG3), KX752383 (Isolate-FED1), KX752327 (Isolate-ADT10), KX752271 (Isolate-INS5), KX752412 (Isolate-TEP5) and KU587712 (*M.hapla*-ZEW1) (see Table 3.3). *Pratylenchus penetrans* was used as an out-group.

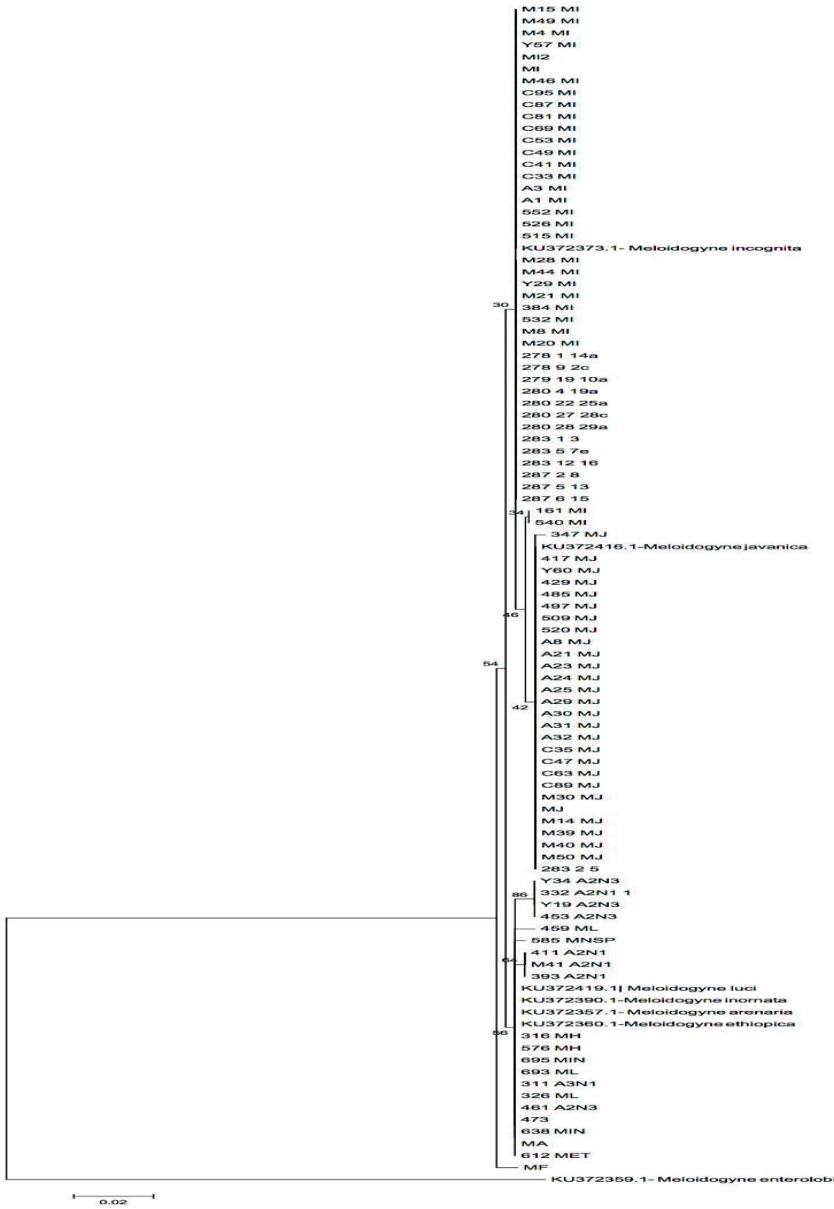

Figure 3.9. A phylogenetic tree inferred from the mitochondrial Nad5 gene fragment of 13 *M. incognita* Ethiopian populations 278 1 14a (***GUR2***), 278 9 2c (***ALM4***), 279 19 10a (***JIT6***), 280 4 19a (***KUL3***), 280 22 25a (***MRT4***), 280 27 28c (***ZEW9***), 280 28 29a (***ZEW10***), 283 1 3 (***ALM3***), 283 5 7e (***TNF5***), 283 12 16 (***TIB10***), 287 2 8 (***JIT1***), 287 5 13 (***HUR5***), 287 6 15 (***TIB5***) and one *M. javanica* population 283 2 5 (***BGS5***). *Meloidogyne enterolobii* was used as an out-group. The codes inside the brackets can be used to get the accession number of each isolate as presented in Table 3.3

Table 3.3. Identification of Ethiopian RKN isolates using different DNA-based and isozyme profiles

Isolate Code	Locality	SCAR Primer	Band length amplified	mtDNA Region	Band length amplified	EST	MDH	Identified species	GenBank Accession number
ADT1	Adami Tullu	Fjav/Rjav	760bp					*M. javanica*	KX752287
ADT2	Adami Tullu	Fjav/Rjav	760bp					*M. javanica*	KX752325
ADT3	Adami Tullu	Finc/Rinc	1200bp			Yes	Yes	*M. incognita*	-
ADT4	Adami Tullu	Finc/Rinc	1200bp			Yes	Yes	*M. incognita*	KX752340
ADT5	Adami Tullu	Finc/Rinc	1200bp			Yes	Yes	*M. incognita*	KX752338
ADT6	Adami Tullu	Far/Rar	420bp					*M. arenaria*	KX752348
ADT7	Adami Tullu	Fjav/Rjav	760bp					*M. javanica*	KX752362
ADT8	Adami Tullu	Fjav/Rjav	760bp					*M. javanica*	KX752322
ADT9	Adami Tullu			NAD5F2/R1	610bp			*M. incognita*	KX752326
ADT10	Adami Tullu	Fjav/Rjav	760bp					*M. javanica*	KX752327
ALM1	Alemtena	Finc/Rinc	1200bp					*M. incognita*	KX752289
ALM2	Alemtena	Fjav/Rjav	760bp					*M. javanica*	KX752329
ALM3	Alemtena			NAD5F2/R1	610bp			*M. incognita*	KX752330
ALM4	Alemtena			NAD5F2/R1	610bp			*M. incognita*	KX752414
ALM5	Alemtena	Fjav/Rjav	760bp					*M. javanica*	KX752413
BEF1	Babile Erer	Finc/Rinc	1200bp					*M. incognita*	KX752332
BEF2	Babile Erer	Fjav/Rjav	760bp					*M. javanica*	KX752333
BEF3	Babile Erer	Fjav/Rjav	760bp					*M. javanica*	KX752341
BEF4	Babile Erer	Fjav/Rjav	760bp			Yes	Yes	*M. javanica*	KX752347
BEF5	Babile Erer	Fjav/Rjav	760bp					*M. javanica*	KX752334
BEF6	Babile Erer	Finc/Rinc	1200bp					*M. incognita*	KX752331
BEF7	Babile Erer	Finc/Rinc	1200bp					*M. incognita*	KX752270
BEF8	Babile Erer	Finc/Rinc	1200bp					*M. incognita*	KX752267
BEF9	Babile Erer	Fjav/Rjav	760bp			Yes	Yes	*M. javanica*	KX752328
BEF10	Babile Erer	Fjav/Rjav	760bp					*M. javanica*	KX752324
BGS1	Babile Gende Sudan	Fjav/Rjav	760bp					*M. javanica*	KX752381

BGS2	Babile Gende Sudan	Finc/Rinc	1200bp					*M. incognita*	KX752382
BGS3	Babile Gende Sudan	Finc/Rinc	1200bp					*M. incognita*	KX752374
BGS4	Babile Gende Sudan	Fjav/Rjav	760bp					*M. javanica*	KX752375
BGS5	Babile Gende Sudan			NAD5F2/R1	610bp			*M. javanica*	KX752367
BGS6	Babile Gende Sudan	Finc/Rinc	1200bp					*M. incognita*	KX752386
BGS7	Babile Gende Sudan	Fjav/Rjav	760bp					*M. javanica*	KX752373
BGS8	Babile Gende Sudan	Fjav/Rjav	760bp					*M. javanica*	KX752376
BGS9	Babile Gende Sudan	Fjav/Rjav	760bp			Yes	Yes	*M. javanica*	KX752377
BGS10	Babile Gende Sudan	Finc/Rinc	1200bp	NAD5F2/R1	610bp			*M. javanica*	KX752371
BTL1	Bati Lijamo	Finc/Rinc	1200bp					*M. incognita*	KX752364
BTL2	Bati Lijamo	Fjav/Rjav	760pb					*M. javanica*	KX752366
BTL3	Bati Lijamo	Far/Rar	420bp					*M. arenaria*	KX752312
BTL4	Bati Lijamo			NAD5F2/R1	610bp			*M. incognita*	KX752275
CHL1	Chele	Finc/Rinc	1200bp					*M. incognita*	KX752393
CHL2	Chele	Far/Rar	420bp					*M. arenaria*	KX752355
CHL3	Chele	Finc/Rinc	1200bp					*M. incognita*	KX752352
CHL4	Chele	Fjav/Rjav	760bp					*M. javanica*	KX752370
CHL5	Chele	Finc/Rinc	1200bp			Yes	Yes	*M. incognita*	KX752359
ERG1	Erer Gota	Fjav/Rjav	760bp					*M. javanica*	KX752305
ERG2	Erer Gota	Finc/Rinc	1200bp					*M. incognita*	KX752317
ERG3	Erer Gota	Finc/Rinc	1200bp			Yes	Yes	*M. incognita*	KX752274
ERG4	Erer Gota	Fjav/Rjav	760bp					*M. javanica*	KX752301
ERG5	Erer Gota	Finc/Rinc	1200bp					*M. incognita*	KX752411
FED1	Fedis	Finc/Rinc	1200bp					*M. incognita*	KX752383
FED2	Fedis	Fjav/Rjav	760bp					*M. javanica*	KX752378
FED3	Fedis	Fjav/Rjav	760bp					*M. javanica*	KX752380
FED4	Fedis	Fjav/Rjav	760bp					*M. javanica*	KX752379
FED5	Fedis	-	-	-	-	-	-	Mspp	KX752372
GUR1	Gursum	Finc/Rinc	1200bp					*M. incognita*	KX752361
GUR2	Gursum			NAD5F2/R1	610bp			*M. incognita*	KX752401
GUR3	Gursum	Finc/Rinc	1200bp			Yes	Yes	*M. javanica*	KX752392

GUR4	Gursum	Fjav/Rjav	760bp			Yes	Yes	*M. javanica*	KX752396
GUR5	Gursum	Finc/Rinc	1200bp					*M. incognita*	KX752394
GUR6	Gursum	Finc/Rinc	1200bp					*M. incognita*	KX752406
GUR7	Gursum	Finc/Rinc	1200bp					*M. incognita*	KX752408
GUR8	Gursum	Fjav/Rjav	760bp					*M. javanica*	KX752356
GUR9	Gursum	Fjav/Rjav	760bp					*M. javanica*	KX752360
GUR10	Gursum	-	-	-	-	-	-	Mspp	KX752407
HUR1	Hurso	Finc/Rinc	1200bp					*M. incognita*	KX752405
HUR2	Hurso	Finc/Rinc	1200bp			Yes	Yes	*M. incognita*	KX752315
HUR3	Hurso	Fjav/Rjav	760bp					*M. javanica*	KX752320
HUR4	Hurso	Finc/Rinc	1200bp			Yes	Yes	*M. incognita*	KX752351
HUR5	Hurso			NAD5F2/R1	610bp			*M. incognita*	KX752397
INS1	Insano	Fjav/Rjav	760bp					*M. javanica*	KX752292
INS2	Insano	Finc/Rinc	1200bp					*M. incognita*	KX752284
INS3	Insano	Far/Rar	420bp					*M. arenaria*	KX752410
INS4	Insano	Far/Rar	420bp					*M. arenaria*	KX752344
INS5	Insano	Fjav/Rjav	760bp					*M. javanica*	KX752271
JIT1	Jittu			NAD5F2/R1	610bp			*M. incognita*	KX752272
JIT2	Jittu	Fjav/Rjav	760bp			Yes	Yes	*M. javanica*	KX752400
JIT3	Jittu	Far/Rar	420bp					*M. arenaria*	KX752282
JIT4	Jittu			NAD5F2/R1	610bp	Yes	Yes	*M. incognita*	KX752353
JIT5	Jittu	Finc/Rinc	1200bp					*M. incognita*	KX752395
JIT6	Jittu			NAD5F2/R1	610bp			*M. incognita*	KX752398
JIT7	Jittu	Fjav/Rjav	760bp					*M. javanica*	KX752365
JIT8	Jittu	Finc/Rinc	1200bp			Yes	Yes	*M. incognita*	KX752306
JIT9	Jittu	Finc/Rinc	1200bp			Yes	Yes	*M. incognita*	KX752369
JIT10	Jittu	Finc/Rinc	1200bp					*M. incognita*	KX752290
KER1	Kersa	Finc/Rinc	1200bp					*M. incognita*	KX752319
KER2	Kersa	Fjav/Rjav	760bp					*M. javanica*	-
KER3	Kersa			NAD5F2/R1	610bp			*M. incognita*	KX752368
KER4	Kersa	Finc/Rinc	1200bp			Yes	Yes	*M. incognita*	KX752363

KER5	Kersa	Fjav/Rjav	760bp					*M. javanica*	KX752346
KOK1	Koka	Fjav/Rjav	760bp					*M. javanica*	KX752390
KOK2	Koka	Fjav/Rjav	760bp					*M. javanica*	KX752391
KOK3	Koka	Finc/Rinc	1200bp					*M. incognita*	KX752387
KOK4	Koka	Finc/Rinc	1200bp			Yes	Yes	*M. incognita*	KX752402
KOK5	Koka	Finc/Rinc	1200bp			Yes	Yes	*M. incognita*	KX752403
KOK6	Koka	Fjav/Rjav	760bp					*M. javanica*	KX752384
KOK7	Koka	Fjav/Rjav	760bp					*M. javanica*	KX752388
KOK8	Koka			NAD5F2/R1	610bp			*M. javanica*	KX752385
KOK9	Koka	Far/Rar	420bp					*M. arenaria*	KX752295
KOK10	Koka	Fjav/Rjav	760bp					*M. javanica*	KX752389
KUL1	Kulubi	Fjav/Rjav	760bp					*M. javanica*	-
KUL2	Kulubi	Fjav/Rjav	760bp					*M. javanica*	KX752307
KUL3	Kulubi			NAD5F2/R1	610bp			*M. incognita*	KX752269
KUL4	Kulubi			NAD5F2/R1	610bp			*M. incognita*	KX752318
KUL5	Kulubi	Finc/Rinc	1200bp			Yes	Yes	*M. incognita*	KX752283
MRT1	Merti			NAD5F2/R1	610bp			*M. incognita*	KX752293
MRT2	Merti	Fjav/Rjav	760bp					*M. javanica*	KX752314
MRT3	Merti	Far/Rar	420bp					*M. arenaria*	KX752354
MRT4	Merti			NAD5F2/R1	610bp			*M. incognita*	KX752316
MRT5	Merti	Finc/Rinc	1200bp			Yes	Yes	*M. incognita*	KX752313
SHN1	Shiniele	Finc/Rinc	1200bp					*M. incognita*	KX752335
SHN2	Shiniele	Finc/Rinc	1200bp			Yes	Yes	*M. incognita*	KX752337
SHN3	Shiniele	Fjav/Rjav	760bp					*M. javanica*	KX752336
SHN4	Shiniele	Fjav/Rjav	760bp					*M. javanica*	-
TEP1	Tepo Choronke	Finc/Rinc	1200bp					*M. incognita*	KX752294
TEP2	Tepo Choronke	Fjav/Rjav	760bp					*M. javanica*	KX752278
TEP3	Tepo Choronke	Fjav/Rjav	760bp					*M. javanica*	KX752279
TEP4	Tepo Choronke	Far/Rar	420bp					*M. arenaria*	KX752268
TEP5	Tepo Choronke	-	-	-	-	-	-	Mspp	KX752412
TEP6	Tepo Choronke	Finc/Rinc	1200bp					*M. incognita*	KX752273

TEP7	Tepo Choronke			NAD5F2/R1	610bp	Yes	Yes	*M. incognita*	KX752280
TEP8	Tepo Choronke	Fjav/Rjav	760bp					*M. javanica*	KX752409
TEP9	Tepo Choronke	Finc/Rinc	1200bp			Yes	Yes	*M. incognita*	KX752321
TEP10	Tepo Choronke	Finc/Rinc	1200bp			Yes	Yes	*M. incognita*	KX752298
TIB1	Tibila	Far/Rar	420bp					*M. arenaria*	KX752277
TIB2	Tibila	Fjav/Rjav	760bp					*M. javanica*	KX752300
TIB3	Tibila	Fjav/Rjav	760bp					*M. javanica*	KX752309
TIB4	Tibila	Fjav/Rjav	760bp					*M. javanica*	KX752310
TIB5	Tibila	Fjav/Rjav	760bp	NAD5F2/R1	610bp			*M. incognita*	KX752303
TIB6	Tibila	Finc/Rinc	1200bp					*M. incognita*	KX752308
TIB7	Tibila	Finc/Rinc	1200bp			Yes	Yes	*M. incognita*	KX752299
TIB8	Tibila	Fjav/Rjav	760bp					*M. javanica*	KX752304
TIB9	Tibila	-	-	-	-	-	-	*M* spp	KX752276
TIB10	Tibila			NAD5F2/R1	610bp			*M. incognita*	KX752311
TKW1	Tikur Wuha			NAD5F2/R1	610bp	Yes	Yes	*M. incognita*	KX752350
TKW2	Tikur Wuha			NAD5F2/R1	610bp			*M. incognita*	KX752349
TKW3	Tikur Wuha	Fjav/Rjav	760bp					*M. javanica*	KX752357
TKW4	Tikur Wuha	Finc/Rinc	1200bp					*M. incognita*	KX752345
TKW5	Tikur Wuha	Fjav/Rjav	760bp					*M. javanica*	KX752346
TNF1	Tony Farm	Finc/Rinc	1200bp			Yes	Yes	*M. incognita*	KX752288
TNF2	Tony Farm	Fjav/Rjav	760bp					*M. javanica*	KX752339
TNF3	Tony Farm	Fjav/Rjav	760bp					*M. javanica*	KX752302
TNF4	Tony Farm	Finc/Rinc	1200bp					*M. incognita*	KX752297
TNF5	Tony Farm			NAD5F2/R1	610bp			*M. incognita*	KX752323
ZEW1	Zeway	Jmv1-5s/Jmv hapla	440bp					*M. hapla*	KU587712
ZEW2	Zeway	Fjav/Rjav	760bp					*M. javanica*	KX752296
ZEW3	Zeway	Finc/Rinc	1200bp			Yes	Yes	*M. incognita*	KX752358
ZEW4	Zeway	Far/Rar	420bp					*M. arenaria*	KX752399
ZEW5	Zeway	Fjav/Rjav	760bp					*M. javanica*	-
ZEW6	Zeway	Fjav/Rjav	760bp					*M. javanica*	KX752286
ZEW7	Zeway	Finc/Rinc	1200bp			Yes	Yes	*M. incognita*	KX752342

ZEW8	Zeway	-	-	-	-	-	-	Mspp	KX752343
ZEW9	Zeway			NAD5F2/R1	610bp			*M. incognita*	KX752404
ZEW10	Zeway			NAD5F2/R1	610bp			*M. incognita*	KX752285

Table 3.4. The 28S rDNA sequence identity of *M. hapla* population (accession number: KU587712) originated Zeway location Ethiopia during the 2012/13 growing season with accessions deposited from different countries in GenBank.

Accessions	Country of Origin	Source	Query length (bp)	Query Cover	Variable position	Identity (%)	Reference
DQ328685	Moldova	-	638	100	0	100	Subbotin *et al*. 2006
GQ130139	China	Peanut root gall	1043	100	2	99	Wang *et al*. 2009 unpublished
KF430798	China	*Rosa rugosa*	694	68	0	100	Zaho L., unpublished
KJ645432	Ethiopia	*Rosa* sp.	711	69	3	99	Meressa *et al*., 2014
KJ755183	China	Peanut	763	69	1	99	Liao & Zhang, 2014 unpublished
KP306532	South Korea	Strawberry	1036	99	2	99	Park *et al*., 2014 unpublished
KP306534	South Korea	Strawberry	1037	99	2	99	Park *et al*., 2014 unpublished
KU587712	***Ethiopia, Zeway***	***Tomato root gall***	***1006***				***Seid et al., 2016 unpublished***

3.3.4. Farmers Knowledge and Factors Associated with Root-Knot Nematode damage

The effect of eight independent variables (previous crop, soil texture, fertilizer used, source of irrigation water, owner of the field, seedling source, awareness about RKN and pesticides used) on the dependant variable RKN damage on tomato roots was investigated using binary logistic regression analysis (Table 3.5).

Out of the 646 respondents, 280 (43.3%) reported to have RKN damage symptoms when shown the symptoms of RKN, while 366 (56.7%) did not report damage. Even though all the independent variables had a significant ($P < 0.01$) effect on RKN damage, the highest effect on the dependent variable RKN damage on tomato roots comes from the previous crop and soil texture.

Out of the 646 respondents, 242 (37.5%), 247 (38.2%), 9 (1.3%) and 148 (22.9%) indicated that their farm in the previous season was grown with solanaceous, non-solanaceous, kept fallow and subjected to natural flood respectively. From 242 respondents that used solanaceous as previous crop, 233 (96.3%) reported to have the RKN damage on their tomato production while 9 (3.7%) did not report nematode damage in their tomato field. From 148 respondents with farms subjected to natural flood, 2 (1.4%) reported to have nematode damage on their tomato production while 146 (98.6%) did not report nematode damage in their field. Out of the 646 respondents, 271 (41.9%), 265 (41.1%) and 110 (17%) reported to have sandy, loam and clay soil texture in their farm respectively. From 271 respondents with sandy soil texture farms, 233 (86%) reported to have RKN damage on their tomato production while 38 (14%) did not report nematode damage on their field. Out of 110 respondents with clay soil texture farms, 2 (1.8%) reported to have RKN damage on their tomato production while 108 (98.2%) did not report nematode damage in their field.

Table 3.5. Binary logistic regression analysis between the dependant variable (RKN damage symptom) and eight independent variables based on the crude odds ratio (COR) at *P <0.05* significance level.

Independent variable	RKN Damage Symptom		COR [a]	95% CI [b]	P value
	Yes	NO			
Previous crop	**n=280**	**n=366**			
Solanaceous	233 (96.3)	9 (3.7)	20.8	2.55-170	<0.001
Non solanaceous	43 (17.4)	204 (82.6)	1.890	402.70-8.86	<0.001
Fallow	2 (22.2)	7 (77.8)	15.387	3.68-64.53	<0.001
Flooded	2 (1.4)	146 (98.6)	1.00		
Soil Texture	**n**=280	**n**=366			
Sandy	233 (86.0)	38 (14.0)	331.11	78.44-1.39	<0.001
Loam	45 (17)	220 (83.0)	11	2.63-46.38	<0.001
Clay	2 (1.8)	108 (98.2)	1.00		
Fertilizer used	**n**=280	**n**=366			
Organic	7 (26.9)	19 (73.1)	0.468	0.19-1.13	<0.01
Inorganic	273 (44.0)	347 (56.0)	1.00		
Source of irrigation	**n**=280	**n**=366			
River	38 (56.7)	29 (43.3)	2.963	1.48-5.89	0.002
Underground water	219 (43.5)	285 (56.5)	1.737	1.03-2.92	0.038
Lake	23 (30.7)	52 (69.3)	1.00		
Owner of the field	**n**=280	**n**=366			
Farmer	78 (24.1)	245 (75.9)	0.099	0.06-0.147	<0.001
State owned	34 (33.0)	69 (67.0)	0.153	0.09-0.25	<0.001
Investors (foreign/domestic)	168 (76.4)	52 (23.6)	1.00		
Seedling source	**n**=280	**n**=366			
Same field	157 (33.2)	316 (66.8)	0.20	0.14-0.29	<0.001
Separated nursery	123 (71.1)	50 (28.9)	1.00		
Awareness about PPN	**n**=280	**n**=366			
Yes	195 (87.4)	28 (12.6)	27.69	17.44-43.96	<0.001
No	85 (20.1)	338 (79.9)	1.00		
Pesticide used	**n**=280	**n**=366			
Nematicides	21(100)	0 (0)	1.451E9	0.08-0.23	<0.001
Insecticides	23 (13.5)	147 (86.5)	0.141		
Herbicides	0 (0)	7 (100)	0.000		
Fungicides	236 (52.7	212 (47.3)	1.00		

[a] Crude odds ratio
[b] 95% confidence interval
[c] P-value at 5% level of significance

3.4. Discussion

In this study, the occurrence of *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* alone, or in mixed populations from samples collected across 40 tomato production localities, clearly showed that RKN are widespread in major tomato growing areas of Ethiopia. The occurrence of *M. incognita* and *M. javanica* co-infesting almost all the positive samples detected in the present study is not a surprise. *Meloidogyne incognita* and *M. javanica* have previously been reported as the most common and widespread species on vegetable production in Ethiopia (Tadele & Mengistu, 2000; Mandefro & Dange, 2000; Mandefro &

Mekete, 2002). These two species were also cited as the most common and widespread species on food plants in East Africa particularly in Kenya, Uganda and Tanzania (Whitehead, 1969; IITA 1981; Bridge *et al*., 1991; CABI 2002; Nono-Womdim *et al*., 2002; Coyne *et al*., 2003; Onkendi *et al*., 2014; Kimani *et al*., 2015). The occurrence of *M. arenaria* and *M. hapla* is less documented in Ethiopia and based on the obtained results it should be given due attention especially in determining its economic importance for tomato production.

Accurate identification of the population and genetic diversity of *Meloidogyne* species present in a given tomato field is the first step in designing proper pest management programs (Powers *et al*., 2005). This can be achieved through a regular, comprehensive and accurate survey of *Meloidogyne* species across tomato growing regions (Moleleki & Onkendi, 2013). However, a single identification technique will not always be dependable especially when processing large number of samples. Proper identification of RKN requires a combination of morphological (perineal pattern, J2 morphometrics), cytological, isozyme and DNA-based tools. Quite recently, several DNA based reliable identification tools for different *Meloidogyne* species are being developed such as the new qPCR tool for *M. enterolobii* (Braun-Kiewnick *et al*., 2016), using mitochondrial haplotype to identify *M. haplanaria* (Joseph *et al*., 2016) and using Nad5 gene fragment for tropical RKN or MIG group (Janssen *et al*., 2016).

In our study, we used the 28S rDNA region partial sequence homology, SCAR primers developed by Zijlstra *et al*. (2000) for the detection of *M. incognita*, *M. javanica* and *M. arenaria* and Nad5 gene fragment sequence for *M. javanica* and *M. incognita* by Janssen *et al*. 2016; Wishart *et al*. (2002) to check for *M. hapla, M. chitwoodi, M. fallax* and *M. enterolobii*. To our knowledge, this is the first comprehensive molecular characterization of RKN populations originating from agricultural crop fields (specifically from tomato) in Ethiopia.

Based on the 28S rDNA partial sequences all the isolates studied fall under Clade I of the tropical RKN species except the single *M. hapla* isolate. Sequence analysis of the PCR products generated with the D2A and D3B primers showed the highest similarity between *M. incognita, M. javanica* and *M. arenaria*. Diagnostic resolution of D2-D3 expansion segments of 28S rDNA is insufficient to discriminate between some of the most closely related, problematic and economically damaging tropical RKN species (Naz *et al*., 2012; McClure *et*

al., 2012; Landa *et al*., 2008). The complete 28S rDNA dataset of our isolates only confirmed that this region could not be used for identification of tropical RKN species. For these reasons, SSP were tried and found well suited for the sampled areas in Ethiopia. A higher proportion of molecular identification of *M. incognita* and *M. javanica* from our PCR results over the other species of RKN were found indicating the higher prevalence of these species in the study localities. SSP are simple to perform and can successfully be used to any developmental stage of the nematode (Blok & Powers, 2009). Despite SCAR primers being simple to use, there are still some challenges associated with this method such as lower sensitivity or specificity of SPP, lack of reproducibility, the need to use large amount of DNA and appearance of weak bands (Adam *et al*., 2007; Blok & Powers, 2009; Onkendi *et al*., 2014). The recently developed method by Janssen *et al*. (2016) using Nad5 gene fragment reliably identified *M. incognita* and *M. javanica* isolates. However, as reported by Janssen *et al* (2016) the relatively uncommon, closely related linages of *Meloidogyne incognita* group (MIG) such as *M. ethiopica* and some populations of *M. arenaria* were found clustered in one Nad5 haplotype indicating lack of identification resolution for these species. Biochemical identification can successfully fill the deficiencies of DNA-based identification techniques and can be used as a confirmation tool when weak bands amplified using SSP. However, this method is female age dependent (egg laying young female) and sometimes it is difficult to determine and differentiate band sizes between different species (Onkendi *et al*., 2014). In this study, the esterase phenotype (EST) was more clearly amplified than the malate dehydrogenase (MDH) phenotype, which was almost always found with an additional unspecified double band. The EST phenotype (J3) for *M. javanica* populations and the EST phenotypes (I1) for *M. incognita* were diagnostic (Carneiro *et al*., 2000) and helped to confirm samples that showed weak amplifications with the SSP. The unspecified double band obtained after MDH showed that the gels were double stained for EST and MDH. The amount of extraction buffer loaded (0.6 µl) to the 8 well sample applicator might have been low given the fact that Karssen *et al*. (1995) used this volume for the smaller 12 well sample applicator. Nevertheless, there was no reproducibility problem, bands were observed and confirmed the weak bands obtained using SSP.

The higher prevalence and incidence (100%) of RKN in samples collected from Adami Tullu, Babile, Jittu, Tikur Wuha, Tepo Choronke, Zeway and Koka is probably due to the fact that these areas have long been known for their intensive tomato production. These areas also have a sandy soil which is known to be favourable for *Meloidogyne* species (Karssen *et*

al., 2013) as supported in the questionnaire. The high prevalence and incidence of RKN suggests their importance as a potential threat in tomato production (Naz *et al*., 2012). Several reasons could be mentioned for the high incidence of RKN in these areas: tomato monoculture, favourable climate (especially temperature), and use of susceptible rotation crops or intercropping with other *Solanaceous* crops.

The information obtained from growers indicated that most of the fields sampled had been under vegetable cultivation for several years. Localities such as Meki, Jeju and Nura Era are also known for their intensive tomato production along the Rift Valley and Upper Awash basin. However, RKN were not detected from these areas during the survey. These areas were subjected to natural flood for more than 3 months during 2011/12 cropping season. Fields with minimum infestation of RKN had silt loam and clay soil types as indicated by growers in the questionnaire survey and the climate in surrounding areas were dry and at times very hot. The lack of visible root damage symptoms by *Meloidogyne* spp. on a given plant is not necessarily an indication of its absence from the rhizosphere soil. The appearance of damage symptoms on an indicator tomato plant after the bioassay test performed on soil samples from which their respective root samples collected directly from field were not found infected proved this.

The highly damaging northern RKN, *M. hapla* was reported in Ethiopia in open tomato field at Zeway location. It is not clear if *M. hapla* is distributed in other tomato growing areas or if it is only restricted to Zeway. A systematic sampling approach, specifically in Zeway area, is needed to find this out. According to Whitehead (1969), *M. hapla* only flourishes at altitudes above 1829 m.a.s.l in East Africa (Kenya, Tanzania and Uganda), despite the abundance of host plants at lower altitudes. In this study, *M. hapla* is reported at a significantly lower altitude 1620 m.a.s.l indicating that *M. hapla* can flourish at lower altitudes too. There is no adequate information about its economic importance on tomato crops in Ethiopia. However, of concern is the fact that *M. hapla* was identified in a farm where it has the history of intensive vegetables production including tomato and pepper, thus this is likely to have considerable consequences for vegetables production at large and on tomato in particular. In 2014, *M. hapla* was reported in protected greenhouses in rose plantation in Ethiopia (Meressa *et al*., 2014). To our knowledge, this is the first report of *M. hapla* in farmer's open tomato production field in Ethiopia. The presence of this species in an Ethiopian protected rose plantation was suspected to be due to the introduction of infected planting materials imported

from abroad. However, its presence in an open tomato field suggests that this nematode may be indigenous in Ethiopia or was introduced in the past. Hence, this species should be given due attention given the location from which it was found is known for its intensive tomato production.

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Chapter 4: Resistance screening of breeding lines and commercial tomato cultivars for *Meloidogyne incognita* and *M. javanica* populations (Nematoda) from Ethiopia

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Awol Seid, Chemeda Fininsa, Tesfamariam Mekete, Wilfrida Decraemer, and Wim M. L. Wesemael, (2016). Resistance screening of breeding lines and commercial tomato cultivars for *Meloidogyne incognita* and *M. javanica* populations (Nematoda) from Ethiopia. *Under Review Euphytica.*

Abstract

Soil and root samples were collected from major tomato growing areas of Ethiopia during the 2012/2013 growing season to identify RKN problems. DNA-based and isozyme techniques revealed that *Meloidogyne incognita* and *M. javanica* were the predominant *Meloidogyne* species across the sampled areas. The aggressiveness of different populations of these species was assessed on tomato cultivars *Marmande* and *Moneymaker*. The two most aggressive populations of each species were selected and further tested on 33 tomato genotypes. The resistance screening and mechanism of resistance was performed after inoculation with 100 freshly hatched (<24 hr.) second-stage juveniles (J2). After eight weeks of inoculation, the number of egg masses produced on each cultivar was assessed. For the resistance mechanism study, J2 penetration and their subsequent development inside the tomato roots were examined at 1, 2, 4 and 6 weeks after inoculation. On both cultivars, *Marmande* and *Moneymake*r, all *M. incognita* and *M. javanica* populations formed a high number of egg masses indicating highly aggressive behaviour. Populations from 'Jittu' and 'Babile' for *M. incognita* and 'Jittu' and 'Koka' for *M. javanica* were selected as most aggressive. None of the 33 tomato genotypes were immune for these *M. incognita* and *M. javanica* populations. However, several tomato genotypes were found to have a significant effect ($p < 0.05$) on the number of egg masses produced indicating possible resistance. For *M. javanica* populations there were more plants from cultivars or breeding lines on which no egg masses were found compared to *M. incognita* populations. The lowest number of egg masses for both populations of *M. incognita* was produced on cultivars *Bridget40, Galilea,* and *Irma* while for *M. javanica* it was on *Assila, Eden, Galilea, Tisey, CLN-2366A, CLN-2366B* and *CLN-2366C*. Tomato genotypes, time (weeks after inoculation) and their interaction were significant sources of variation ($p < 0.05$) for J2 penetration and their subsequent development inside the tomato roots. Differential penetration was found in breeding lines such as *CLN-2366A, CLN-2366B* and *CLN-2366C*, but many of the selected tomato genotypes resistance for the tested *M. incognita* and *M. javanica* populations were expressed by delayed nematode development. Therefore, developing a simple screening technique to be used by local farmers or extension workers is crucial to facilitate selection of a suitable cultivar.

Key words: Aggressiveness, Breeding, Differential Penetration, Root-Knot Nematodes, Resistance, Screening

4.1. Introduction

In Ethiopia, tomato (*Solanum lycopersicum*) is a widely grown vegetable crop ranking 8th in terms of annual national production (Jiregna *et al*., 2013). However, its production is threatened by many insect pests and diseases, which are damaging both quality and quantity of tomato production. Among them, PPN represent an important constraint on the delivery of global food security (Jones *et al*., 2013). One of the major obstacles to the production of adequate supplies of food in many developing nations is damage caused by RKN *(Meloidogyne* spp.) (Sasser, 1980; Coyne *et al*., 2009). Particularly *Meloidogyne incognita* and *M. javanica* are economically important on tomato (Belan *et al*., 2009).

Owing to their polyphagous nature, management of RKN is a difficult task. Chemical nematicides, resistant varieties and rootstocks are commonly used to manage RKN (Devran *et al*., 2010). Environmental safety, high costs and problems regarding nematicide use forced many scientists to look for alternative methods for control (McSorley, 2011). Plant resistance is considered an alternative environment-friendly method to manage soil-borne pathogens including RKN. Cultivated tomato varieties are naturally susceptible to *Meloidogyne* spp. but some accessions of the related tomato species, *Solanum peruvianum* possess a single dominant gene (*Mi*) which confers resistance to *M. incognita*, *M. javanica* and *M. arenaria* (Roberts & Thomason 1986). This *Mi*-gene has been incorporated into many commercially available tomato cultivars (Devran *et al*., 2010) and is used against RKN for tomato plants grown in home gardens, fresh market, and processing tomato cultivars (Roberts & Thomason, 1989). For over 70 years, it has been the only source of resistance in all commercial tomato cultivars (Seid *et al*., 2015). It has been effective to manage RKN especially when used in combination with other management techniques such as rotation and sanitation (Roberts, 1992). Despite its effectiveness and profitability, the resistance conferred by this gene has some critical limitations. Planting a resistant crop for several consecutive years can increase the risk of selection of virulent nematode populations (Verdejo-Lucas *et al*., 2009). The *Mi* mediated resistance breaks down when the soil temperature is at and above 28 °C (Dropkin, 1969). Moreover, resistant tomato cultivars with the *Mi*-gene are not available for SSF and sub-tropical countries because of the high cost and due to temperature limitations and poor adaptation to local commercial production (Roberts *et al*., 1998). Its performance varies across different local climatic conditions. Searching for a resistance gene (s) from local cultivars that are adapted to the prevailing climatic conditions is therefore crucial. Hence, the objectives of this work were, to evaluate the host suitability of 23 commercial tomato

cultivars including the most common (such as *Chochoro*, *Marglobe*, *Melkasalsa*, *Melkashola* and *Fetane*) and 10 breeding lines to aggressive *M. incognita* and *M. javanica* populations from Ethiopia, and to determine root penetration rates and subsequent development of these populations on selected tomato cultivars and breeding lines to further understand the mechanisms of resistance.

4.2. Materials and Methods

4.2.1. Selection of Aggressive *M. incognita* and *M. javanica* Populations

During the 2012/2013 growing season, soil and root samples were collected from major tomato growing areas of Ethiopia. All of the populations collected were maintained on a susceptible tomato cultivar *Marmande* at HU greenhouse located at 'Raree' experimental station. *Meloidogyne* species were identified using DNA-based and isozyme based (esterase and malate dehydrogenase) techniques. *Meloidogyne incognita* and *M. javanica* were found to be the predominant RKN species across the sampled areas (see chapter 3). Consequently, the aggressiveness of 26 *M. incognita* and 25 *M. javanica* populations was assessed on two susceptible tomato cultivars *Marmande* and *Moneymaker*. The tomato seedlings of the susceptible cultivars were raised separately in a plastic tray (54 cm x 28 cm x 6 cm). A single seedling was transplanted at four-leaf stage into 200 cm^3 plastic cups (8 cm x 4 cm x 6.3 cm). Each plastic cup was inoculated with 200 infective second-stage juveniles (J2) (<24 h old) of all the *M. incognita* or *M. javanica* populations with five replications in a completely randomized design at HU 'Raree' greenhouse. Plants were watered daily as required using an atomiser. At eight weeks after inoculation, the cups with the plants were soaked in water and the soil was removed gently from the roots. The roots were then submerged in a Phloxin 'B' solution (0.15g/L tap water) for 15-20 min to stain the gelatinous matrix of the egg masses produced by the female RKN on the root surfaces (Daykin & Hussey, 1985). After 15-20 min of staining, the roots were rinsed with tap water to get rid of residual stain on the roots. The number of egg masses per plant was counted using a stereomicroscope. The two most aggressive populations from each *M. incognita* and *M. javanica* i.e. those with the highest mean number of egg masses on both susceptible cultivars, were selected and further used for resistance screening of locally available commercial tomato genotypes and breeding lines.

4.2.2. Resistance Screening of 33 Tomato Genotypes

4.2.2.1. Sources of tomato cultivars and breeding lines

The two most aggressive populations of *M. incognita* ('Jittu' and 'Babile') and *M. javanica* ('Jittu' and 'Koka') were used to screen 23 commercial tomato cultivars and 10 tomato breeding lines for resistance. The tomato cultivar *Marmande* was included as a susceptible

control. The list and sources of the tomato genotypes, growth habit and their pedigree are described in Table 4.1.

4.2.2.2. Experimental approach for the resistance screening

For each cultivar 30 plants were screened. Each plant was raised individually in 66 cm^3 volume plastic tubes (16 cm x 2.5 cm, low density with UV stabilizers, Stuewe & Sons, Inc. USA). The experimental set up was blocked per cultivar and per population assuming that the environmental factors are stable in a controlled growth chamber. Individual tubes were filled with sterilized (100 °C, 16h) sandy soil (74% sand, 17% sandy loam, 3% loam, 6% clay). In each tube, two seeds were sown; thinning was performed when both germinated to keep one seedling per tube. The tubes were kept in a growth chamber (18-27°C) with a daily 16 h light period. The temperature was recorded every hour using a data logger (Testo 175T2) throughout the experiment. At the four-leaf stage, each individual plant was inoculated with 100 J2 (<24 h old) of *M. incognita* 'Jittu' or 'Babile' populations and *M. javanica* 'Jittu' or 'Koka' populations. At eight weeks after inoculation, the number of egg masses produced per plant was counted using a stereomicroscope as described above. Designation of resistance was made based on the egg mass production on all the cultivars and breeding lines tested. '*S' (susceptible)* was used for plants on which mean egg mass production was more than 50% of the egg mass production on the known susceptible cultivar *Marmande.* '*SR*' (*slightly resistant*) was used for plants on which mean egg mass production was 25-50% compared to cv. *Marmande*; '*MR*' (*moderately resistant*) was used for plants when egg mass production was 10-25%; '*VR*' (*very resistant*) when egg mass production was 1-10%; '*HR*' (highly resistant) when egg mass production was <1%; and '*IM*' (*immune*) when nematodes entered the roots but did not reproduce or were killed (Hadisoeganda & Sasser, 1982). The degree-days (DD) for *M. incognita* and *M. javanica* populations to complete their life cycle were calculated using a base temperature of 10.1 °C and 13.1°C respectively (Ploeg & Maris, 1999). Then the base temperature of the respective species was subtracted from the hourly-recorded actual temperature. The daily average degree-days (DD) were calculated and later the daily average temperature was summed up for the entire eight weeks.

Table 4.1. List of tomato genotypes, their sources, growth habit and pedigree used for resistance screening against *Meloidogyne incognita* and *M. javanica* populations originating from Ethiopia.

Tomato cultivars/breeding lines[1]	**Sources**	**Growth Habit**	**Pedigree**
Metadel	MARC[2]	Indeterminate	Caraibo
Miya	MARC	Determinate	Floralou
Moneymaker	MARC	Indeterminate	Unknown
Chali	MARC	Determinate	Rio Grande
Marglobe	MARC	Indeterminate	Unknown
Chochoro	MARC	Determinate	Pacesetter
Melkashola	MARC	(Semi) determinate	Red Pear
Fetane	MARC	Determinate	Picador
Eshete	MARC	Indeterminate	Calypso
Bishola	MARC	Determinate	Floradade
Roma VF	MARC	Determinate	Unknown
ARP-Tomato d2	MARC	Determinate	Unknown
H-1350	MARC	Determinate	Unknown
Melkasalsa	MARC	Determinate	Serio
Marmande-Control	MARC	Determinate	Unknown
Virgilio F1 Hybrid	Clause	Determinate	Unknown
Irma	Israel	Determinate	Unknown
Bridget40	Israel	Determinate	Unknown
Galilea (***Mi***)	Israel	Semi-determinate	Unknown
Assila (***Mi***)	Seminis	Determinate	Unknown
Eden (***Mi***)	Seminis	Determinate	Unknown
Mersa	SARC[3]	Determinate	Unknown
Tisey (***Mi***)	Seminis	Determinate	Unknown
VL-642 (***TYLCV***)	Seminis	Determinate	Unknown
CL5915-D4-2-2-0 (***Tm2a***)	AVRDC[4]	Semi-determinate	[[(VC48-1 x Tamu Chico III) x (ahTm-2a x VC11-1UG)] x [ahTm-2a x (VC11-1-21B x Saturn)] x [[(TR/VC9-1)-4-2-5F5 x Plum)] x 71-483N]]

CL5915-206-D4-2-2 ***(Tm2a, Bwr-12)***	AVRDC	Indeterminate	[[(VC48-1 x Tamu Chico III) x (ahTm-2a x VC11-1UG)] x [ahTm-2a x (VC11-1-21B x Saturn)] x [[(TR/VC9-1)-4-2-5F5 x Plum)] x 71-483N]]
CLN-2366B ***(Beta, Tm2a)***	AVRDC	Indeterminate	[(Orion F1 x (CLN698BC1F1F2-358-4-13 x CLN1314B1F3-51-25-16-6-5)] x (UC204A x CLN1314B1F3-51-25-16-6-5B-8)
CLN-2037B ***(Ph-3, Tm2a)***	AVRDC	(Semi) determinate	CLN657BC1F2-285-0-21-0 x CLN1776BC1F1 (Moneymaker x L3708)
CLN-2037A ***(Ph-3, Tm2a)***	AVRDC	Indeterminate	CLN657BC1F2-285-0-21-0 x CLN1776BC1F1 (Moneymaker x L3708)
CLN-2037H ***(Ph-3, Tm2a)***	AVRDC	Indeterminate	CLN657BC1F2-285-0-21-0 x CLN1776BC1F1 (Moneymaker x L3708)
CLN-2366A ***(Beta, Tm-22)***	AVRDC	Indeterminate	[(Orion F1 x (CLN698BC1F1F2-358-4-13 x CLN1314B1F3-51-25-16-6-5)] x (UC204A x CLN1314B1F3-51-25-16-6-5B-8)
CLN-2366C ***(Beta, Tm-22)***	AVRDC	Indeterminate	[(Orion F1 x (CLN698BC1F1F2-358-4-13 x CLN1314B1F3-51-25-16-6-5)] x (UC204A x CLN1314B1F3-51-25-16-6-5B-8)
CLN-2037C ***(Ph-3, Tm2a)***	AVRDC	Indeterminate	CLN657BC1F2-285-0-21-0 x CLN1776BC1F1 (Moneymaker x L3708)
CL5915-206-D4 ***(Tm2a)***	AVRDC	Indeterminate	[[(VC48-1 x Tamu Chico III) x (ahTm-2a x VC11-1UG)] x [ahTm-2a x (VC11-1-21B x Saturn)] x [[(TR/VC9-1)-4-2-5F5 x Plum)] x 71-483N]]

[1]Cultivars with known resistance sources for a plant pathogen are mentioned within a bracket and with bold. Where *Mi*- resistant for root knot nematodes (*M. incognita*, *M. javanica* and *M. arenaria*), *Beta* – resistant for late and early blight of tomato, *Bwr-12*- resistant for bacterial wilt in tomato, *Tm2a*- resistant for tomato mosaic virus, *Ph-3*- resistant for late blight in tomato, *Tm-22* –Tomato mosaic virus and tobamoviruses, *TYLCV*- resistant to tomato yellow leaf curling viruses

[2]*Melkassa Agricultural Research Centre (MARC), Ethiopia*

[3]*Sirinka Agricultural Research Centre (SARC), Ethiopia*

[4]*Asian Vegetable Research Development Centre (AVRDC)*

4.2.3. Assessment of Resistance Mechanisms

Four commercial tomato cultivars and seven breeding lines were selected to monitor the penetration and development of the respective *M. incognita* 'Babile' and *M. javanica* 'Jittu' and 'Koka' populations to which they possessed potential resistance (Table 4.2). The criteria of selection was a combination of many factors such as the result of the screening experiment, availability of enough tomato seeds to initiate the experiment, availability of enough inoculum, farmers preference for specific cultivars and a future potential for genetic improvement (more breeding lines were checked over the commercial cultivars). *Meloidogyne incognita* 'Babile' population was used due to its virulence behaviour on tomato cultivars with the *Mi-gene* during the resistance screening work. Most of the breeding lines were found VR or HR for 'Koka' *M. javanica* population while 'Jittu' *M. javanica* population was included for comparison purposes. Breeding lines that only showed resistance for one population of the same species (*M. incognita* or *M. javanica*) were included to check for their possible difference between the two populations of the same species in penetration and development. Cultivar *Marmande* was used as a susceptible cultivar. For each cultivar/breeding line 40 plants were grown per population per species in plastic tubes as mentioned above. The experiment was blocked per cultivar/breeding line per population. When seedlings reached the four-leaf stage, each individual plant was inoculated with 100 freshly hatched J2 (<24 h old) of *M. incognita* or *M. javanica.* At 1, 2, 4 and 6 weeks after inoculation, 10 plants per cultivar/ breeding lines were taken to monitor the presence and the developmental stages of *M. incognita* and *M. javanica* inside the roots. Plants were submerged in water and the adhering soil was gently removed from the roots. The nematodes inside the roots were stained with acid fuchsin using the method described by Byrd *et al*. (1983) for the detection of nematodes inside plant tissues. After staining, root fragments were kept at 4 °C in glycerol in a small petri dish until counting. The stained nematodes were counted using a stereomicroscope. The nematodes were classified into: vermiform (J2), swollen (J2, J3 and J4) juveniles and adult females (young females included). The total number of nematodes/per plant was calculated to check if there was any emigration of J2 after penetration between the time periods checked.

4.2.4. Data Analysis

The screening data were analysed using Genstat 13th Edition, VSN International Ltd software 2010. Mean numbers of egg masses were statistically compared using Fisher's unprotected LSD at $P < 0.05$. Nematode penetration and their development assessment data were analysed using SAS 9.4 software and the penetration counts were Log 10 (x+1) transformed for

analysis to fulfil the criteria for normality. Mean separation for juvenile penetration and their subsequent development was done using Fisher's unprotected LSD at $P < 0.05$.

Table 4.2. The overview of tomato cultivars/ breeding lines used for the mechanism study against one *M. incognita* and two *M. javanica* populations.

Tomato cultivars or breeding lines	*M. incognita* 'Babile'	*M. javanica* 'Jittu'	*M. javanica* 'Koka'
Chochoro	✓	✓	
Roma VF	✓	✓	
Marglobe			✓
Melkashola			✓
CLN-2366A		✓	✓
CLN-2366B		✓	✓
CLN-2366C		✓	✓
CLN5915-206-D4-2-2		✓	✓
CLN-2037A		✓	✓
CLN-2037B		✓	✓
CLN-2037H		✓	✓
Marmande	✓	✓	✓

4.3. Results

4.3.1. Selection of Aggressive *M. incognita* and *M. javanica* Populations

All populations of *M. incognita* and *M. javanica* reproduced well on both susceptible cultivars. The mean minimum and maximum number of egg masses produced by *M. incognita* populations on the cultivars *Moneymaker* ranged from 66 to 148 and on *Marmande* from 67 to 168, respectively. The mean minimum and maximum number of egg masses produced by *M. javanica* population ranged from 67 to 132 on *Moneymaker* and from 79 to 158 on *Marmande*, respectively. In general, for all tested populations more egg masses were found on cv. *Marmande* but there was no significant difference with cv. *Moneymaker*. *Meloidogyne incognita* populations 'Babile' (BGS6) and 'Jittu' (JIT6) and *M. javanica* populations 'Jittu' (JIT7) and 'Koka' (KOKA8) were highly aggressive on both susceptible cultivars *Marmande* and *Moneymaker* as shown by the high number of egg masses formed

(Fig 4.1 & 4.2). Therefore, these populations were chosen as the most aggressive and maintained continuously on cv. *Marmande* for the subsequent screening work.

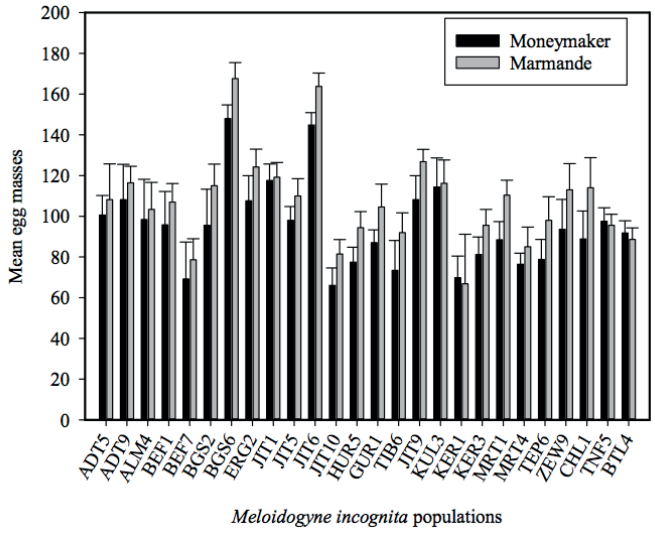

Figure 4.1. Mean number of egg masses of 26 *M. incognita* populations collected across the major tomato growing areas of Ethiopia on two susceptible tomato cultivars: '*Marmande*' and '*Moneymaker*' eight weeks after inoculation with 200 J2.

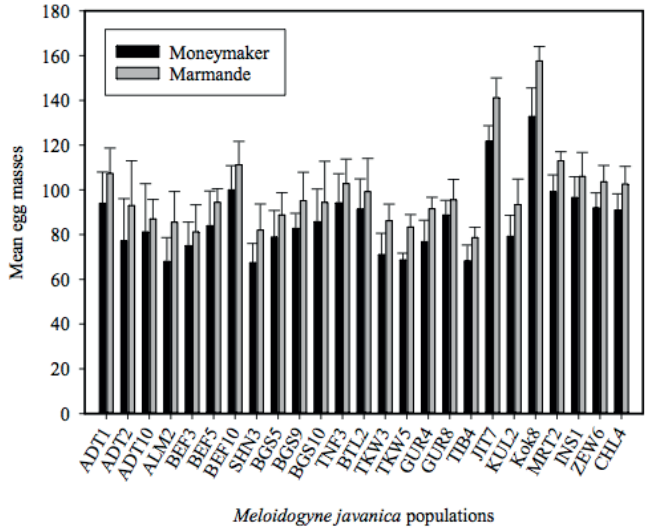

Figure 4.2. Mean number of egg masses of 25 *M. javanica* populations collected across the major tomato growing areas of Ethiopia on two susceptible tomato cultivars: '*Marmande*' and '*Moneymaker*' eight weeks after inoculation with 200 J2.

4.3.2. Resistance Screening of 33 Tomato Genotypes

The mean number of egg masses of 'Jittu' and 'Babile' *M. incognita* and 'Jittu' and 'Koka' *M. javanica* populations per plant eight weeks after inoculation with 100 J2 are shown in Table 4.3. None of the 33 tomato genotypes were immune for any of the *M. incognita* and *M. javanica* populations tested. However, several tomato genotypes had a significant effect ($P < 0.05$) on the number of egg masses produced. Four commercial tomato cultivars (*Metadel*, *Miya*, *H-1350* and *Melkasalsa*) and one breeding line (*CLN-2037C*) were found equally susceptible for the four populations tested. Among the tested tomato genotypes, *Tisey* was the only cultivar found resistant for the four populations tested.

Fifteen tomato genotypes were found susceptible, four moderately resistant (*Assila*, *Eden*, *Mersa* and *VL-642*) and three very resistant (*Bridget40*, *Galilea* and *Irma*) against 'Jittu' *M. incognita* population while 23 susceptible and two highly resistant (*Bridget40* and *Irma)* against 'Babile' *M. incognita* population. The 'Jittu' *M. incognita* population produced the highest number of egg masses (53) on the control *Marmande* and on the genotype *Miya* (47) while the lowest number (2) was found on the genotype *Galilea*. The 'Babile' *M. incognita* population produced the highest number of egg masses (43) on the clone *CL5915-206-D4* while the lowest number (1) was produced on the genotypes *Bridget40* and *Irma*. *Galilea* was found susceptible for 'Babile' *M. incognita* population.

Seventeen tomato genotypes were found susceptible, two moderately resistant (*Mersa* and *VL-642*), six very resistant (*CLN-2037B*, *CLN-2037H*, *CLN-2366A*, *CLN-2366B*, *CLN-2366C*, *CL5915-206-D4-2*) and five highly resistant (*Assila*, *CLN-2037A, Eden*, *Galilea* and *Tisey*) against 'Jittu' *M. javanica* population while thirteen susceptible, one moderately resistant (*Irma*), three very resistant (*Bridget40*, *Galilea*, and *Marglobe)* and eight highly resistant (*Assila, CLN-2366A*, *CLN-2366B*, *CLN-2366C*, *Eden, Melkashola, Tisey* and *VL-642*) against 'Koka' *M. javanica* population. The 'Jittu' *M. javanica* population produced the highest number of egg masses (53) on the genotype *Marglobe* while the lowest number (1) was found on the genotypes *Assila*, *Eden*, *Galilea*, *Tisey* and *CLN-2037A*. The 'Koka' *M. javanica* population produced the highest number of egg masses (45) on the clone *CLN-2037A* while the lowest number (1) was found on the genotypes *Assila*, *Eden*, *Vl-642, CLN-2366A*, *CLN-2366B* and *CLN-2366C*.

The highest percentages of plants without egg masses were found on very resistant and highly resistant tomato genotypes. The 'Koka' *M. javanica* population produced no egg

masses on 93% of the plants of the tomato cultivar *Assila* and 90% plants of *Eden*, *VL-642* and *CLN-2366B*. The 'Jittu' *M. javanica* population produced no egg masses on 70% plants of the tomato cultivar *Assila* and *Tisey* and 67% plants of *Eden* (Table 4.3). The maximum percentage of plants (17%) without egg masses by 'Jittu' *M. incognita* population was on *Galilea*. In general, for *M. javanica* populations there were more plants on which no egg masses were found compared to *M. incognita* populations. The DD for *M. javanica* and *M. incognita* during the screening experiment were 505.16 and 672.41, respectively.

Table 4.3. The mean number of egg masses per plant, percentage of plants without egg masses, and designation of resistance for *Meloidogyne incognita* 'Jittu' and 'Babile' and *M. javanica* 'Jittu' and 'Koka' populations on 33 tomato (*Solanum lycopersicum* L.) genotypes eight weeks after inoculation with 100 J2.

Genotypes	***Meloidogyne incognita* populations**						***Meloidogyne javanica* populations**					
	'Jittu'			**'Babile'**			**'Jittu'**			**'Koka'**		
	PP (%)[a]	**AEM[b]**	**DR[c]**	**PP**	**AEM**	**DR**	**PP**	**AEM**	**DR**	**PP**	**AEM**	**DR**
Metadel	0	31 ef	S	0	38 b	S	0	39 bc	S	7	29 d	S
Miya	0	47 b	S	0	16 klmno	S	0	25 fgh	S	0	22 fg	S
Moneymaker	0	47 b	S	0	23 ef	S	0	31 de	S	10	16 hi	SR
Chali	0	30 f	S	0	19 ghijk	S	0	17 jk	SR	0	14 ij	SR
Marglobe	0	43 bc	S	3	18 ijklm	S	0	53 a	S	70	3 kl	VR
Chochoro	3	23 ghij	SR	3	12 opq	SR	0	20 ij	S	0	18 h	S
Melkashola	0	27 fgh	S	0	15 lmnop	SR	0	23 hi	S	77	1 l	HR
Fetane	0	29 f	S	0	15 nop	SR	0	29 ef	S	0	13 ij	SR
Eshete	0	32 ef	S	0	12 pq	SR	0	28 efg	S	0	33 bc	S
Bishola	0	40 cd	S	0	15 mnop	SR	0	22 hi	S	0	27 de	S
Roma VF	0	20 ijk	SR	0	10 q	SR	0	14 k	SR	0	13 ij	SR
ARP-Tomato d2	0	35 de	S	0	20 efghij	S	0	20 ij	S	0	12 j	SR
H-1350	0	27 fg	S	0	19 hijk	S	0	26 fgh	S	0	30 cd	S
Melkasalsa	0	30 f	S	0	19 ijkl	S	0	32 de	S	0	43 a	S
Marmande (Control)	0	53 a	S	0	31 c	S	0	35 cd	S	0	35 b	S
Virgilio F1 Hybrid	0	38 d	S	0	32 c	S	0	25 gh	S	0	11 j	SR
Irma	7	4 pq	VR	60	1 r	HR	0	25 gh	S	13	6 k	MR
Bridget40	10	4 q	VR	67	1 r	HR	0	25 fgh	S	7	3 kl	VR
Galilea	17	2 q	VR	0	16 lmno	S	40	1 m	HR	13	3 kl	VR
Assila	0	9 nop	MR	0	23 efgh	S	70	1 m	HR	93	1 l	HR

Eden	0	7 opq	MR	0	20 efghij	S	67	1 m	HR	90	1 l	HR
Mersa	0	13 lmn	MR	0	19 fghij	S	7	4 lm	MR	10	12 j	SR
Tisey	0	14 lm	SR	0	11 jklmn	SR	70	1 m	HR	50	1 l	HR
VL-642	0	11 mno	MR	0	20 efghij	S	13	7 l	MR	90	1 l	HR
CL5915-D4-2-2-0	0	24 ghi	SR	0	23 efg	S	0	16 jk	SR	0	23 f	S
CL5915-206-D4-2-2	0	22 hij	SR	0	27 d	S	33	2 m	VR	0	24 ef	S
CLN-2366B	0	18 jkl	SR	0	22 efghi	S	23	3 m	VR	90	1 l	HR
CLN-2037B	0	21 ij	SR	0	22 efghi	S	27	2 m	VR	0	29 d	S
CLN-2037A	0	21 ij*	SR	0	32 c	S	57	1 m	HR	0	45 a	S
CLN-2037H	0	15 klm	SR	0	9 q	SR	37	2 m	VR	0	29 d	S
CLN-2366A	0	22 ij	SR	0	20 efghij	S	13	3 m	VR	67	1 l	HR
CLN-2366C	0	20 ijk	SR	0	23 de	S	27	2 m	VR	80	1 l	HR
CLN-2037C	0	37 d	S	0	36 b	S	0	39 b	S	0	19 gh	S
CL5915-206-D4	0	40 cd	S	0	43 a	S	0	41 b	S	0	14 ij	SR

[a] *PP is percentage of plants*

[b] *AEM is Average Egg Mass; Data are means of 30 replicates (untransformed data)*

[c] *DR is designation of resistance, S (Susceptible) egg mass production more than 50% of that on the susceptible cultivar 'Marmande'; SR (Slightly Resistant) with egg mass production 25-50%; MR (Moderately Resistant) when egg mass production 10-25%; VR (Very Resistant) when egg mass production 1-10%; HR (Highly Resistant) when egg mass production < 1%; and IM (immune) plants on which nematodes did not reproduce or were killed based on Hadisoeganda & Sasser (1982).*

[*] *Means in columns followed by a similar letter are not statistically different at 5% level by Fisher's unprotected least significance difference.*

4.3.3. Assessment of Resistance Mechanism

Tomato genotypes (cultivars and breeding lines) and time (weeks after inoculation) had a significant effect ($p < 0.05$) on J2 penetration and their subsequent development inside the root tissue. A significant interaction was found between the two factors for the detection of the majority of different developmental stages inside the root tissue for all the three populations tested. The mean numbers of vermiform juveniles (VJ), swollen juveniles (SJ) (J2, J3 & J4) and adult females (AF) (young females considered) of *M. incognita* 'Babile' and *M. javanica* 'Jittu' and 'Koka' populations that were detected in the roots of tomato cultivars and breeding lines at different weeks after inoculation are presented Fig 4.5 - 4.7.

4.3.3.1. *Meloidogyne incognita* 'Babile' population

The tomato genotypes and time had a significant effect on the numbers of VJ ($F_{genotypes} = 3.433$, $p = 0.036$; $F_{time} = 815.598$, $p < 0.01$), SJ ($F_{genotypes} = 36.617$, $p < 0.01$; $F_{time} = 141.399$, $p < 0.01$) and AF ($F_{genotypes} = 39.395$, $p < 0.01$; $F_{time} = 549.368$, $p < 0.01$) of *M. incognita* 'Babile' population detected inside the plant roots. The interaction between both was significant for SJ ($F = 6.810$, $p < 0.01$) and AF ($F = 7.395$, $p < 0.01$). The J2 of *M. incognita* 'Babile' population penetrated, and further developed into egg laying adult females on the two genotypes tested (Fig 4.5).

4.3.3.2. *Meloidogyne javanica* 'Jittu' and 'Koka' populations

The tomato genotypes and time had a significant effect on the numbers of VJ ($F_{genotypes} = 37.896$, $p < 0.01$; $F_{time} = 312.933$, $p < 0.01$), SJ ($F_{genotypes} = 65.095$, $p < 0.01$; ($F_{time} = 77.962$, $p < 0.01$) and AF ($F_{genotypes} = 109.018$, $p < 0.01$; $F_{time} = 364.193$, $p < 0.01$) of *M. javanica* 'Jittu' population detected inside the plant roots. The interaction between both was significant for VJ ($F = 21.621$, $p < 0.01$); SJ ($F = 4.856$, $p < 0.01$) and AF ($F = 15.169$, $p < 0.01$). Similarly, tomato genotypes and time had a significant effect on the numbers of VJ ($F_{genotypes} = 52.058$, $p < 0.01$; $F_{time} = 812.262$, $p < 0.01$), SJ ($F_{genotypes} = 62.293$, $p < 0.01$; ($F_{time} = 45.356$, $p < 0.01$) and AF ($F_{genotypes} = 201.619$, $p < 0.01$; $F_{time} = 849.461$, $p < 0.01$) of *M. javanica* 'Koka' population detected inside the plant roots. The interaction between both was significant for VJ ($F = 34.844$, $p < 0.01$); SJ ($F = 9.345$, $p < 0.01$) and AF ($F = 27.788$, $p < 0.01$). The tomato breeding lines *CLN-2366A*, *CLN-2366B* and *CLN-2366C* were effective in reducing J2 penetration rate and their subsequent development for both *M. javanica* 'Jittu' and 'Koka' populations as compared to the cv. *Marmande* (Fig 4.3A & Fig 4.4).

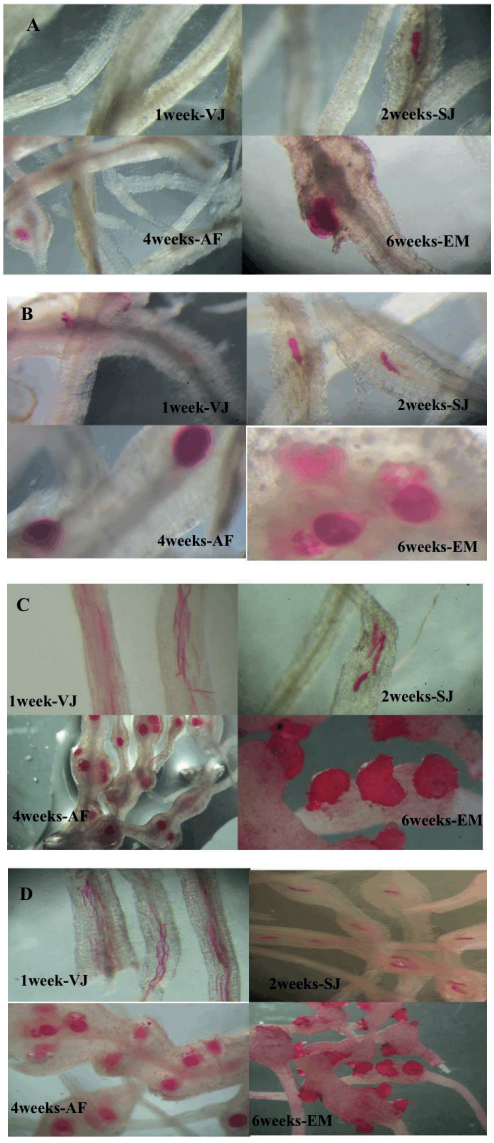

Figure 4.3. A comparison of penetration and developmental stages of *Meloidogyne incognita* and *M. javanica* populations detected inside the tomato roots after 1, 2, 4 and 6 weeks of inoculation with 100J2 in a growth chamber experiments. A) *CLN-2366A*, *CLN-2366B* & *CLN-2366C* breeding lines after inoculation with *M. javanica* 'Jittu' and 'Koka' populations; B) the cv. '*Melkashola*' after inoculation with *M. javanica* 'Koka' population; C) the cv. '*Chochoro*' after inoculation with *M. incognita* 'Babile' population; and D) the cv. '*Marmande*' (susceptible control) after inoculation with *M. javanica* 'Jittu' and 'Koka' and *M. incognita* 'Babile' population showed similar reactions.

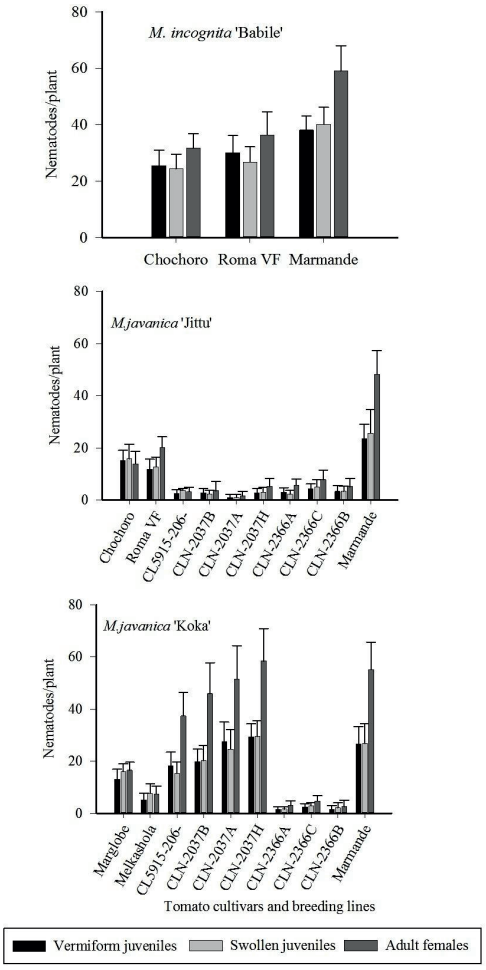

Figure 4.4. Mean numbers of total nematodes/ plant of different developmental stages (vermiform juveniles, swollen juveniles and adult females) of *M. incognita* 'Babile' and *M. javanica* 'Jittu' and 'Koka' populations inside roots of selected tomato genotypes. The bars on the graphs indicate the standard deviation of the mean.

In general, at 4 and 6 weeks after inoculation there were no VJ detected in none of the tomato genotypes studied against *M. incognita* 'Babile' population and 'Jittu' and 'Koka' *M. javanica* populations (Fig 4.5-4.7). At 1 week after inoculation, females from these tested populations were not detected in all genotypes including on the cv. *Marmande*. At 2 weeks after inoculation, a few females were able to develop on a few genotypes (Fig 4.5 - 4.7). Swollen juveniles were found at all times 1, 2, 4 and 6 weeks after inoculation though on the first week it was only detected in few genotypes (Fig 4.6 & Fig 4.7).

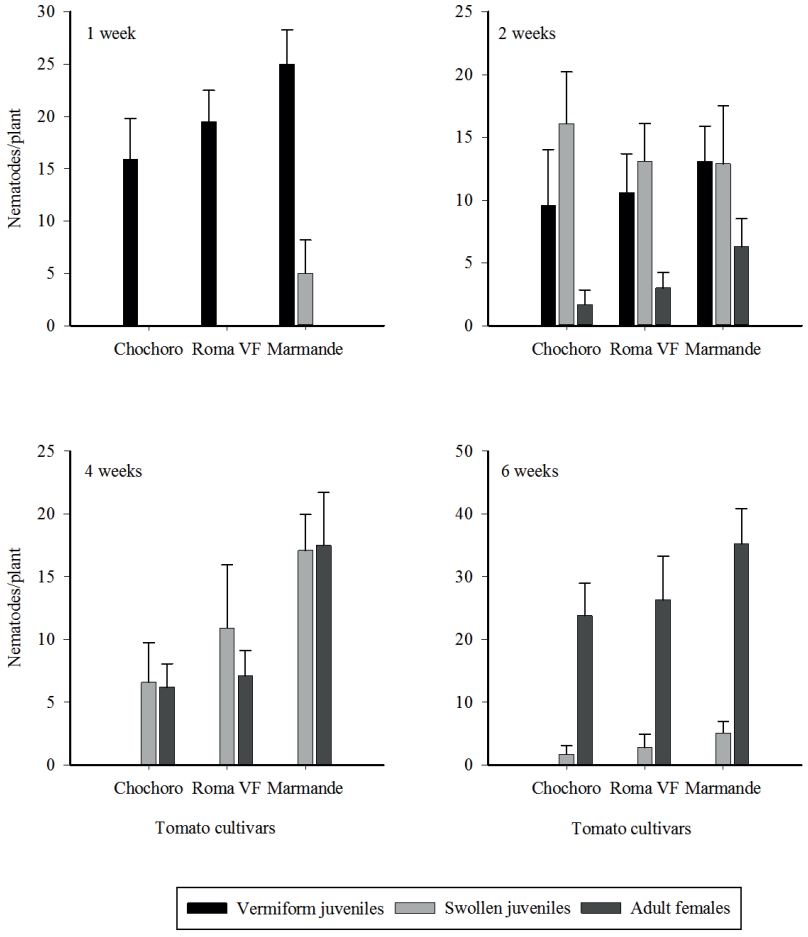

Figure 4.5. Mean number (nematodes/plant) of different developmental stages of *M. incognita* 'Babile' population inside roots of three tomato cultivars at different times after inoculation (n = 10).

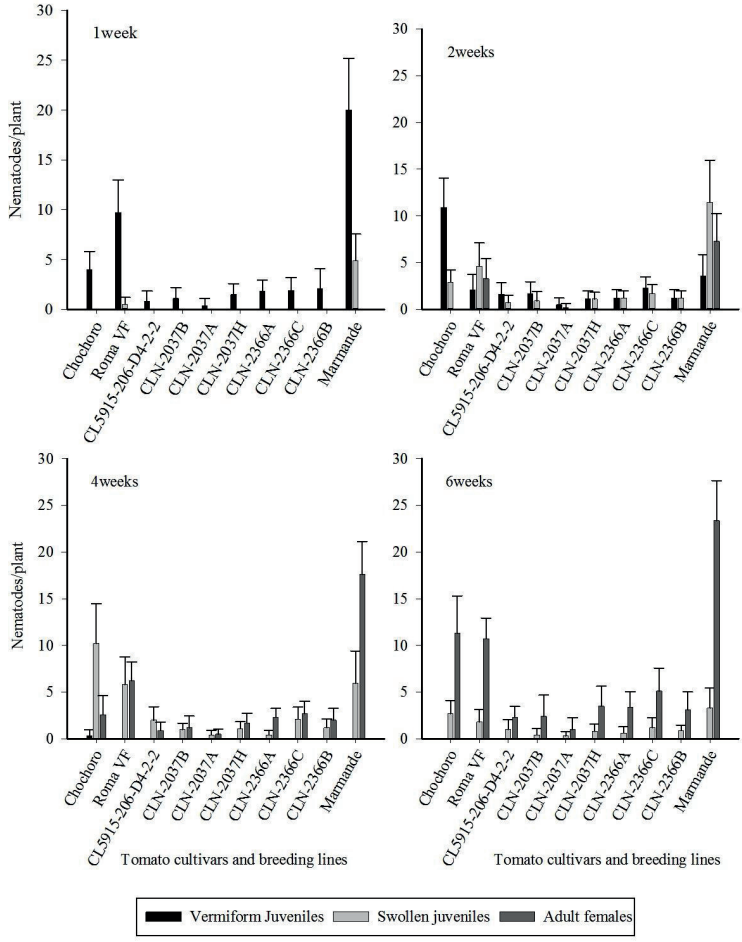

Figure 4.6. Mean number (nematodes/plant) of developmental stages of *M. javanica* 'Jittu' population inside roots of ten tomato cultivars and breeding lines at different times after inoculation (n = 10).

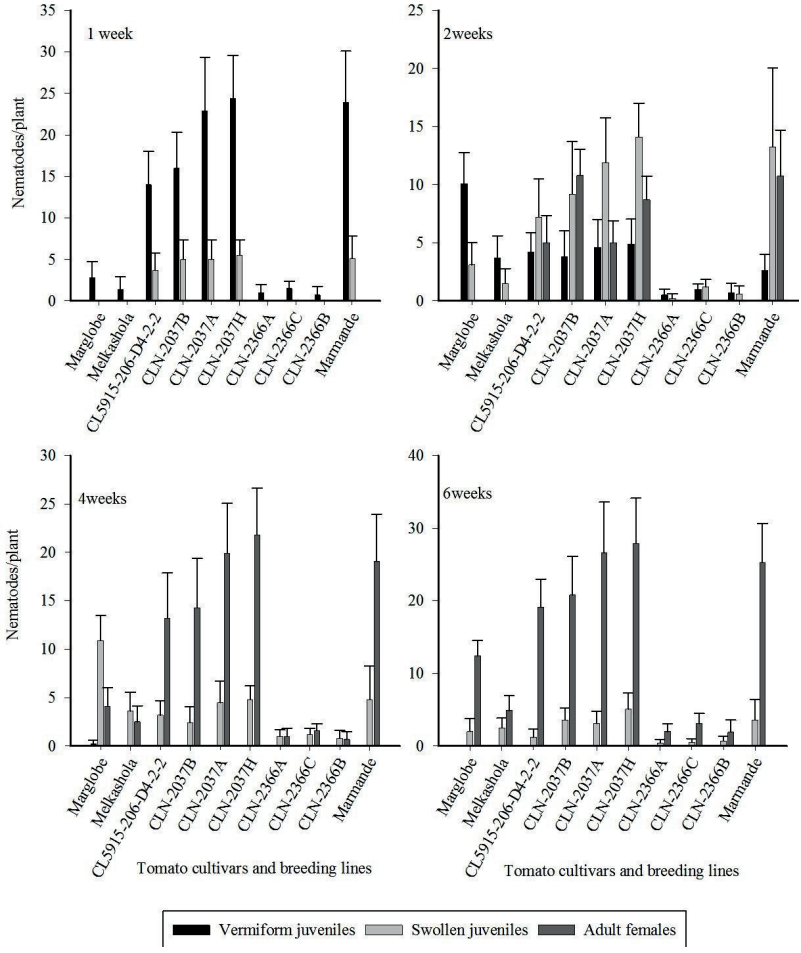

Figure 4.7. Mean number (nematodes/plant) of developmental stages of *M. javanica* 'Koka' population inside roots of ten tomato cultivars and breeding lines at different times after inoculation (n = 10).

4.4. Discussion

In this study, the host suitability and mechanism of resistance of 33 commercial tomato cultivars and breeding lines against Ethiopian local aggressive RKN populations were examined. Several of the tested tomato genotypes had varying degrees of resistance against the selected populations of *M. incognita* and *M. javanica*. *Tisey* (with *Mi*-gene) has shown a spectrum of resistance against the four populations tested, which indicates the performance of the contained resistance genes. However, in all the other tomato genotypes a variation for

resistance was found against the different populations of the same species that may emphasis the importance of population based nematode management strategies. The tomato cultivars (*Metadel*, *Miya*, *H-1350* and *Melkasalsa*) frequently used by SSF of Ethiopia were found equally susceptible for the four tested populations. Care should be given not to grow these cultivars in nematode infested soils. Interestingly, *CLN-2037C* was also found equally susceptible for the four populations tested while other breeding lines with the same pedigree (*CLN-2037H* and *CLN-2037B*) were found having different levels of resistance. The tomato cultivars that are commonly grown by SSF of Ethiopia (mainly in the Rift Valley and Upper Awash areas) such as *Marglobe* and *Melkashola* were found susceptible for the two populations of *M. incognita* and 'Jittu' *M. javanica* population but these cultivars were found very resistant and highly resistant for 'Koka' *M. javanica* respectively. This proves the need to consider population based differences while recommending resistance cultivars as a management tool. The tomato cultivars and breeding lines such as *Assila* (with *Mi*-gene), *CLN-2366A*, *CLN-2366B*, *CLN-2366C*, *Eden* (with *Mi*-gene), *Mersa*, *VL-642* (with *TYLCV* resistance gene with some degree of nematode resistance) and *Galilea* (with *Mi*-gene) were found resistant (though with different levels) for 'Jittu' *M. incognita* and 'Jittu' and 'Koka' *M. javanica* populations but surprisingly they all were found susceptible for 'Babile' *M. incognita* population. This may indicate that 'Babile' *M. incognita* population might have been dominated by virulent individuals which were able to overcome or break the contained resistance genes. The cultivars *Bridget40* and *Irma* showed resistance against both populations of *M. incognita* and 'Koka' *M. javanica* population but both were found susceptible for 'Jittu' *M. javanica* population. The cultivar '*Virgilio F1 Hybrid'* that was reported to be resistant for several isolates of *M. incognita*, *M. javanica*, *M. arenaria* and some unidentified isolates of *Meloidogyne* species from Uganda (Mwesige, 2013) showed susceptible reaction for the tested four populations of *M. incognita* and *M. javanica* from Ethiopia. Thus, searching for a possible resistance in tomato cultivars or breeding lines should therefore be against population levels rather than at species level. The current study is in agreement with earlier studies conducted on host suitability of tomato genotypes for *M. incognita* and *M. javanica* (Hadisoeganda & Sasser, 1982; Singh & Khurma, 2007; Khah *et al*., 2011; Kesba *et al*., 2015).

Due to the polyphagous nature of *Meloidogyne* species, decisions on the choice of crops for crop rotations in infested fields are difficult and have to be taken carefully (Wesemael *et al*., 2011). This screening experiment showed that the tested genotypes of tomato were poor to

very good hosts to both *M. incognita* and *M. javanica* populations used. The information generated is important in designing crop rotation schemes and cropping systems to avoid yield losses due to nematode infested soil.

The presence of egg masses on all tomato genotypes indicates that none of them is immune for the tested populations of *M. incognita* and *M. javanica*. Nevertheless, significant differences in the number of egg masses produced on the tomato genotypes indicate different degrees of susceptibility. The degree of susceptibility is controlled by the presence of resistance genes such as *Mi* gene and genetic background of the tomato genotypes (Castagnone-Sereno, 2006). The heterozygous or homozygous state of the *Mi* locus has been reported to affect the degree of resistance to *Meloidogyne* species with the genotypes having the heterozygous form of the *Mi* gene being more susceptible over homozygous genotypes (Seid *et al*., 2015; Kesba *et al*., 2015). The variation in the degree of susceptibility in this study to the tested RKN populations in all the 33 tomato genotypes is likely to be due to the genetic differences between the genotypes (Singh & Khurma, 2007; Kesba *et al*., 2015). Knowing the genetic difference present in the genotypes will have important implications on the yield and economic returns and thereby to farmers while selecting the tomato varieties for planting on *Meloidogyne spp*. infested fields. As observed in some genotypes used in this study and supported by other researchers, several plants may have numerous galls (*Bridget40* and *Irma*) caused by RKN with little or no reproduction (could be used as a trap crop) and much reproduction may occur on some plants with few or no galls (*Chali* and *Bishola*)(data not shown) (Fassuliotis, 1979; Cook & Evans, 1987).

In the resistance screening experiment, variation in resistance was found between different commercial tomato cultivars and breeding lines even between different populations of the same species. There were tomato cultivars or breeding lines with a high percentage of plants on which no egg masses were produced. The absence of egg masses does not necessarily imply J2 penetration failure J2 may enter the plant root but fail to develop into adult females or after penetration they may be killed by hypersensitive reaction or they may emigrate out of the root to search for a new host (Silva *et al*., 2013). In order to elucidate this more, the resistance screening study was followed by resistance mechanism study. Moreover, knowing the proper mechanism of resistance of cultivars or breeding lines will also help in designing appropriate management strategies. Mechanisms for nematode resistance in plants are of two types, pre-infection resistance, in which the nematodes cannot enter the plant roots possibly

due to the presence of pre-formed chemicals such as toxic or antagonistic to nematodes (Bendezu & Starr, 2003) and post-infection resistance, which is manifested after nematodes penetrated roots due to failed development (Anwar & Mckenry, 2000) and the later being the most common type (Fassuliotis, 1979). The applicability of statistical significance values in practical terms is limited when we use the ANOVA analysis result, however, this method detects small difference in egg mass production between tomato genotypes. Resistance designation using scales or ranges is more applicable but it is not robust enough to take into consideration the small differences in egg masses production.

From the two (tomato genotypes and time) main effects, time had the highest significant effect on the number of VJ, SJ and AF detected inside the tomato roots and this information will have a practical management significance. Their interaction effect was also found significant but it was less important compared to the main effects. In our study differential penetration was found on breeding lines such as *CLN-2366A, CLN-2366B* and *CLN-2366C* as compared to the control *Marmande*. Many of the selected tomato genotypes resistance against the *M. incognita* and *M. javanica* population used was expressed by delayed nematode development rather than differential penetration such as in *Marglobe* and *Melkashola* against the *M. javanica* 'Koka' population. Similar penetration of resistant and susceptible cultivars by infective J2 of *Meloidogyne spp*. has also been found in cucumber (Walters *et al*., 2006), coffee (Silva *et al*., 2013), common bean (Sydenham *et al*., 1996; Wesemael & Moens, 2012), alfalfa (Reynolds *et al*., 1970), maize (Windham & Williams, 1994), cotton (Creech *et al*., 1995), tobacco (Schneider, 1991), tomato (Hadisoeganda & Sasser, 1982), and soybean (Herman *et al*., 1991).

The most common and preferred tomato commercial cultivar *Chochoro* by the SSF of Ethiopia was found susceptible for both *M. javanica* populations but slightly resistant for both *M. incognita* populations. Both *M. incognita* and *M. javanica* multiplied on this cultivar though with different degrees and therefore appropriate crop rotation schemes need to be designed to minimize losses for the subsequent crops. The tomato breeding lines *CLN-2366A, CLN-2366B* and *CLN-2366C* were found highly resistant. Further breeding efforts (preferably used as a rootstock) to improve these breeding lines will allow their use in RKN infested soil, particularly in areas where *M. javanica* populations are prevalent such as 'Babile locations' in eastern Hararghe districts. Simple screening techniques that suit SSF are vital. Planting different tomato cultivars on selected nematode hot spot areas to serve as demonstration plots

so that farmers can easily compare the performance of different tomato cultivars in their local settings is recommended. For the future work on assessment of crop resistance against RKN populations, the inclusion of number of eggs produced per eggmass as one parameter on top of number of egg masses produced per plant will have a practical significance and a reliable prediction of the nematode populations present in the soil at the end of a cropping season and thereby in designing crop rotation schemes. There should also be an effort to study the genetic background of the crop under consideration for resistance screening.

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Chapter 5: Heat stability of resistance in selected tomato breeding lines against *Meloidogyne incognita* and *Meloidogyne javanica* populations under elevated soil temperatures

Awol Seid, Chemeda Fininsa, Tesfamariam Mekete, Wilfrida Decraemer, And Wim M. L. Wesemael, (2016). Heat stability of resistance in selected tomato breeding lines against *Meloidogyne incognita* and *M. javanica* populations under elevated soil temperatures. Prepared to be submitted for the *Russian Journal of Nematology*.

Abstract

In tomato, the only commercially available source of resistance to RKN is the *Mi-1* gene that confers resistance to *Meloidogyne incognita*, *M. javanica*, and *M. arenaria*. However, its effectiveness was found limited in higher soil temperatures. A study was initiated with the objective to check the durability of the potential resistance genes found in some tomato breeding lines after screening in controlled greenhouse conditions ≤ 27°C by exposing them into higher soil temperatures at 28, 32 and 36°C for 24 and 48hrs period. The aggressive Jittu and Babile *M. incognita* and Jittu and Koka *M. javanica* populations originally collected from Ethiopia were used. When seedlings reached the four-leaf stage, each tube was inoculated with 50 freshly (≤ 24hrs) hatched infective second-stage juveniles (J2). Immediately after inoculation, the seedlings were exposed continuously for 24 and 48hrs in a warm water bath at 28, 32 and 36°C respectively. A control was kept separately in ambient temperature (24°C ± 2°C). The external ambient temperature and the soil temperature inside the tube while in the water bath were simultaneously recorded using a TESTO data logger. Temperature, tomato breeding lines and time had a significant ($P < 0.01$) effect on the number of J2 of *M. incognita* Jittu and Babile and Jittu and Koka *M. javanica* populations penetrated the plant roots. The utility of the potential resistance found in our breeding lines during the controlled growth chamber resistance screening experiment was found limited at higher soil temperatures especially at 32 and 36°C. At 36°C there was no significant difference found on the mean number of penetrated J2 of Jittu and Babile *M. incognita* and Jittu and Koka *M. javanica* populations inside the roots of all the tested breeding lines compared to *Marmande* after 48hrs of heat exposure after inoculation. More J2 were found in the roots of the tested breeding lines after 48hrs compared with 24hrs heat exposure after inoculation for each soil temperature level tested and for both populations of *M. incognita*. It is clear from our observations that local tomato breeding lines with resistance potential can be used when soil temperatures remain below 32°C. Differences were observed between breeding lines depending on the RKN population at higher temperatures and this knowledge can help in further optimizing the development of sustainable resistance under local Ethiopian circumstances.

Key words: resistance, breeding lines, *Mi-1*, heat stability, soil temperature, *and Meloidogyne* species

5.1. Introduction

Tomato (*Solanum lycopersicum*) is among the most valuable agricultural crops worldwide (Carvalho *et al*., 2015). A considerable portion of the tomato production takes place in warm and hot climates (Verdejo-Lucas *et al*., 2013) where RKN are important endoparasitic pests (Ammiraju *et al*., 2003) and its infestation causes serious crop losses (Carvalho *et al*., 2015). Tomato yield reduction as severe as 100% have been reported (Seid *et al*., 2015) (see chapter 2).

Management of RKN is a challenging task due to its wider host range which hinders the practice of crop rotation (Chen *et al*., 2006). Soil fumigants, systemic and contact nematicides, resistant rootstocks, resistant cultivars and cultural practices are commonly employed to control RKN (Devran *et al*., 2010, Seid *et al*. 2015). After the withdrawal of the effective and widely used soil fumigant methyl bromide from the market due to its negative effect on stratospheric ozone (Rosskopf *et al*., 2005) and risks for non-target organisms (Ploeg, 2002; Devran *et al*., 2010), HPR appeared as a powerful and sustainable tool for crop protection including the management of RKN (Devran *et al*., 2010).

In tomato, the single dominant gene *Mi-1* confers resistance but not immunity to the three most damaging species: *Meloidogyne incognita*, *M. javanica*, and *M. arenaria* (Milligan *et al*., 1998) with *M. incognita* and *M. javanica* reported in the Ethiopian agriculture (Mandefro & Mekete, 2002). This gene has been the only commercially available source of resistance to RKN for the last 70 years globally (Seid *et al*., 2015). *Mi-1* gene was found in *Solanum peruvianum* and interogressed into *S. lycopersicum* (Rodriguez, 2013). The effectiveness of the *Mi-1* gene varies with RKN species and population, tomato cultivar, and environmental conditions, particularly soil temperature (Devran *et al*., 2010; Verdejo-Lucas *et al*., 2013; Seid *et al*., 2015). Even though there is inconsistency in literature, increased gall formation has been reported in plants exposed to soil temperatures above 28°C (Haroon *et al*., 1993; Wang *et al*., 2009; Devran *et al*., 2010; Verdejo-Lucas *et al*., 2013). The *Mi-1* resistance was lost after 4 days at ≥ 33°C in 1 to 3 days old seedlings exposed to heat treatment after inoculation with *M. incognita* and subsequently held at 27°C for 1 month (Dropkin, 1969). Climatic heterogeneity is a general characteristics of Ethiopia and in areas where tomato is largely produced soil temperature at times rises above 28°C (Alemayehu, 2002). Under field conditions, it is possible that plants are subjected to environmental and daily soil temperature fluctuations (Verdejo-Lucas *et al*., 2013). Consequently, the expression of the resistance

phenotype may or may not be similar to that observed under constant (generally < 28 °C) temperature conditions. The incorporation of heat-stable resistance to *Meloidogyne* spp. would be a valuable genetic improvement in tomato. Thus, checking the heat stability of the potential resistance gene in higher soil temperatures after screening tomatoes against RKN populations in a controlled environment is crucial. Therefore, the objective of this study was to check the durability of the potential resistance genes found in some tomato breeding lines after screening in controlled greenhouse conditions ≤ 27°C by exposing them to higher soil temperatures at 28, 32 and 36 °C for 24 and 48 hrs period.

5.2. Materials and Methods

5.2.1. Tomato Breeding Lines

Based on the results of the screening experiment (see chapter 4) and the availability of enough seeds to initiate the experiment, seven tomato breeding lines (*CLN-2366A, CLN-2366B, CLN-2366C, CLN-2037H, CLN-2037A, CLN-2037B* and *CL5915-206-D4-2-2*) obtained from AVRDC were selected to check the heat stability of their potential resistance at 28, 32 and 36°C soil temperatures. The tomato cultivar *Marmande* was used as a susceptible control.

5.2.2. *Meloidogyne incognita* and *Meloidogyne javanica* Culture

The aggressive Jittu and Babile *M. incognita* and Jittu and Koka *M. javanica* populations originally collected from Ethiopia (see chapter 4) were used. The culture of these populations was maintained on tomato cv. *Marmande* in the glasshouses at ILVO. Inoculum was prepared from the infected tomato roots. Therefore, roots were carefully washed and cut into smaller pieces and placed on a Baermann funnel in a mistifier. Freshly hatched (≤ 24hrs) infective J2 were collected and used as inoculum.

5.2.3. Heat Stability Experiment

A plastic tube (15 ml volume, 95mm height, 15mm diameter) was filled with sterilized (100°C, 16hrs) soil (74% sand, 14% sandy loam, 6% clay, 5% loam, 1% organic matter content and a neutral pH). A single seed was allowed to grow per tube. Plants were watered daily and maintained at 23°C ± 2°C (11hrs day), 20°C ± 1°C (13hr night), with 60 to 65% relative humidity for three consecutive weeks in a growth chamber at ILVO. When the seedlings reached the four-leaf stage, they were brought to the laboratory and kept in the ambient temperature overnight. Then, each tube was inoculated with 50 freshly (≤ 24hrs) hatched infective J2. Immediately after inoculation, the seedlings were exposed continuously for 24hrs and 48hrs in a warm water bath at 28, 32 and 36°C, respectively. A control was

kept separately in ambient temperature (24°C ± 2°C). The external ambient temperature and the soil temperature inside the tube while in the water bath were simultaneously recorded using a data logger (Testo 175T2) (Fig 5.1). After the exposure period, the seedlings were returned to the growth chamber. The experiment was arranged in a completely randomized design with eight replications. Eight days after inoculation, the nematodes inside the root were stained with acid fuchsin using the method described by Byrd *et al*. (1983).

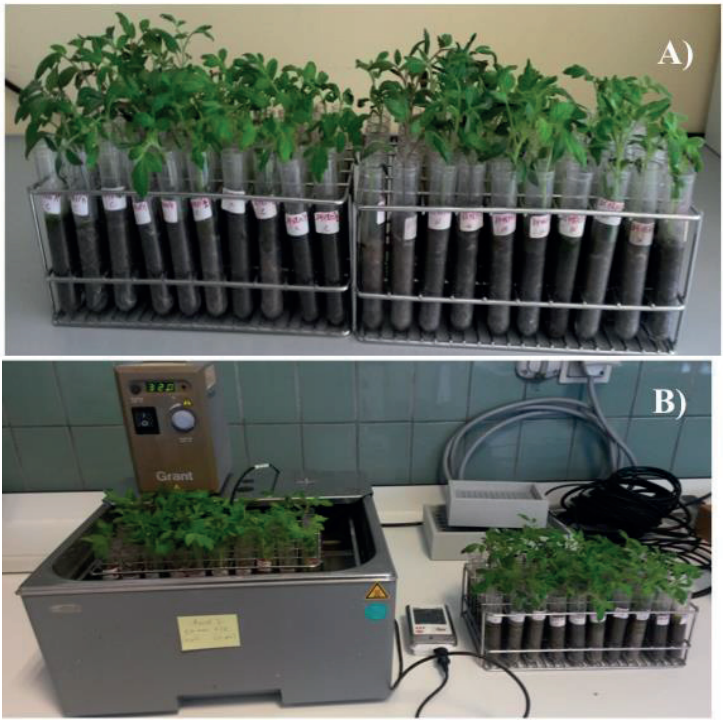

Figure 5.1. A) Plastic tubes with tomato seedlings; B) the experimental set up for the heat stability test in a awarm water bath with simultanously recording of the soil temperatures using adata longer (TESTO).

5.2.4. Data Analysis

The heat stability data were analysed using Statistica Version7 Software. Mean numbers of J2 that penetrated the tomato roots after being exposed to different soil temperature regimes (25, 28, 32 and 36°C) and time (24 and 48hrs) was subjected to factorial ANOVA and statistically compared using Fisher's unprotected LSD at $P < 0.05$. One-way ANOVA was used to analyse the penetration of J2 in the roots of each clone separately for each temperature after inoculation followed by a post-hoc test using Tukey HSD at $P < 0.05$. J2

penetration counts were Log 10 (x+1) transformed for analysis to fulfil the criteria for normality. All graphs were drawn using Sigmaplot13 statistical software.

5.3. Results

5.3.1. Jittu and Babile *M. incognita* Populations

5.3.1.1. The effect of temperature, breeding lines and time

Temperature, tomato breeding lines and time had a significant effect on the numbers of J2 ($F_{\text{temperature}} = 118.06$, $p < 0.01$; $F_{\text{breeding lines}} = 41.30$, $p < 0.01$ and $F_{\text{time}} = 23.01$, $p < 0.01$) of *M. incognita* Jittu population that penetrated the plant roots. The interaction between breeding lines*temperature, temperature*time and breeding lines*temperature* time was significant for *M. incognita* Jittu J2 penetration ($F = 2.26$, $p < 0.01$; $F = 5.96$, $p < 0.01$ and $F = 1.63$, $p < 0.01$ respectively) but the effect was less evident compared to the main effects. Similarly, temperature, breeding lines and time had a significant effect on the numbers of J2 ($F_{\text{temperature}} = 134.35$, $p < 0.01$; $F_{\text{breeding lines}} = 47.17$, $p < 0.01$ and $F_{\text{time}} = 26.44$, $p < 0.01$) of *M. incognita* Babile population that penetrated the plant roots. The interaction between breeding lines *temperature, breeding lines*time, temperature*time and breeding lines*temperature*time was significant for *M. incognita* Babile population J2 penetration ($F = 4.12$, $p < 0.01$; F= 2.05, $p < 0.05$; $F = 8.75$, $p < 0.01$ and $F = 1.92$, $p < 0.01$, respectively) but the effect was again less compared to the main effects.

5.3.1.2. The effect of temperature levels

After 24hrs heat exposure to 28°C, the number of J2 of *M. incognita* Babile population inside the roots of *CLN-2366C* and *CLN-2366A* were significantly higher compared to the control (25°C). The number of J2 of this population inside the roots of *CLN-2366C*, *CLN-2366A*, *CLN-2037A*, *CLN-2037B* and *CL5915-206-D4-2-2* were significantly higher after 48hrs exposure to 28°C compared to 25°C. For the Jittu *M. incognita* population, the number of J2 detected in the roots of *CLN-2366B*, *CLN-2366A* and *CLN-2037H* after 24hrs exposure to 28°C were significantly higher compared to 25°C. The number of J2 found inside the roots of all the tested breeding lines after 48hrs exposure to 28°C were significantly higher compared to 25°C for Jittu *M. incognita* population.

The number of J2 for Babile *M. incognita* population detected inside the roots of all the tested tomato breeding lines was considerably higher after 24 and 48hrs exposure to 32°C compared to 25°C (Fig 5.3.). Remarkably higher number of J2 were found inside the roots of all the

tested breeding lines after inoculation with Jittu *M. incognita* population and 24hrs exposure to 32°C compared to 25°C with the exception of *CLN-2037A*. A significantly higher number of J2 entered the roots of all the tested breeding lines for *M. incognita* Jittu population after 48hrs heat exposure to 32°C compared to 25°C (Fig 5.2). A higher number of J2 was found in roots of all the tested breeding lines for both *M. incognita* populations after 24 and 48hrs exposure to 36°C compared to 25°C. Except on *CLN-2366C,* significantly higher number of J2 were entered in the roots of all the tested breeding lines after inoculation with Babile *M. incognita* population and exposure to 32°C compared with the exposure to 28°C. For the Babile *M. incognita* population, the number of J2 in the roots of *CLN-2366B*, *CLN-2366A*, *CLN-2037H* and *CLN-2037A* after 48hrs exposure to 32°C were significantly higher compared to the exposure at 28°C.

After 24hrs exposure to 36°C, the number of J2 for *M. incognita* Babile population in the roots of *CLN-2366A*, *CLN-2366C*, *CLN-2037H*, *CLN-2037A*, *CLN-2037B* and *CL5915-206-D4-2-*2 were significantly higher compared to the exposure at 28°C. The Babile *M. incognita* population J2 detected inside the roots of *CLN-2366A*, *CLN-2366B*, *CLN-2366C*, CLN-2037H and *CLN-2037A* after 48hrs exposure to 36°C were significantly higher compared with 28°C (Fig 5.3). The number of J2 of *M. incognita* Jittu population that was found in the roots of all the tested breeding lines was significantly higher after both exposure times to 36°C compared to the exposure at 28°C. However, after 24hrs exposure to 36°C, the number of J2 detected inside the roots of *CLN-2366A*, *CLN-2037A* and *CL5915-206-D4-2-2* for *M. incognita* Jittu population was significantly higher compared to the exposure at 32°C. Moreover, the J2 of *M. incognita* Jittu population entered the roots of *CLN-2366A*, *CLN-2366B*, *CLN-2366C*, *CLN-2037H* and *CLN-2037B* with higher number after 48hrs exposure to 36°C compared to the exposure at 32°C (Fig 5.2). The breeding lines *CLN-2366C*, *CLN-2037H* and *CLN-2037B* supported a significantly higher number of Babile *M. incognita* J2 penetration after 24hrs of exposure to 36°C compared to the exposure at 32°C. The J2 of Babile *M. incognita* population found inside the roots of *CLN-2366B*, *CLN-2366C*, *CLN-2037H* and *CLN-2037A* after 48hrs exposure to 36°C were considerably higher compared to the exposure at 32°C.

In general, after 24 and 48hrs of heat exposure to 28, 32 and 36°C, the J2 of both *M. incognita* populations penetrated the roots of all the tested breeding lines with significantly

higher number compared to 25°C (the control temperature). There was no significant difference in J2 penetration to the roots of all the tested breeding lines for both *M. incognita* populations after 24 and 48hrs of exposure to 36°C compared to *Marmande* (susceptible control). A considerably higher number of J2 was detected in the roots of all the tested tomato breeding lines for both *M. incognita* populations after 48hrs heat exposure compared to the 24hrs heat exposure for each soil temperature level studied.

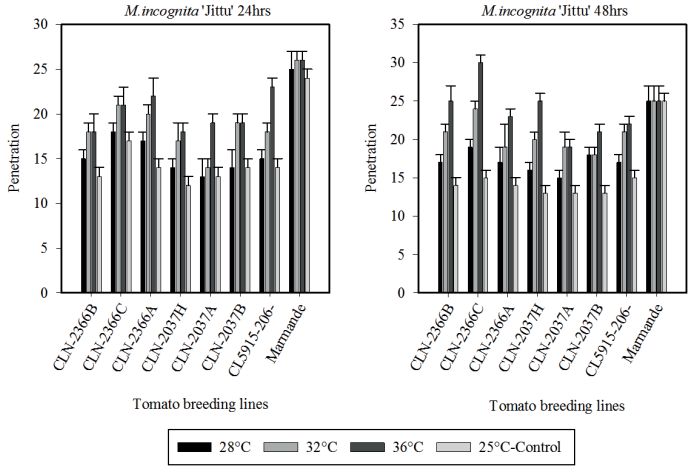

Figure 5.2. Mean number of J2 of *M. incognita* 'Jittu' population penetrated to the tomato roots after 24 and 48hrs of inoculation at soil temperatures of 25, 28, 32 and 36°C.

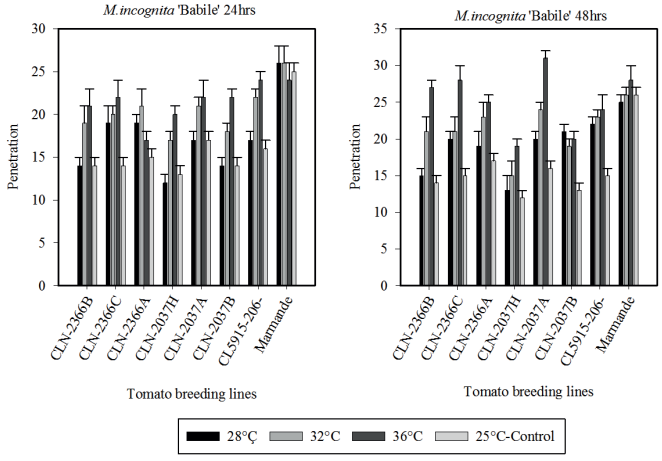

Figure 5.3. Mean number of J2 of *M. incognita* 'Babile' population penetrated to the tomato roots after 24 and 48hrs of inoculation at soil temperatures of 25, 28, 32 and 36°C.

5.3.2. Jittu and Koka *M. javanica* Populations

5.3.2.1. The effect of temperature, cultivar and time

Temperature, tomato breeding lines and time had a significant effect on the numbers of J2 ($F_{temperature}$ = 464.24, $p < 0.01$; $F_{breeding\ lines}$ = 208.40, $p < 0.01$ and F_{time} = 115.60, $p < 0.01$) of *M. javanica* Jittu population that penetrated the plant roots. The interaction between breeding lines*temperature, breeding lines*time, temperature*time and breeding lines*temperature*time was significant for *M. javanica* Jittu penetration (F = 14.49, $p < 0.01$; F = 7.76, $p < 0.01$; F = 32.88, $p < 0.01$ and F = 1.96, $p < 0.01$ respectively) but the effect was again less pronounced compared to the main effects. Likewise, temperature, cultivar and time had a significant effect on the numbers of J2 ($F_{temperature}$ = 218.69, $p < 0.01$; $F_{breeding\ lines}$ = 143.67, $p < 0.01$ and F_{time} = 49.90, $p < 0.01$) of *M. javanica* Koka population that penetrated the plant roots. The interaction between breeding lines*temperature, temperature*time and breeding lines*temperature*time was significant for *M. javanica* Koka population penetration (F = 7.834, $p < 0.01$; F= 5.55, $p < 0.01$ and F = 2.84, $p < 0.01$ respectively) but the effect was less pronounced compared to the main effects.

5.3.2.2. The effect of temperature levels

There was no significant difference found in the number of penetrated J2 for *M. javanica* Jittu population inside the roots of the tested breeding lines (except on *CLN-2037A)* after 24 and 48hrs exposure to 28°C compared to 25°C (Fig 5.4). After 24hrs exposure to 28°C, the number of J2 of *M. javanica* Koka population found inside the roots of all tested breeding lines was not significantly different compared to 25°C. However, after 48hrs exposure to 28°C, the J2 from *M. javanica* Koka population inside the roots of *CLN-2366B*, *CLN-2366C*, *CLN-2037H* and *CL5915-206-D4-2-2* were significantly different compared to 25°C (Fig 5.5). After 24 and 48hrs exposure of both *M. javanica* populations to 32°C, the number of J2 detected in the roots of all tested breeding lines was significantly higher compared at 25°C except on the *CLN-2037H* (after 48hrs exposure with *M. javanica* Koka population). The number of J2 detected inside the roots of all the tested breeding lines was significantly higher for both *M. javanica* populations after both exposure times to 36°C compared to the control (25°C) (Fig 5.4 & 5.5). After both exposure times to 32°C and 36°C, the number of J2 that entered the roots of all the tested breeding lines for both *M. javanica* populations was significantly different compared to the exposure at 28°C. The number of J2 detected inside the roots of all the tested breeding lines was significantly higher for *M. javanica* Jittu population after 24hrs exposure to 36°C compared to 32°C (except on the *CLN-2366B* and

CL5915-206-D4-2-2). Furthermore, the number of J2 found in the roots of all the tested breeding lines (except on *CL5915-206-D4-2-2*) was significantly higher for *M. javanica* Jittu population after 48hrs exposure to 36°C compared to the exposure at 32°C. The roots of *CLN-2366B*, *CLN-2366C* and *CLN-2366A* were found penetrated with a higher number of J2 from both *M. javanica* populations after both exposure times to 36°C compared to the exposure at 32°C (Fig 5.4 & Fig 5.5). Generally, a significantly higher mean number of J2 from both *M. javanica* populations penetrated the roots of all tested breeding lines after 48hrs exposure to 36°C to compared the 24hrs exposure time.

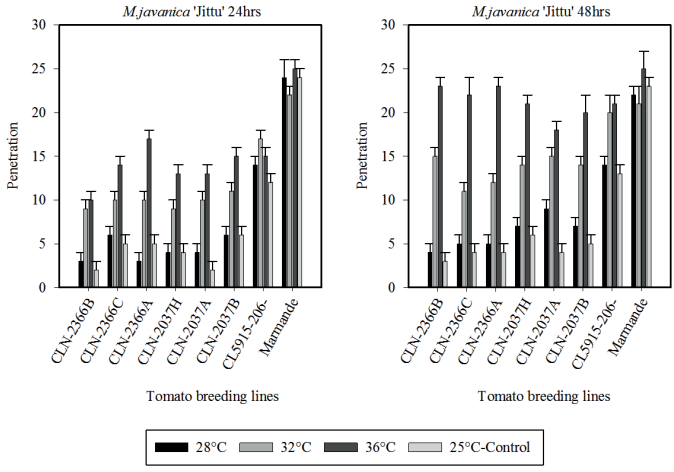

Figure 5.4. Mean number of J2 of *M. javanica* 'Jittu' population penetrated to the tomato roots after 24 and 48hrs of inoculation at soil temperatures of 25, 28, 32 and 36°C.

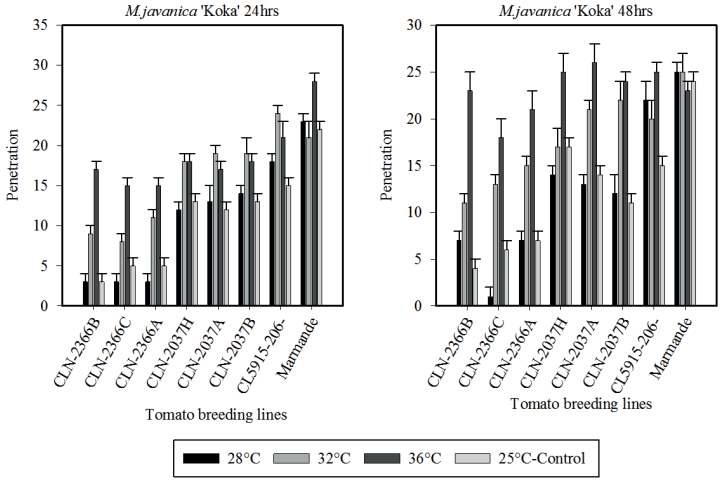

Figure 5.5. Mean number of J2 of *M. javanica* 'Koka' population penetrated to the tomato roots after 24 and 48hrs of inoculation at soil temperatures of 25, 28, 32 and 36°C.

5.4. Discussion

All the tested tomato breeding lines in this study have shown resistance gene inactivation for Jittu and Babile populations of *M. incognita* after exposure to 28, 32 and 36°C during 24 and 48 hrs. For Jittu and Koka populations of *M. javanica* this effect was only seen after 24 and 48hrs at 32 and 36°C. This finding is in agreement with several reports of *Mi-1* gene inactivation characterized by higher egg masses and root gall production above 28°C soil temperature both in greenhouse and field conditions (Dropkin 1969; Ammati *et al*., 1986; Tzortzakakis & Gowen 1996; Noling 2000; Wang *et al*., 2009; Devran *et al*., 2010). The *Mi-1* mediated resistance was found inactivated by *M. incognita* in the cultivar Motelle and VFNT at 32°C (Ammiraju *et al*., 2003). The utility of the potential resistance found in our breeding lines during the controlled growth chamber resistance screening experiment is found limited at higher soil temperatures especially at 32 and 36°C. Hence, we suggest that soil temperatures at 32 and 36°C will reduce the effectiveness of the potential resistance genes contained in the tested tomato breeding lines. Tomato breeding lines such as *CLN-2366A*, *CLN-2366B* and *CLN-2366C* can be used as a potential resistance gene source after genetic improvement especially at soil temperatures below 32°C and in areas where *M. javanica* is the predominant *Meloidogyne* species. In our study, J2 of all *M. incognita* and *M. javanica* populations penetrated the breeding lines tested after 24 and 48hrs heat exposure irrespective of the soil temperature levels. Higher numbers of J2 were found after 48hrs of exposure compared to 24 hrs exposures. This exposure time could be comparable to field-like heat exposures in tropical agriculture (including Ethiopia) during the day and the night. Soil temperatures fluctuate monthly, daily and even at times hourly mainly by variations in air temperature and solar radiations (Cheon *et al*., 2014). In the face of global warming and climate change this is even more evident. A single mid-day heat exposure of 35°C during 3hrs was sufficient to break the *Mi-1* gene resistance in cv. Amelia by an *M. incognita* population (Carvalho *et al*., 2015). Similarly in our study all the tested tomato breeding lines resistance was lost after 24 or 48hrs of exposure to 36°C and there was no significant difference found between the tested breeding lines and the susceptible control *Marmande*. The *Mi-1* resistance was hypothesised either to acclimatize or recovers from exposure to high temperatures (Zacheo *et al*., 1995; Carvalho *et al*., 2015). There is still some inconsistency in the literature and several authors have reported as *Mi-1* conferred resistance was found effective at temperatures ≥ 34°C (Abdul-Baki *et al*., 1996; Verdejo-Lucas *et al*., 2013). The *Mi-9* obtained from the *S. peruvianum* complex and a homologue of *Mi-1* gene was found

unaffected by temperature (Bleve-Zacheo *et al*., 2007) but it is not yet commercially available. As confirmed with the tested tomato breeding lines in this study and reported in different crop species such as bean (Mullin *et al*., 1991), pepper (Thies & Fery, 2000), sweet potato (Jatala & Russell, 1972), alfalfa (Griffin, 1969) and cotton (Carter, 1982) temperature sensitivity is a characteristic of several RKN resistance genes.

Checking the heat stability of resistance genes in time at higher temperatures and assessing whether the resistance genes could be reversed or not is crucial for further development of sustainable cultivars. Adjustment of planting date to avoid planting at the hottest season when monthly soil temperature rises above 28°C is also very important especially in areas with enough irrigation potential in the vicinity. Systematically designed crop rotation schemes in which plants are included that can reduce the soil temperature (i.e. using crops with higher canopy coverage) and the nematode population (a non-host crop) should be developed and used. A mechanism to increase soil aeration and thereby cools down the soil temperature might also be reliable especially till the plant successfully establishes in the field right after transplanting. It is also recommended to soften the soil between the plants without affecting the tomato roots so that soil will be aerated and soil temperature cools down. Most of the tomato growing areas in Ethiopia are characterized by sandy soil (less thermal conductivity and diffusivity) and it can be feasible to look for alternatives to cool down the soil temperatures. We also recommend checking the soil temperature prior to transplanting tomato seedlings using a simple soil thermometer and avoid too dry and too wet extreme field conditions. It is clear from our observations that local tomato breeding lines with resistance potential can be used when soil temperatures remain below 32°C. Differences were observed between breeding lines depending on the RKN population at higher temperatures and this knowledge can help in further optimizing the development of sustainable resistance under local Ethiopian circumstances.

Acknowledgements

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Chapter 6: Tolerance and resistance of selected breeding lines and commercial tomato cultivars in *Meloidogyne* spp. infested fields in Eastern Ethiopia

Abstract

RKN are widely distributed in Ethiopian agriculture and often found dramatically reducing the yield of tomato. One of the best management strategies for RKN is to use resistant crop varieties. However, tomato resistance screening against *Meloidogyne* spp. has often been performed in controlled greenhouses, which does not represent the actual tomato production conditions. A study was initiated to assess the tolerance and resistance of selected tomato cultivars under local conditions on two *Meloidogyne* spp. infested fields (Dire Dawa-Tony farm and Babile-Fethiya Farm) in eastern Ethiopia. A total of seven tomato genotypes (*Assila*, *CLN-2366A*, *CLN-2366B*, *Chochoro*, *Eden*, *Moneymaker* and *Tisey*) were grown in both locations. A randomized complete block design (RCBD) was used with four replications for each cultivar at both locations. The plant parameters that were considered as a measure of varieties tolerance were found significantly ($p < 0.01$) different among the tomato varieties. Except the initial population densities (*Pi*) at planting, all nematode related parameters that were used as a measure of varieties resistance such as the final population density, root galls per root system, egg mass per root system, mean number of eggs per egg mass, root gall index, egg mass index and multiplication rate were found statistically ($p < 0.01$) different among the tested tomato genotypes. Positive correlation of plant data parameters indicated that total number of fruits per plant, fruit set percentage, number of fruits per cluster, number of fruit clusters per plant and shape index were the most important fruit yield components contributing to fruit yield per hectare. A similar performance trend was obtained among the tested tomato genotypes at both locations though the level of damage was found dependant on the initial population densities present in the soil. A cultivar potentially tolerant or resistant at Dire Dawa (Tony farm) was also found performing nearly similar at Babile (Fethiya farm). The tomato varieties *Assila* and *Eden* are recommended in nematode infested areas with strict crop rotation. The tomato cultivar *Tisey* was found to be the most susceptible while the local cultivar *Chochoro* was found to be tolerant. The tomato breeding lines *CLN-2366A* and *CLN-2366B* were found good in reducing the nematode populations but they need some critical genetic improvement (preferably they can be used as a rootstock).

Key words: tolerance, resistance, sustainability, tomato, *Meloidogyne* spp., and hotspot areas

6.1. Introduction

Tomato (*Solanum lycopersicum*) can adapt to diverse environmental conditions (Rice *et al*., 1987). Tomato has also been extensively used as a model plant for resistance studies through genetic and biotechnological approaches (Ercolano *et al*., 2012). In Ethiopia, more emphasis is being given to tomato production both as a source of income and food security (Mulualem & Tekeste, 2014). However, tomato production is constrained by many insect pests and diseases. Plant-parasitic nematodes (PPN) are recognized as one of the major problems to tomato production worldwide. Alone or in combination with other soil borne pathogens, PPN, attack almost every part of the plant including roots, leaves, fruits and seeds (Handoo, 1998). Globally, PPN account for an estimated 14% of yield losses, which is translated into a $ 100 billion dollars annually (Mitkowski & Abawi, 2003). In order to manage such a significant yield loss, an estimated amount of US$500 million was spent on nematode control worldwide (Keren-Zur *et al*., 2000). When this yield loss figure is translated into economic terms, it covers nine years annual budget for Ethiopia (the second most populous African country) based on the 2015/16 fiscal year approved budget. Among the PPN, RKN are regarded as a universal problem due to their polyphagous nature attacking more than 3000 host species (Harris *et al*., 2003; Abad *et al*., 2003; Jones *et al*., 2013). RKN are widely distributed in Ethiopian agriculture and often dramatically reduce the yield of vegetables including tomato (Mandefro & Mekete, 2002). *Meloidogyne* spp. cause severe damage to the roots of tomato and consequently impacting the quantity and quality of marketable yields. Yield reductions as severe as 100% have been reported due to RKN on tomato (see chapter 2).

Nematode management including chemical treatments have been used for years to combat the harmful effects of PPN (Lambert & Bekal, 2002). Without the use of nematicides, many crops cannot be grown economically (Sikora & Fernandez, 2005). However, due to increasing concerns about their adverse effects and environmental impacts, many products have been pulled from the market (Starr *et al*., 2001). Their use has also been limited for subsistence farmers due to high prices (Luc *et al*., 2005). As a result one of the best alternative ways to manage PPN is to use resistant crop varieties. Resistant crops provide an effective and economical method for managing nematodes in both high and low-cash value-cropping systems (Khan & Mukhopadhyay, 2004). Host-plant resistance (HPR) provides a safe solution for nematode problems. With the availability of germplasm that have nematode

resistance genes and high tech molecular transfer techniques, resistant cultivars are becoming a primary management tactic in nematode management (Barker, 1997).

When new diseases emerge or new races of existing RKN become established screening for resistance is a major goal. HPR has been prioritized over other management practices including chemical control because it provides a sustainable, eco-friendly, effective and economical method for managing PPN including RKN in both high and low value cropping systems (Jaiteh *et al*., 2012). It also allows crop rotation to be shortened and to make best use of land (Roberts, 1992). Genetic resistance in tomato against *Meloidogyne* spp. is efficient in reducing their population densities and thereby reducing the need for nematicides application (Khan, 1994). However, in tropical and subtropical countries, most sources of heightened resistance in tomato are not available because of high temperature and poor adaptation to commercial production (Roberts *et al*., 1998). Most often, tomato resistance screening against *Meloidogyne* spp. has been performed in controlled greenhouses, which doesn't represent the actual tomato production conditions. Screening tomato cultivars against aggressive local *Meloidogyne* populations under the actual prevailing environmental and edaphic field conditions will have practical significance. In Ethiopia, no research has been done on tomato resistance screening against local aggressive *Meloidogyne* spp. at farmer's field conditions. There is an increased demand to identify more sources of resistance in tomato cultivars for seed multiplication or breeding against *Meloidogyne* spp. Therefore, this study was initiated with the objective to assess the tolerance and resistance level of selected tomato cultivars against *Meloidogyne* spp. under two Ethiopian field conditions: Dire Dawa (Tony farm) and Babile (Fethiya farm).

6.2. Materials and Methods

6.2.1. Tolerance and Resistance under Field Conditions

Both experimental fields were located in eastern Ethiopia approximately 550km east from Addis Ababa. The first field is situated at Dire Dawa (Tony farm), which is the field research site of HU. Tony farm is located at the eastern escarpment of the Rift Valley 9.6°N 41.8°E while the second site at Babile (Fethiya farm) is situated between 9.2°N and 42.26°E. The altitude of Tony farm and Fethiya farm is 1196 and 1334 m.a.s.l respectively. The distance between these two experimental fields is approximately 70km. The experiment was conducted from October 2015 through May 2016 at both locations. The monthly average temperature and relative humidity during these experimental months are presented in Table 6.1. Six tomato genotypes (*Assila*, *CLN-2366A*, *CLN-2366B*, *Chochoro*, *Eden* and *Tisey*) were selected based on the screening result (see chapter 4), farmer's choice, success stories of

neighbouring countries such as Kenya, Tanzania and Uganda in using some of these cultivars with *Mi*-gene. The cv. *Moneymaker* was included as a susceptible control. The genotypes were raised and nursed in separate plastic trays containing sterilized soil till they reached the four-leaf stage.

Table 6.1. Average monthly maximum and minimum air temperature and relative humidity at Dire Dawa (Tony farm) and Babile (Fethiya farm) during 2015/16 growing season.

Experimental Months	Dire Dawa: Tony farm			Babile: Fethiya farm		
	MaxTemp[a] (°C)	MinTemp[b] (°C)	RH[c] (%)	MaxTemp (°C)	MinTemp (°C)	RH (%)
October 2015	35.2	20.3	61.5	33.4	19.6	59.6
November 2015	31.8	17.6	73.4	32.9	21.4	71.8
December 2015	30.1	16.4	75.7	29.7	17.5	76.3
January 2016	31.4	16.8	80.2	32.1	18.3	75.7
February 2016	31.7	16.4	69.9	30.8	17.4	72.1
March 2016	36.4	21.7	34.0	34.8	20.0	43.6
April 2016	31.9	20.4	55.2	35.6	22.7	58.3
May 2016	35.4	22.3	49.7	36.3	23.9	39.5

[a]*Average monthly maximum temperature in* (°C)**,** [b]*Average monthly minimum temperature in* (°C) and [c]*Average monthly relative humidity in (%).*
Source: *The Tony farm temperature data was obtained from the National Meteorology and Jigjiga Meteorology Directorate while the Fethiya farm temperature data was obtained from Haramaya University, Babile Meteorology Centre.*

The fields were slashed, ploughed, levelled and ridges were made at 75 cm between rows and 60 cm between plants (Fig 6.1). The total surface area (length x width) of the two experimental fields was 184 m^2 for each. Twelve plants per cultivar were transplanted on one row and for each cultivar 4 replicates (= rows) were used. Cultivars were organised in RCBD. Two additional buffer rows were included from the top and bottom edges from which data were not collected. Per row plants number one and twelve were not considered for data collection. Plant number two, five, eight and eleven from each row were uprooted destructively to take the growth and biomass plant data parameters i.e. fresh shoot weight, fresh root weight and dry shoot weight exactly in the same manner from both field locations. Entire yield, yield components and nematode data parameters were considered from plants number three, four, six, seven, nine and ten from each row exactly in the same manner at both

locations. Around the field experimental areas maize was planted to repel some insect pests. Sticky yellow insect traps were placed both around and inside the experimental fields to reduce and detect different insect pest populations on tomato. Plants were watered with furrow irrigation and fertilized with NPK based on the nationally recommended rate i.e. 60-120 kg N, 60-140 kg P_2O_5 and 60-120 kg K_2O per hectare (Balem, 2008). Weeding was done manually on a weekly basis. The initial population (*Pi*) densities were determined from the two field locations one day before transplanting the tomato seedlings. In May 2016 plants were harvested and different plant and nematode parameters collected.

Figure 6.1. (A) Field and ridges preparation (B) Field lay-outing (C) Furrow irrigation before seedling transplanting (D) Irrigated field one day before transplanting

6.2.2. Plant and Nematode Data Parameters Collected

A list of plant data parameters were collected and analysed during this experiment. However, to keep the focus of this chapter on nematode tolerance and resistance only a selected number of plant parameters as a measure of tolerance are discussed from both field locations: Dire Dawa (Tony farm) and Babile (Fethiya farm). The descriptions of all plant parameters recorded and the result of its analysis (both discussed and not discussed in this chapter) are presented in the appendix.

Table 6.2. Selected plant and nematode parameters used as a measure of nematode tolerance and resistance in two field conditions in Eastern Ethiopia during 2015/16 growing season.

Plant parameter used as a measure of nematode tolerance	**Nematode parameter as a measure of resistance**
Plant height (PH)	The initial population densities (*Pi*)
Number of leaves (NL)	The final population densities (*Pf*)
Number of flowers per plant (NFPP)	The reproduction factor (RF)
Total fruit number per plant (TFr)	Number of root galls per plant (Galls/RS)
Marketable fruit number per plant (MFr)	Number of egg masses per plant (EM/RS)
Unmarketable fruit number per plant (UnFr)	Root Gall Index (RGI)
Single fruit weight (SFW)	Egg Mass Index (EMI)
Marketable yield (MY) (ton/ha)	Mean number of eggs per eggmass (Meggs/EM)
Fruit set percentage (FSP)	
Root size (RS)	
Root length (RL)	
Fresh shoot weight (FSW)	
Dry shoot weight (DSW)	
Fresh root weight (FRW)	

Nematode parameters used as a measure of tomato genotypes resistance

The initial population densities (*Pi*): three subsamples were combined per row to make one sample (approximately 1kg) in total 28 separate soil samples to represent 28 planting rows per location (Table 6.3). After homogenizing each sample, nematodes were extracted using a modified Baermann funnel from two (100 cm^3 each) subsamples and counted using a stereomicroscope. The *Pi* was expressed per 100cm^3 soil.

The final population (*Pf*): The final *Pf* was determined from the mean number of J2s and eggs estimated from the whole root system after extracting nematodes from a subsample of 10gram roots per plant and after a proper homogenization of the rhizosphere soil per plant using a sub sample of 100cm^3-rhizosphere soil from the pre-tagged plants per row. The soil sample was extracted using a modified Baermann funnel and it was left for 10 days. Nematodes were collected every two days and counted. Nematodes from root samples were extracted based on Hussey and Barker (1973).

The reproduction factor (RF): this was determined by dividing the *Pf* by *Pi*.

Number of root galls per plant (Galls/RS): the total number of galls present per plant root system was counted and their mean was computed.

Number of egg masses per plant (EM/RS): the total number of egg masses present per plant root system was counted and their mean was computed.

Root Gall Index (RGI) and **Egg Mass Index (EMI):** these indices were determined per plant from the pre-tagged plants based on Taylor and Sasser (1978). Scoring was done on a 0 to 5 scale; where 0 = no galls/egg masses; 1 = 1-2 galls/egg masses; 2 = 3-10 galls/egg masses; 3 = 11-30 galls/egg masses; 4 = 31-100 galls/egg masses and 5 = >100 galls/egg masses.

Mean number of eggs per eggmass (Meggs/EM): three to 5 egg masses (when available) were randomly picked per plant and the number of eggs per egg mass was determined and their mean was computed.

Table 6.3. A) Dire Dawa - Tony farm and (B) Babile –Fethiya farm field plot arrangement and its initial population density (*Pi*) as determined one day before seedling transplanting during 2015/16 growing season.

A. Dire Dawa-Tony Farm			**B. Babile Farm-Fethiya Farm**		
RN[a]	Randomized Cultivars	*Pi* (expressed per 100cm^3 soil) per row	RN	Randomized Cultivars	*Pi* (expressed per 100cm^3 soil) per row
1	*Eden*-R4	5	1	*Tisey*-R4	31
2	*Tisey*-R3	5	2	*Assila*-R2	14
3	*Eden*-R1[b]	10	3	*Chochoro*-R1	19
4	*Chochoro*-R2	10	4	*Tisey*-R2	39
5	*CLN-2366A*-R4	9	5	*Eden*-R4	24
6	*Moneymaker*-R2	8	6	*Chochoro*-R3	26
7	*Chochoro*-R1	8	7	*CLN-2366B*-R2	22
8	*Moneymaker*-R1	6	8	*CLN-2366B*-R4	46
9	*Assila*-R1	7	9	*Eden*-R1	38
10	*Tisey*-R4	8	10	*Tisey*-R1	23
11	*CLN-2366A*-R3	5	11	*CLN-2366A*-R1	42
12	*Tisey*-R2	11	12	*Moneymaker*-R1	19
13	*CLN-2366B*-R2	4	13	*Eden*-R3	36
14	*CLN-2366A*-R1	13	14	*Moneymaker*-R3	42
15	*Eden*-R2	6	15	*Eden*-R2	17
16	*CLN-2366A*-R2	7	16	*Assila*-R1	21

17	*Chochoro*-R4	13	17	*Assila*-R3	35
18	*Tisey*-R1	6	18	*Moneymaker*-R4	15
19	*CLN-2366B*-R1	11	19	*Chochoro*-R2	47
20	*Eden*-R3	11	20	*Chochoro*-R4	49
21	*CLN-2366B*-R4	7	21	*Assila*-R4	41
22	*Moneymaker*-R4	4	22	*Moneymaker*-R2	30
23	*Chochoro*-R3	6	23	*CLN-2366A*-R2	21
24	*Moneymaker*-R3	11	24	*Tisey*-R3	17
25	*Assila*-R4	13	25	*CLN-2366A*-R4	37
26	*Assila*-R3	10	26	*CLN-2366B*-R1	51
27	*CLN-2366B*-R3	12	27	*CLN-2366A*-R3	16
28	*Assila*-R2	6	28	*CLN-2366B*-R3	46

[a]*RN is row number,* [b]*R1=replication1, R2=replication2, R3=replication3, R4=replication4*

6.2.3. Data Analysis

A one-way ANOVA was performed between the tomato varieties for each plant and nematode data parameters used to measure the tolerance and resistance level of the varieties at 5% level of significance using SPSS 22 statistical software package. A Tukey post hoc test was performed for each significant parameter to detect where the significant difference among the studied cultivars lied. A factorial ANOVA was used to compare these parameters between the two locations. Data were log transformed when they failed to satisfy the assumption of ANOVA. Bivariate Pearson's correlation among the plant and nematode data parameters was also analysed. Significant letters assigned in tables of this chapter and the appendix are read per farm not across farm.

6.3. Results

During the survey work, a 100% incidence of RKN was found in Tony farm and Fethiya farm (Babile) locations. Both experimental fields were found infested with the two most predominant RKN species *Meloidogyne incognita* and *M. javanica* based on the DNA-based and isozyme identification (see chapter 3). During the *Pi* determination (using soil samples) prior to seedling transplanting for the field trial both species were detected. Moreover, during the *Pf* determination (from both soil and roots of all cultivars) after field experiments the presence of mixtures of these two species on both fields was confirmed. However, the population density for each species separately was not determined. The *Pi* at Babile was found higher as compared to *Pi* at Tony farm (Table 6.3).

6.3.1. Dire Dawa (Tony farm) Location

6.3.1.1. Plant parameters used as a measure of nematode tolerance

Results for plant parameters used as measure of tolerance are shown in Table 6.4. The PH (F = 20.32) and NL (F = 11.63) were significantly ($p < 0.001$) different between the tested tomato varieties. The mean value for the PH ranged from 60 cm (*Eden*) to 88 cm (*Moneymaker*) while the mean NL laid between 55 (*Chochoro*) to 106 (*Moneymaker*). The NFPP (F = 25.25) was found significantly ($p < 0.001$) different among the tested tomato varieties. The highest mean NFPP (110) was recorded on *CLN-2366A* while the lowest mean NFPP (58) was counted from *Eden*. The TFr (F = 13.08), MFr (F = 14.99), UnFr (F = 23.10), SFW (F = 42.72), MY (F = 37.87) and FSP (F = 54.79) were found significantly ($p < 0.001$) different among the tomato varieties tested. The mean TFr ranged from 36 (*Eden*) to 52 (*Chochoro*). The mean MFr was ranged from 35 (*Eden* and *Moneymaker*) to 51 (*Chochoro*). The highest mean UnFr (6) was obtained from the cultivar *Assila* while the lowest (0) was from *Eden*. The highest mean SFW were 133 g (*Tisey*), 121 g (*Eden*) followed by 118 g (*Chochoro*) while the lowest was obtained from *CLN-2366B* (61 g). The highest mean MY was obtained from *Chochoro* (60 ton/ha) followed by *Tisey* (50 ton/ha) while the lowest was recorded from *CLN-2366B* (26 ton/ha). The highest mean FSP (80%) was found from the tomato cultivar *Chochoro* while the lowest mean FSP (41%) was obtained from *CLN-2366B*. The RS (F = 2.20, p = 0.08) was not found significantly different among the studied tomato varieties. The RL (F = 2.80) was found significantly different at $p < 0.05$ between the tomato varieties. The shortest mean RL (20 cm) was measured from *Tisey* and *Moneymaker* while the longest mean RL (24 cm) was recorded from *Assila*. The FSW (F = 8.99), DSW (F =10.18) and FRW (F = 21.21) were found significantly ($p < 0.001$) different among the tested tomato varieties. The highest mean FSW was recorded from the cultivar *Assila* (103 g) and *Eden* (101 g) while the lowest was recorded from *Tisey* (53 g). The mean DSW ranged from 8 g (*Tisey*) to 19 g (*Assila*). The mean FRW ranged from 6 g (*Tisey*) to 16 g (*Assila*).

The highest mean number of FFP (5) was obtained from *Chochoro* while the lowest mean number of FFP (4) was recorded from the tomato cultivars *Assila*, *Tisey* and *Moneymaker*. A gradual change in the colour, firmness and appearance of fruits was observed. The breaker stage fruits turned to red ripe stage within 4 to 32 days depending on the commercial tomato cultivar or clone. The shelf life of *CLN-2366A* and *CLN-2366B* was found too short (7days) under room temperature. While the shelf life of *Assila* (21days), *Moneymaker* (24days), *Tisey*

(25days), *Chochoro* (27days) and *Eden* (32days) was found in increasing order. The average daily room temperature in the lab was recorded as 26 ± 2°C.

Figure 6.2. Fruit diversity of the tested tomato cultivars. A) *Tisey* while in the field, B) *Eden* C) CLN-2366A D) *Assila*-branched fruits quite often observed, E) fully matured *Tisey* and F) *CLN-2366B*

6.3.1.2. Nematode parameters as a measure of varieties resistance

Results for nematode parameters are summarized in Table 6.5. At Dire Dawa (Tony farm) the *Pi* was not found statistically different between the tomato varieties tested (F = 0.22, p = 0.96). The *Pf* (F = 84.39), RF (F = 15.85), Galls/RS (F = 10.22), EM/RS (F = 8.08), RGI (F = 8.82), EMI (F = 8.30) and Meggs/EM (F = 80.56) were found significantly different at $p <$ 0.001 between the different tomato varieties used. The mean number of *Pi* ranged from 7 (J2 + eggs)/100 cm^3 soil (*Moneymaker*) to 9 (J2 + eggs)/100cm^3 soil (*Chochoro*). The mean number of *Pf* of nematodes among the varieties tested was 262 (*Assila*), 280 (*CLN-2366A*), 328 (*Eden*), 347 (*CLN-2366B*), 662 (*Chochoro*), 2006 (*Tisey*) and 3525 (J2 + eggs)/100 cm^3 soil (*Moneymaker*) in an increasing order. The highest RF (*Pf*/*Pi*) was found on *Moneymaker* (567) followed by *Tisey* (276) while the lowest was calculated from cultivar *Assila* (29). The mean number of galls/RS ranged from 2 (*Assila*) to 21 (*Moneymaker*) while the RGI ranged from 1 (*Assila*) to 3 (*Moneymaker*). The mean number of EM/RS ranged from 1 (*Assila*) to

15 (*Moneymaker*) while EMI was found in a range of 1 (*Assila*) to 3 (*Moneymaker*). The highest mean number of eggs/EM was found in *Tisey* (185) while the lowest was in *CLN-2366B* (60).

6.3.2. Babile (Fethiya farm) Location

6.3.2.1. Plant parameters used as a measure of nematode tolerance

Results for plant parameters used as a measure of nematode tolerance are shown in Table 6.4. The PH (F = 53.57) and NL (F = 46.91) were significantly ($p < 0.001$) different between the tested tomato varieties. The mean value for the PH ranged from 58 (*Eden*) to 83 cm (*CLN-2366A*) while the mean NL laid between 57 (*Chochoro*) to 96 (*Moneymaker*). The number of flowers per plant (NFPP) (F = 54.80) was found significantly ($p < 0.001$) different among the tested tomato varieties. The highest mean NFPP (106) was recorded on the *CLN-2366A* while the lowest mean NFPP (59) was counted from *Eden*. The TFr (F = 12.57), MFr (F = 15.18), UnFr (F = 4.59), SFW (F = 63.67), MY (F = 38.27) and FSP (F = 44.69) were found significantly ($p < 0.001$) different among the tomato varieties. The mean TFr ranged from 33 (*Moneymaker*) to 50 (*Chochoro*). The mean MFr ranged from 31 (*Moneymaker*) to 49 (*Chochoro*). The highest mean UnFr (2) was obtained from the cultivars *Assila* and *Moneymaker* while the lowest (1) was from *Eden* and *CLN-2366B*. The highest mean SFW were 130 g (*Tisey*), 119 g (*Eden*) followed by 113 g (*Chochoro*) while the lowest was obtained from *CLN-2366B* (60 g). The highest mean MY was obtained from *Chochoro* (55 ton/ha) followed by *Tisey* (45 ton/ha) while the lowest was recorded from *CLN-2366B* (24 ton/ha). The highest mean FSP (76%) was found from cultivar *Chochoro* while the lowest mean FSP (39%) was obtained from *CLN-2366B*. The highest mean number of FFP (5) was obtained from *Chochoro* while the lowest mean number of FFP (4) was recorded from the cultivars *Assila*, *Tisey* and *Moneymaker*. The RS (F = 0.96, p = 0.47) and RL (F = 1.42, p = 0.25) were not found significantly different among the studied tomato varieties. The FSW (F = 4.26), DSW (F = 4.08) and FRW (F = 4.79) were found significantly ($p < 0.05$) different among the tested tomato varieties. The highest mean FSW was recorded from the cultivar *Assila* (93 g) and *Eden* (90 g) while the lowest was recorded from *Tisey* (53 g). The mean DSW ranged from 8 g (*Tisey*) to 15 g (*Assila*). The mean FRW ranged from 6 g (*Tisey*) to 13 g (*Assila*). The mean number of days to 50FL ranged from 32 (*Chochoro*) to 42 (*Moneymaker*) while the mean number of days to first harvest (DFrH) was ranged from 67 (*CLN-2366A* and *CLN-2366B*) to 79 days (*Moneymaker*).

6.3.2.2. Nematode parameters as a measure of varieties resistance

Results for nematode parameters used as a measure of resistance are summarized in Table 6.5. The *Pi* (F = 0.36) was not found significantly different (p = 0.90) between the tomato varieties tested. The final *Pf* (F = 11.89), RF (F = 142.01), Galls/RS (F = 18.46), EM/RS (F = 10.47), RGI (F = 14.46), EMI (F = 7.00) and Meggs/EM (F = 12.58) were found significantly different at $p < 0.001$ between the different tomato varieties. The mean *Pi* ranged from 27 (J2 + eggs)/100 cm^3 soil (*Moneymaker*) to 36 (J2 + eggs)/100cm^3 soil (*CLN-2366B*). The mean *Pf* of nematodes among the varieties tested was 517 (*Assila*), 695 (*CLN-2366A*), 787 (*Eden*), 818 (*CLN-2366B*), 1117 (*Chochoro*), 2662 (*Tisey*) and 4358 (J2 + eggs)/100 cm^3 soil (*Moneymaker*) in an increasing order. The highest mean RF was found on *Moneymaker* (170) followed by *Tisey* (95) while the lowest was calculated from cultivar *Assila* (18). The mean number of galls/RS was found ranged from 3 (*Assila*) to 30 (*Moneymaker*) while the RGI ranged from 2 (*Assila*) to 4 (*Moneymaker*). The mean number of EM/RS ranged from 4 (*Assila*) to 26 (*Moneymaker*) while EMI was found in a range of 2 (*Assila* and *CLN-2366A*) to 3 (*Moneymaker*). The highest Meggs/EM was found in *Moneymaker* (158) while the lowest was in *CLN-2366A* and *CLN-2366B* (125).

Figure 6.3. A) Representatives of farmer groups personnel from the agricultural offices of eastern Hararghe visiting the Tony farm experimental plot, (B) The researcher is explaining the aim, why he is doing the research and how, (C) Checking the damage and population dynamic studied on bigger pots (see chapter 7) and (D) A visitor taking pictures to show for farmers group.

Table 6.4. Plant parameters used as a measure of tomato varieties tolerance against a mixture of *Meloidogyne incognita* and *Meloidogyne javanica* populations at Dire Dawa (Tony Farm) and Babile (Fethiya Farm) locations during 2015/16 growing season

Location	Tomato Genotypes	PH	NL	NFPP	TFr	MFr	UnFr	SFW	MY	FSP	RS	RL	FSW	DSW	FRW
Dire Dawa (Tony farm)	*Assila*	65a	64 a	69 a	47 b	42 b	6 b	96 b	40 b	68 c	21a	24 b	103 c	19 c	16 c
	CLN-2366A	83 b	85 b	110 b	47 b	46 b	1 a	76 a	35 b	43 a	17 a	22 ab	71 b	9 a	10 b
	CLN-2366B	83 b	78 b	108 b	43 a	42 b	1 a	61 a	26 a	41 a	18 a	21 ab	72 b	12 b	9 b
	Chochoro	62 a	55 a	65 a	52 c	51 c	1 a	118 c	60 d	80 d	19 a	22 ab	66 b	10 a	13 bc
	Eden	60 a	61 a	58 a	36 a	35 a	0 a	121 c	43 b	62 c	18 a	22 ab	101 c	15 bc	11 bc
	Moneymaker	88 b	106 c	71 a	37 a	35 a	2 ab	105 b	37 b	53 b	19 a	20 a	67 b	12 b	8 b
	Tisey	81 b	70 b	68 a	39 a	38 a	1 a	133 d	50 c	58 b	18 a	20 a	53 a	8 a	6 a
Babile (Fethiya farm)	*Assila*	61 a	64 a	66 a	42 b	40 b	2 b	95 b	38 b	64 c	19 a	22 a	93 c	15 c	13c
	CLN-2366A	83 c	82 b	106 b	44 b	43 b	1 a	73 a	32 b	42 a	17 a	20 a	66 b	9 a	9 b
	CLN-2366B	83 c	79 b	106 b	41 b	40 b	1 a	60 a	24 a	39 a	18 a	20 a	66 b	11 b	9 b
	Chochoro	59 a	57 a	65 a	50 c	49 c	1 a	113 c	55 d	76 d	21 a	20 a	64 b	11 b	10 b
	Eden	58 a	60 a	59 a	36 a	35 a	1 a	119 c	41 b	61 c	20 a	21 a	90 c	14 c	11bc
	Moneymaker	82 c	96 c	70 a	33 a	31 a	2 b	102 b	31 b	48 b	21 a	19 a	67 b	9 a	7 a
	Tisey	71 b	70 b	66 a	36 a	35 a	1 a	130 d	45 c	56 b	19 a	19 a	53 a	8 a	6 a

PH-plant height in (cm), NL-number of leaves, NFPP-number of flower per plant, TFr-total fruit number per plant, MFr-marketable fruit number per plant, UnFr-unmarketable fruit number per plant, SFW-single fruit weight, MY-marketable yield per hectare (ton/ha), FSP-fruit set percentage, RS-root size (g), RL- root length (cm), FSW-fresh shoot weight (g), DSW-dry shoot weight (g) and FRW-fresh root weight (g). Means within the same column that shared the same letter (only per farm not across farm) are not significant at $p \leq 0.05$ based on Tukey HSD.

Table 6.5. Nematode data parameters used as a measure of tomato varieties resistance potential to RKN at Dire Dawa (Tony farm) and Babile (Fethiya Farm) field experiments during 2015/16 growing season

Locations	Tomato genotypes	*Pi*	*Pf*	RF (*Pf*/*Pi*)	Galls/RS	RGI	EM/RS	EMI	Meggs/EM
Dire Dawa (Tony farm)	*Assila*	9 a	262 a	29 a	2 a	1 a	1 a	1 a	85 a
	CLN-2366A	9 a	280 a	33 a	3 a	2 a	2 a	1 a	65 a
	CLN-2366B	9 a	347 a	44 a	5 a	2 a	3 a	1 a	60 a
	Chochoro	9 a	662 a	73 b	6 a	2 a	2 a	1 a	130 b
	Eden	8 a	328 a	40 a	4 a	2 a	2 a	1 a	86 a
	Moneymaker	7 a	3525 c	567 d	21 b	3 b	15 b	3 b	184 c
	Tisey	8 a	2007 b	276 c	8 a	2 a	5 a	2 ab	185 c
Babile (Fethiya farm)	*Assila*	28 a	517 a	18 a	3 a	2 a	4 a	2 a	129 a
	CLN-2366A	29 a	695 a	23 a	6 a	2 a	5 a	2 a	126 a
	CLN-2366B	36 a	818 ab	23 a	6 a	2 a	6 ad	2 ab	125 a
	Chochoro	35 a	1117 b	32 a	8 a	2 a	8 ad	2 ab	135 a
	Eden	29 a	787 ab	27 a	7 a	2 a	5 ad	2 ab	133 a
	Moneymaker	27 a	4358 d	170 c	30 c	4 b	26 ce	4 cd	158 b
	Tisey	28 a	2662 c	95 b	18 b	3 ab	17 bde	3 bd	153 b

Pi-initial population density in the soil, Pf-final population density at harvest, a (Pf/Pi)-reproduction rate, galls/RS-number of galls per root system, RGI-root gall index, EM/RS- number of egg masses per root system, EMI- egg mass index, Meggs/EM- mean number of eggs per egg mass. Means within the same column that shared the same letter are not significant at $p \leq 0.05$. Significant difference letters should be read only per location not across location.

6.3.3. Plant and Nematode Data Parameters Compared between Locations

The PH (F = 9.19), TFr (F = 9.83), MFr (F = 9.74), MY (F = 14.11), FSP (F = 9.36) and RL (F = 10.30) were found significantly ($p < 0.01$) different between the study locations. The FRW (F = 4.92) was also found statistically significant at $p < 0.05$. All the other plant data parameters studied were not found significant at $p < 0.05$ between the two locations. However, all the nematode data parameters *Pi* (F = 79.12), *Pf* (F = 9.45), RF (F= 40.30), mean number of Galls/RS (F = 13.68), RGI (F = 10.71), EM/RS (F = 24.40), EMI (F = 30.94) and Meggs/EM (F = 77.90) that were considered as a measure of resistance were found statistically ($p < 0.01$) different between both locations.

Both a positive and negative correlation was found between the parameters studied (see appendix for the detailed presentation). However, here, only the correlation between selected plant and nematode parameters is presented. A positive significant correlation was found between the PH and Galls/RS (r = 0.42) and EM/RS (r = 0.43); NL and *Pf* (r = 0.43), Galls/RS (r = 0.54) and EM/RS (r = 0.54); DFrH and *Pf* (r = 0.76), Galls/RS(r = 0.82) and EM/RS(r = 0.77); RS and *Pi* (r = 0.72); Pf with Galls/RS(r = 0.96), EM/RS (r=0.98); Galls/RS with EM/RS (r = 0.98) and Meggs/EM with Pf (r = 0.69), Galls/RS (r =0.71) and EM/RS (r = 0.77). There was also a significant negative correlation found between the parameters checked at both Dire Dawa and Babile farms. The full detailed Pearson's correlation result of twenty plant and nematode data parameters performed for both field locations are presented in the Appendix (Table A3).

6.4. Discussion

The majority of the quantitative characters used as a measure of tolerance and resistance were found more affected in Babile (Fethiya farm) compared to Dire Dawa (Tony farm). This might be allocated to the fact that initial *Meloidogyne Pi* was higher at Babile location as compared to Dire Dawa. A similar performance trend was obtained among the tested tomato breeding lines or cultivars at both locations though the level of damage was found dependant on the *Pi* present in the soil. A cultivar potentially tolerant or resistant at Dire Dawa (Tony farm) was also found performing the same at Babile. In this study, for the majority of the plant data parameters used a highly significant ($p < 0.01$) difference was found among the studied breeding lines and commercial tomato cultivars. This is in agreement with several studies that reported significant differences for all characters studied among the tomato genotypes used (Pradeepkumar *et al*., 2001; Mohanty & Prusti, 2001; Golani *et al*., 2007; Regassa *et al*., 2012; Chernet & Zibelo, 2014).

The cultivar *Tisey* was found having quality fruit (Fig 6.2) characteristics such as highest SFW, higher MY per hectare and longer SL. However, *Tisey* productivity will be hampered in areas infested with mixtures of *M. incognita and M. javanica* species which is largely the case in field situations and this even with smaller *Pi* value. Although a significant difference was obtained between the cultivar *Tisey* and *Moneymaker* (the susceptible control) towards supporting reproduction, the absolute values of the *Pf* on *Tisey* were consistently high as on *Moneymaker* at both locations. On this cultivar RL, FSW, RS (at maturity) and FRW were found highly affected by the *Pi* compared to the other tomato cultivars studied indicating the importance of these parameters as a measure to nematode tolerance. A reduced rate of nutrient and water uptake was observed due to nematode damage on the root system (Anwar & Din, 1986). Plants top growth was found substantially reduced due to reduced nutrient and water uptake (Davis *et al*., 2003) and root length was reported to have a direct impact on foliage growth (Kamran, 2013). *Tisey* (out of the 33 tomato genotypes screened for resistance) was the only cultivar found resistant for the four aggressive populations screened under the growth chamber experiment. *Tisey* (with *Mi*-gene) was found from SR to HR during the growth chamber screening experiment for the tested populations (see chapter 4). However, the reaction of *Tisey* was found susceptible in both field trials (locations). The higher number of *Pf*, Galls/RS, EM/RS and more importantly Meggs/EM measured its susceptibility. The following four main reasons could be cited: firstly, the resistance found in the growth chamber experiment was only when it was challenged by a single population of

M. incognita or *M. javanica* species but in the field trials there were mixtures of these two species which could have led to synergistic reaction; secondly, the *Mi*-resistance in *Tisey* might have been broken due to the fact that the two field experimental locations were found with reasonably constant air temperatures above 28°C (threshold limit for the *Mi*-gene is 28°C soil temperature); thirdly, virulent individuals could have been present in the field and subsequently overcome the *Mi*-gene present in *Tisey*; and fourthly, apart from the nematode there are also many other biotic and abiotic factors involved in field conditions, which are impossible to control. Thus, with its current sensitive performance in *M. incognita* and *M. javanica* species mixtures *Tisey* is not a preferred cultivar to recommend for SSF of Ethiopia. However, *Tisey* has a very good fruit quality (for both consumption and commercial production) and it can be used as a scion with a nematode resistance rootstock.

The tomato cultivars *Assila* and *Eden* were found having the same resistance reaction for the four aggressive *M. incognita* and *M. javanica* populations tested under a growth chamber test. Both cultivars (with *Mi*-gene) had MR, S, HR and HR reactions against Jittu (*M. incognita*), Babile (*M. incognita*), Jittu (*M. javanica*) and Koka (*M. javanica*) populations respectively (see chapter 4). However, on the field trials, the Pf, RF, Galls/RS, RGI, EM/RS and Meggs/EM were slightly higher on the cultivar *Eden* over *Assila* even though there were no statistical differences between these two cultivars. Despite the contained *Mi* gene in these two cultivars they were found susceptible for the *M. incognita* Babille population during the growth chamber experiments (see chapter 4). However, in the Babile field trial (from which the Babile *M. incognita* population originated) it seems that this population was not found virulent as it had been found aggressive under the growth chamber trials for both cultivars. It could be that there might have been antagonistic reaction during the invasion of these two cultivars from other populations of the same species or other species. The cultivars *Assila* and *Eden* were found potential tomato cultivars to be promoted for tomato growers in Ethiopia. These cultivars can easily adapt to climatic and edaphic conditions of Ethiopia as these cultivars have already been successfully used in the neighbouring countries such as Kenya, Uganda and Tanzania through Seminis (MONSANTO). These cultivars were found resistant to the tomato yellow leaf-curling virus (TYLCV), which causes a common bottleneck for tomato production in Ethiopia (Eyasu, 2010). However, from our study, the cultivar *Assila* was observed with a higher unmarketable fruit number as compared to other cultivars. The problem of blossom end rot on their fruit and being preferred by birds over the other tomato cultivars are major reasons. It should therefore be recommended with extreme care and done

along with a soil fertility test. The blossom end rot is often linked to Calcium deficiency. Owing to the higher fruit quality and nematode resistance potential we recommend the cultivar *Assila* to be used for commercial greenhouse production (to get rid of the damage by birds and deficiency of calcium in soil). Thus, in areas with lower calcium soil content, *Assila* will be of limited importance especially if meant for commercial production. The cultivar *Eden* was with a higher MFr with no UnFr. The cultivar *Eden* could be promoted along with some crop rotation advices since it was not found resistant it supported nematode reproduction with reasonably high Meggs/EM.

The cultivar *Chochoro* was found supporting a medium level of nematode reproduction as measured by the *Pf*, Galls/RS, EM/RS and Meggs/EM in comparison with the susceptible check. *Chochoro* was found with higher MFr number and the highest MY per hectare despite supporting a good nematode reproduction. However, the MY was lower in Babile (Fethiya farm) as compared to Dire Dawa (Tony farm). There could be one main reason for the relative lower yield (55 ton/ha) at Babile compared to Dire Dawa (60 ton/ha). During the identification work (see chapter 3) on Babile location (Babile Erer and Babile Gende Sudan) *M. javanica* was found dominant over *M. incognita* though they both were also found co-infesting some samples. During the resistance screening in a growth chamber (see chapter 4) *Chochoro* was found susceptible for both *M. javanica* populations while it was SR for both *M. incognita* populations. Thus, the dominance of *M. javanica* might have brought the lower yield of *Chochoro* at Babile. Ethiopian SSF are widely producing *Chochoro* and representatives of Eastern Hararghe farmer's also recognized *Chochoro* during their visit (Fig 6.3). In agreement with the current study, this cultivar was previously found with higher MFr, longer SL, SFW and less UnFr (Chernet & Zibelo, 2014). Nevertheless, some advises should be forwarded to the growers not to grow '*Chochoro*' continuously without proper crop rotation practice with *Meloidogyne* non-host plants. This cultivar was found tolerant but not resistant (reasonable Meggs/EM was produced) (see chapter 4 and 7).

The breeding lines *CLN-2366A* and *CLN-2366B* were found potentially resistant as they allowed lower nematode multiplication and hampered the final *Pf* next to the cultivar *Assila*. During the growth chamber resistance screening these two breeding lines were found having resistance for the three populations tested except against Babile *M. incognita* population for which they were both susceptible (see chapter 4). However, more genetic improvement work is needed for these breeding lines. The FSP was far lower as compared to the commercial

tomato cultivars used. It has a shorter SL as its fruit was observed rotting 4 days after harvest if kept in the field conditions. A longer shelf life from 15 to 40days in different tomato lines was recently reported (Sinha *et al*., 2014). These breeding lines have a significantly lower SFW and FFP was almost daily and there was a minimal period between days to first harvest and final harvest. According to Chernet *et al*. (2014) these breeding lines were placed in Cluster I which was characterized by less number of fruit clusters per plant, low fruit set percentage, lower number of pickings, less single fruit weight and low total fruit yield per hectare. In our study, these breeding lines were also found sensitive to the aerial temperature in field conditions and needed a continuous supply of irrigation water. Generally, these breeding lines need to be genetically improved further before they go for commercial production and should fit to the tropical agriculture production conditions (climatic and edaphic) such as to Ethiopia. One of the greatest contributions of modern plant breeding is the genetical improvement of tomato varieties with increased disease resistance potential (Foolad, 2007). Some future genetic works are required to confirm the nature of resistant genes present in these breeding lines using a molecular marker and further transfer into the potential cultivars such as *Chochoro*. These two breeding lines can also be used as a rootstock on which a higher yielding tomato variety such as *Tisey* can be grafted.

In general, the parameters used to measure resistance seem to be straightforward as compared to parameters used to assess the tolerance of a cultivar. Nematode parameters can easily be used to measure the resistance potential of a tomato genotype. Particularly the mean number of eggs/EM was found a good measure of resistance from this study. The cultivar *Tisey* had a higher Meggs/EM while Assila, *CLN-2366A* and *CLN-2366B* had the lowest Meggs/EM compared to the susceptible check in both field locations. Surprisingly, there seems to be a variation in Meggs/EM between locations. For example except *Tisey* and *Moneymaker*, all the other five tomato genotypes were found having a higher Meggs/EM in Fethiya farm as compared to Tony farm. From these field trials, we have learnt that there is an urgent need to properly choose plant parameters that could reliably be used to measure the tolerance of a given cultivar for nematode infection. The nematode parameters used to measure resistance of the cultivar *Tisey* and the breeding lines *CLN-2366A* and *CLN-2366B* were straightforward comparing the reproduction on these genotypes. To assess their tolerance level, it was hard to find a straightforward plant parameter that could be used reliably across different tomato cultivars. If we consider for example the MY per hectare as a measure of tolerance, it will truly be misleading as it depends on the MFr number and SFW. The SFW of *Tisey* is much

higher compared to SFW of *CLN-2366A* and *CLN-2366B*. The difficult task on the measure of tolerance is at least from two directions: difficulty of assessing the genetic differences already present in a genotype and controlling the effect of other biotic and abiotic factors on the plant parameters used to assess the tolerance of a genotype for nematode infection. Especially, if we do not compare across locations, it will be challenging to single out the genotypes genetic difference per farm alone. From this study, the MY, PH, RL, FRW, FSP, TFr and MFr were found different across locations which may indicate the importance of these parameters as a measure of nematode tolerance. These parameters were found reduced in Babile (Fethiya farm) compared to Dire Dawa (Tony farm) which could be linked with the higher *Pi* at Babile.

Positive correlation of plant data parameters indicated that total number of fruits per plant, fruit set percentage, number of fruits per cluster, number of fruit clusters per plant and shape index are the most important fruit yield components which contribute to fruit yield per hectare (see Appendix Table). Thus, in tomato improvement programs fruit yield selection based on these data parameters is useful (Chernet & Zibelo, 2014). A highly positive correlation of RS and *Pi*, RL with FSW and FRW, FSW and FRW, *Pf* with Galls/RS and EM/RS, Galls/RS with EM/RS and Meggs/EM with *Pf*, Galls/RS and EM/RS at both locations that may indicate the importance of these characters as a measure of nematode tolerance and/or resistance. Even though there was a variation between the *Pi* values in the soil at the two locations, we did not find a significant difference towards nematode reproduction on the tested tomato cultivars. This has been shown by the inverse relationship between the *Pi* in the soil and nematode reproduction on the host plants (Charganei *et al*., 2012). However, several studies reported a positive relationship between the *Pi* of the nematode in the soil and the damage caused to host plants (Greco & Di Vito, 2009). This is also confirmed in our study where some plant parameters were found more affected in Babile compared to Dire Dawa. This field experiment was replicated once in time but more replications in time are necessitated as observed from a few data parameters that had a higher standard error. To sum up, tomato production in infested fields can be possible if further efforts are made towards resistance and tolerance breeding.

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6.5. References

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Chapter 7: Damage potential of *Meloidogyne incognita* populations on selected tomato genotypes in Ethiopia

Abstract

In Ethiopia, no information is available on the effect and relationship between initial population densities (*Pi*) of *M. incognita* and damage to tomato cultivars. Reliable data are required to relate the effect of a range of *Pi* on plant growth, biomass and yield for specific crop-nematode associations under local conditions. The effect of a series of *Pi* of Babile and Jittu *Meloidogyne incognita* populations on four tomato cultivars (*Assila*, *Chochoro*, *Moneymaker* and *Tisey*) and one clone (*CLN-2366B*) for growth and yield and the relationship with final population densities (*Pf*) were studied. Each tomato cultivar was inoculated with a geometric series of *Pi* (0, 0.125, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 and 256 J2/ 100 gram of dry soil) and was allowed to grow till the crop reaches senescing. The relationship between *Pi* and *Pf* was fitted to the Seinhorst population dynamics model ($Pf = (M * Pi) / (Pi + M/a)$) while the effect of *Pi* on different plant parameters considered was fitted to the Seinhorst yield model ($Y = Ymax*(m + (1 - m)* Z^((Pi-T)/T))$). Based on the damage model fitted to the data all the tested plant parameters were found negatively affected by both populations of *M. incognita.* The Jittu *M. incognita* population was found having more effect on the majority of parameters compared to Babile *M. incognita* population. As the reproduction factors (RF) obtained for the tested tomato cultivars were high the tested cultivars were considered as good host for both populations of *M. incognita*. The highest RF for *M. incognita* populations was obtained at lower *Pi* (0.125 J2/100 gram of dry soil) and reduced with increasing *Pi* on all the tested cultivars. Severity of root galling and number of egg masses per root system were increased with increasing inoculum levels of both populations of *M. incognita*. The tomato genotypes, *M. incognita* population and initial population density (*Pi*) had a highly significant ($P < 0.001$) effect on all the plant and nematode data parameters considered. The seedlings of all the tested tomato genotypes were dead at the higher *Pi* value (256 J2/100 gram of dry soil) except for the cultivar *Assila* of which seedlings survived even with the higher *Pi* values for both *M. incognita* populations. Among all the cultivars tested *Tisey* was found highly susceptible to both Babile and Jittu populations and all the seedlings were dead at ($Pi \geq 16$ J2/100 gram of dry soil) which was worse than the susceptible control *Moneymaker* where seedlings died at $Pi \geq 64$ J2/100 gram of dry soil. For all the plant parameters studied *Tisey* was found to have a lower damage threshold *T* while *Assila* (except for the parameter root weight) was having a higher *T*. A difference was observed for the tested tomato genotypes on their minimum yield (m) for the different plant parameters studied against Babile and Jittu *M. incognita* populations. Determination of *T* and *m* of a given crop variety for the prevailing *Meloidogyne* species in fields to be planted (local setting) is vital.

Keywords: damage, extrapolation, local setting, tolerance limit, minimum yield, *Meloidogyne incognita*, initial population density

7.1. Introduction

In Ethiopia, tomato (*Solanum lycopersicum*) is widely cultivated in both the rainy and dry season (using irrigation), on a range of farms (Mekete *et al*., 2003) and represents among the most profitable vegetable crops for small-scale farmers (Lemma *et al*., 1992). Nonetheless, the major RKN (*Meloidogyne incognita*, *M. javanica* and *M. ethiopica)* are found associated with tomato and often dramatically reduce its yield (Mandefro & Mekete, 2002; Abebe *et al*., 2015). However, little work has been done to determine the relationship between initial population densities (*Pi*) of *M. javanica* and damage to tomato and pepper in Ethiopia in small pots (Mekete *et al*. (2003). This finding also did not provide any information on the effect of these nematodes on yield and yield components of tomato genotypes. Information on crop-nematode relationship is vital for farmers to decide on economically feasible management strategies within their own crop production systems (Kamran *et al*, 2013). Such information is a prerequisite to design nematode management strategies and advisory programs (Barker & Nusbaum, 1971). Hence, reliable data are required to relate the effects of a range of initial nematode densities to plant growth, biomass and yield for specific crop-nematode associations under local conditions. Therefore, the objectives of this study were: 1) to determine the damage thresholds of *Meloidogyne incognita* populations originating from Babile and Jittu locations of Ethiopia on tomato cultivars and one clone for growth and yield reduction, and 2) to study the initial (*Pi*) and final population (*Pf*) relationship of *M. incognita* populations on different tomato genotypes quantitatively.

7.2. Materials and Methods

7.2.1. Tomato genotypes and Soil Solarisation

Tomato genotypes *Assila*, *CLN-2366B*, *Chochoro* and *Tisey* were chosen based on their resistance potential from the growth chamber experiment assay (see chapter 4), preference by SSF of Ethiopia and the field tolerance and resistance trial. The tomato cv. *Moneymaker* was used as a susceptible control. For this experiment a field soil was collected, placed on and covered with a large polythene sheet for 10 consecutive weeks for solarisation at Tony farm, Dire Dawa. The soil was inverted and homogenized every two weeks and covered again with the polythene sheet. The average daily soil temperature inside the polythene sheet was calculated as 38°C during this period. After solarisation, the presence or absence of any PPN were checked after extracting 10 different subsamples (100gram). The absence of live PPN in the soil was confirmed before commencing the actual experiment.

7.2.2. Culturing and Inoculation of *Meloidogyne incognita* Populations

The aggressive 'Jittu' and 'Babile' populations of *M. incognita* (see chapter 4) were used to examine damage potential and *Pi-Pf* relationship on selected tomato genotypes. A stock culture of these *M. incognita* populations originating from Ethiopia were reproduced on tomato (*Solanum lycopersicum*) cv. *Moneymaker* in bigger pots (8000 cm^3 capacity) containing steam sterilized soil (121°C for 2hrs) and maintained in a greenhouse adjusted to 23 ± 3°C for ten weeks. Then, inoculum was prepared from heavily galled tomato roots after chopping them into smaller pieces (approximately 2cm) being placed on a modified Baermann funnel using locally available plastic pans. Every 24hrs, hatched J2 were collected for 6 subsequent days and stored at 10°C in the refrigerator until use. Every 24hrs the water in the pan was replenished with fresh tap water to maintain the J2s fitness and facilitate aeration. Tomato seedlings were raised on a seedling tray using steam sterilized soil (121°C for 2hrs). The four leaf-stage tomato seedlings were transplanted into 6000cm^3 soil volume capacity pots. Two seedlings were transplanted per pot and later thinning was performed when both established to obtain one plant per pot. Plants were watered as required. The pots were kept in the open field Tony Farm (Dire Dawa). The average maximum temperature recorded during the experimental period was 36.4 °C (see chapter 6). Ten days after transplanting, the pots were inoculated at a geometric series of 12 nematode densities (*Pi*) of *M. incognita* 'Jittu' and 'Babile' populations ranging from 0, 0.125, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 to 256 J2s per 100 gram of dry soil (Norshie *et al*., 2011). Three holes were bored around the four-leaf stage tomato plant and 15 ml of the nematode suspension per plant was directly injected into the three holes using a calibrated pipette. Control plants received a similar volume of fresh tap water. The pots were arranged in a completely randomized design. Each *Pi* was replicated four times for both populations of *M. incognita* on each tomato genotype.

7.2.3. Data Collection

The following nematode and plant data parameters were recorded:

Nematode data parameters:-

Final population density (*Pf*): The final *Pf* was estimated from organic (root) and mineral fraction (soil) per pot. The mean number of J2 and eggs in the roots was estimated from the whole root system after extracting nematodes from a subsample of 5gram roots per plant based on Hussey and Barker (1973. The mean number of J2 and eggs from the 6000cm3 soil was estimated after proper homogenization of the soil using a sub-sample of 100cm^3 soil per pot. Nematodes from soil samples were extracted using a modified Baermann funnel technique. It was expressed as (J2+eggs) per 100 gram of dry soil.

Reproduction factor (RF): determined by dividing *Pf* by *Pi*.

Number of root galls per plant (Galls/RS): the total number of galls present per plant root system per pot was counted.

Number of egg masses per plant (EM/RS): the total number of egg masses present per plant root system per pot was counted.

Root gall index (RGI) and **egg mass index (EMI):** these indices were determined per plant from each pot and based on Taylor & Sasser (1978) scored from 0 to 5 scale; where 0 = no galls/egg masses; 1 = 1-2 galls/egg masses; 2 = 3-10 galls/egg masses; 3 = 11-30 galls/egg masses; 4 = 31-100 galls/egg masses and 5 = >100 galls/egg masses.

Plant parameters:-

Plant height (PH): measured from the soil level to the main apex of the plant and mean values were expressed in centimetres.

Number of flowers per plant (NFPP): the total number of flowers per plant per pot was counted.

Root weight (RW): the total weight of roots per plant was taken after removing the adhering soil and expressed in grams. RW was measured after the final fruit harvesting time.

Root length (RL): the adhering soil from the roots was gently washed away using tap water and excess water was removed after blotting with tissue paper. The root length per plant was measured from the soil level to the tip of roots and expressed in centimetres.

Total fruit number per plant (TFr): the total number of fruits (including marketable and unmarketable fruits) per plant per pot was counted.

Marketable fruit number per plant (MFr): the number of healthy fruits that were free from any visible damage symptom starting from the first harvest to the final harvest per plant per pot was counted and later their mean was computed.

Single fruit weight (SFW): three tomato fruits per plant per pot were randomly picked, their weight was measured separately using a sensitive balance and their mean was expressed in grams.

Number of seeds per fruit (NSPF): the three randomly picked fruits to determine SFW were used to extract seeds and counted separately and later their mean was calculated.

7.2.4. Data Analysis and Model Fitting

Non-linear regression analysis was carried out to estimate parameters of the yield, damage and *Pi-Pf* relationship using a script written in Tinn-R version 4.0.2.1 and run in R version 3.2.2. The relationship between ranges of initial population densities (*Pi*) and the plant growth damage of the two (Babile and Jittu) aggressive *M. incognita* populations was

described using the Seinhorst yield loss model (Seinhorst, 1986; Schomaker & Been, 2013). The model (Equ.1) was fitted to the data using least square methods to estimate the parameters of plant damage.

Y = Ymax*(m + (1 - m)* Z^((*Pi*-T)/T)) when $Pi \geq T$, and Y= Ymax ($Pi \leq T$)…. (Equation 1)

Where 'Y' is the relative average value of plant weight; '*m*' the relative minimum value of Y at a very large *Pi*; '*T*' the tolerance limit (the initial nematode density below which plant growth is not affected); and 'Z' a constant < 1 indicating nematode damage in which $Z^{-T} = 0.95$. The coefficient of determination (R^2) adjusted for degrees of freedom (df) was used to indicate the goodness-of-fit of the model. The population dynamics model for migratory nematodes with multiple generation developed by Seinhorst (1970) and as described by Schomaker & Been (2013) was used to fit the model (Equ.2) to the data of the final population density (*Pf*) and estimate population dynamics parameters; the maximum multiplication rate (*a*) and maximum population density (*M*) using the least square methods.

The population dynamics model used is $Pf = (M * Pi) / (Pi + M/a)$…(Equation 2).

A one-way ANOVA was performed between the tomato varieties and each growth, biomass, yield, yield component and nematode data parameter to measure the damage of the tomato varieties and population dynamics of *M. incognita* populations at 5% level of significance using SPSS 22 statistical software package. A factorial ANOVA was also performed between tomato genotypes, *M. incognita* populations and initial population densities (*Pi*) at 5% level of significance. Data were log transformed when they failed to satisfy the assumption of ANOVA.

7.3. Results

7.3.1. Nematode Parameters

The tomato genotype, *M. incognita* populations and initial population densities (*Pi*) had a highly significant effect on *Pf* ($F = 1121.74$, $p < 0.001$; $F = 358.63$, $p < 0.001$; $F = 950.83$, $p < 0.001$), RF ($F = 3110.68$, $p < 0.001$; $F = 652.92$, $p < 0.001$; $F = 2212.00$, $p < 0.001$), Galls/RS ($F = 629.09$, $p < 0.001$; $F = 204.28$, $p < 0.001$; $F = 313.30$, $p < 0.001$) and EM/RS ($F = 1005.14$, $p < 0.001$; $F = 202.54$, $p < 0.001$; $F = 321.13$, $p < 0.001$) respectively. The interaction effect was significantly lower compared to the main effects.

The highest RF for both *M. incognita* populations was obtained on all the tested cultivars at lower *Pi* (0.125 J2/ 100 gram of soil) and reduced with increasing *Pi.* The RF of Babile *M.*

incognita population ranged from 1.80 (at *Pi* = 256 J2/100 gram of soil to 62.87 (*Pi* = 0.125J2/100 gram of soil) for *Assila*, 5.80 (*Pi* = 128 J2g) to 103.30 (*Pi* = 0.125J2) for *CLN-2366B*, 2.00 (*Pi* = 128J2) to 145.50 (*Pi* = 0.125) for *Chochoro*, 33.30 (*Pi* = 32 J2) to 990.30 (*Pi* = 0.125J2) for *Moneymaker* and 63.00 (Pi = 16J2) to 793.00 (*Pi* = 0.125J2) for the cultivar *Tisey*. Similarly, the RF of Jittu *M. incognita* population was found in a range of 3.00 (*Pi* = 256J2/100 gram of soil to 148.50 (*Pi* = 0.125J2/100 gram of soil) for *Assila*, 6.00 (*Pi* = 128 J2g) to 280.30 (*Pi* = 0.125J2) for *CLN-2366B*, 10.00 (*Pi* = 64 J2) to 256.00 (*Pi* = 0.125) for *Chochoro*, 37.00 (*Pi* = 32 J2) to 1358.30 (*Pi* = 0.125J2) for *Moneymaker* and 122.00 (*Pi* = 8 J2) to 1229.50 (*Pi* = 0.125J2) for the cultivar *Tisey*. The highest value for all the nematode data parameters considered (Pf, number of Galls/RS, number of EM/RS, RGI and EMI) were found at their respective higher *Pi* values while the lowest was recorded from the lower *Pi* (0.125J2/100gram of dry soil) value. The severity of root galling and number of egg masses per root system were found increased with increasing inoculum levels of both populations of *M. incognita*. The RGI (= 5) and EMI (= 5) were found to be highest in *Tisey* (at $Pi \geq 8$ for Babile and at $Pi \geq 4$ for Jittu population) and *Moneymaker* (at $Pi \geq 4$ for both populations). The RGI (= 4) and EMI (= 4) were found high on *Assila* and *CLN-2366B* at $Pi \geq 128$ for both Jittu and Babile populations of *M. incognita*. The values of the population dynamics parameters (*a* and *M*) are presented in (Table 7.1). The population dynamics model fitted to the data are presented in Fig 7.1. Based on the population dynamics model curve all the tested tomato cultivars are a host for the studied populations.

Table 7.1. Parameter estimations of the population dynamics model for *Meloidogyne incognita* (Babile and Jittu) populations on five selected tomato varieties

Tomato Cultivar	*M. incognita* populations	N	*a*	*M*	SE_a	SE_M	R^2	df
Assila	Babile	11	12.59	356.62	1.83	81.63	0.96	9
	Jittu	11	36.94	371.75	5.39	59.00	0.96	9
CLN2366B	Babile	10	23.26	556.41	3.62	154.38	0.96	8
	Jittu	10	89.24	372.87	11.00	41.28	0.97	8
Chochoro	Babile	9	56.16	278.73	6.87	37.64	0.97	7
	Jittu	9	68.96	311.53	9.75	46.72	0.96	7
Moneymaker	Babile	8	235.04	274.03	84.08	64.98	0.75	6
	Jittu	8	276.82	274.32	106.32	64.49	0.72	6
Tisey	Babile	7	151.89	395.96	31.24	95.84	0.92	5
	Jittu	6	294.23	348.26	91.04	100.88	0.84	4

'*N*', number of observations; '*a*' maximum rate of multiplication; '*M*' maximum population density; SE_a, standard error for *a*; SE_M, standard error for *M*; 'R^2'-coefficient of determination and 'df'-degree of freedom

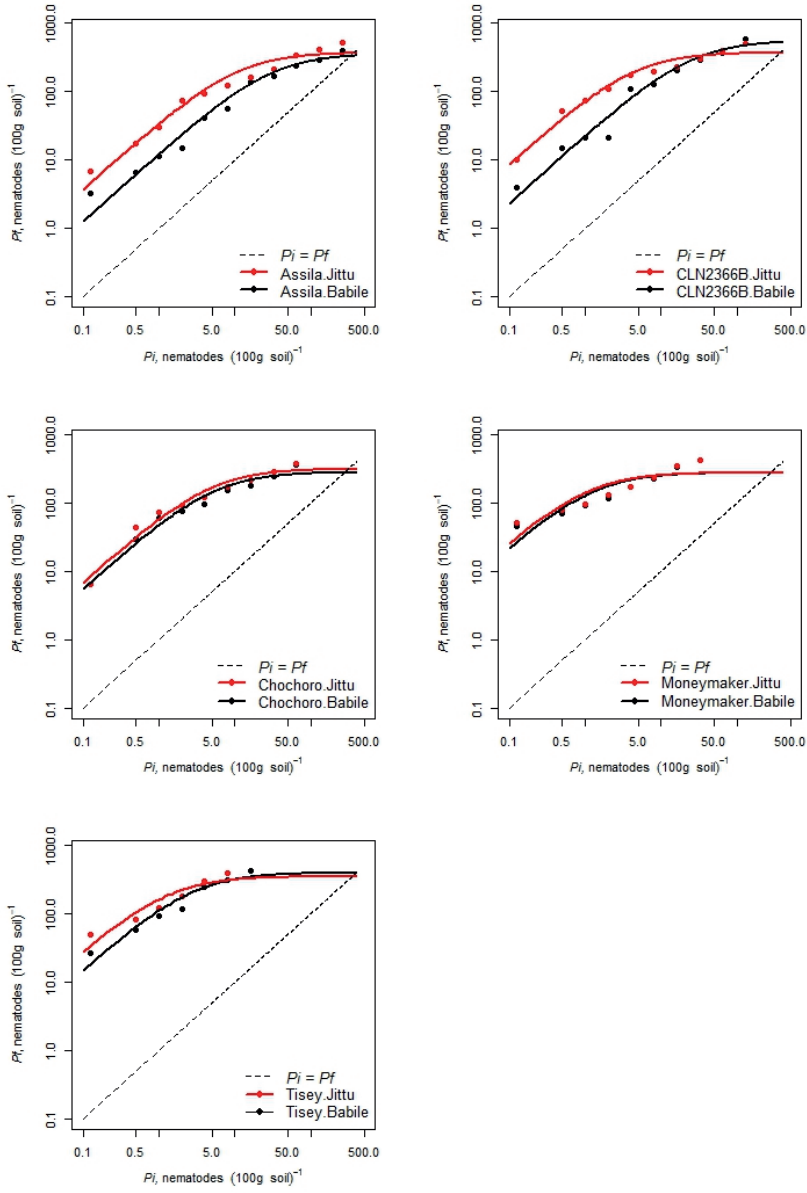


Figure 7.1. The relationship between *Pi* and *Pf* of *Meloidogyne incognita* (Babile and Jittu) populations on five tomato genotypes: *Assila*, *CLN-2366B*, *Chochoro*, *Moneymaker* and *Tisey*. The data were fitted to the equation $Pf = (M * Pi) / (Pi + M/a)$, where '*Pf*' is the final population density, '*M'* the maximum population density and '*a*' maximum multiplication rate of the nematode. Solid lines in each graph represent 50% quintile of the *M. incognita* (Babile and Jittu) populations as marked with different colours. The dotted line is the equilibrium line where *Pf =Pi*.

7.3.2. Plant Parameters

The tomato genotypes, *M. incognita* populations and initial population densities (*Pi*) had a significant effect on PH ($F = 236.29$, $p < 0.001$; $F = 98.64$, $p < 0.001$; $F = 409.37$, $p < 0.001$), NFPP ($F = 723.85$, $p < 0.001$; $F = 43.49$, $p < 0.001$; $F = 237.28$, $p < 0.001$), RW ($F = 344.01$, $p < 0.001$; $F = 7.79$, $p < 0.001$; $F = 484.89$, $p < 0.001$); RL ($F = 110.84$, $p < 0.001$; $F = 0.30$, $p = 0.586$; $F = 382.39$, $p < 0.001$), TFr ($F = 134.71$, $p < 0.001$; $F = 29.78$, $p < 0.001$; $F = 276.56$, $p < 0.001$), MFr ($F = 181.84$, $p < 0.001$; $F = 34.88$, $p < 0.001$; $F = 273.59$, $p < 0.001$), SFW ($F = 474.75$, $p < 0.001$; $F = 4.61$, $p < 0.05$; $F = 295.49$, $p < 0.001$) and NSPF ($F = 29.38$, $p < 0.001$; $F = 0.16$, $p = 0.691$; $F = 24.60$, $p < 0.01$) respectively.

The interaction effect of these factors was not significant for some of the plant parameters, however, when found it was much lower compared to the main effects. The effect of the *Pi* ranges and *M. incognita* populations on the different plant parameters of the tested tomato cultivars are presented from Fig 7.2 through Fig 7.9. Based on the damage model fitted to the data all the tested plant parameters were found negatively affected by both populations of *M. incognita.* The Jittu *M. incognita* population was found having more effect on the majority of parameters compared to Babile *M. incognita* population.

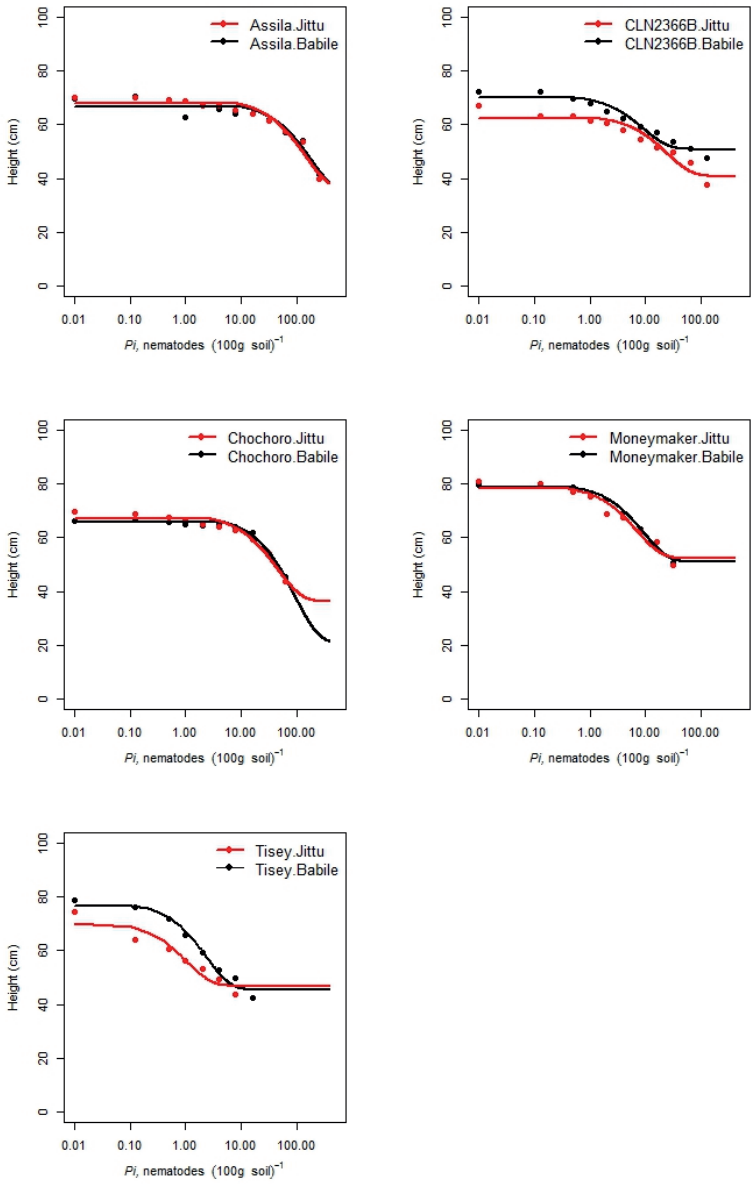


Figure 7.2. The relationship between the initial population density *(Pi)* of *Meloidogyne incognita* populations and tomato cultivars: plant height (PH) in centimetres (cm). Plants were grown in big pots in the open field experimental station at Tony farm, Dire Dawa. Plants were harvested after 90days and each data point in the graph represents a mean of four plants and the line is the predicted function obtained when the data were fitted to the Seinhorst Model (Y = Ymax*(m + (1 - m)* Z^((*Pi*-T)/T)), where 'Ymax' is the maximum yield at $Pi \leq T$ and 'Y', is yield in terms of any weight, '*m'* the minimum relative yield, '*T'* the tolerance limit and 'Z' a constant <1.

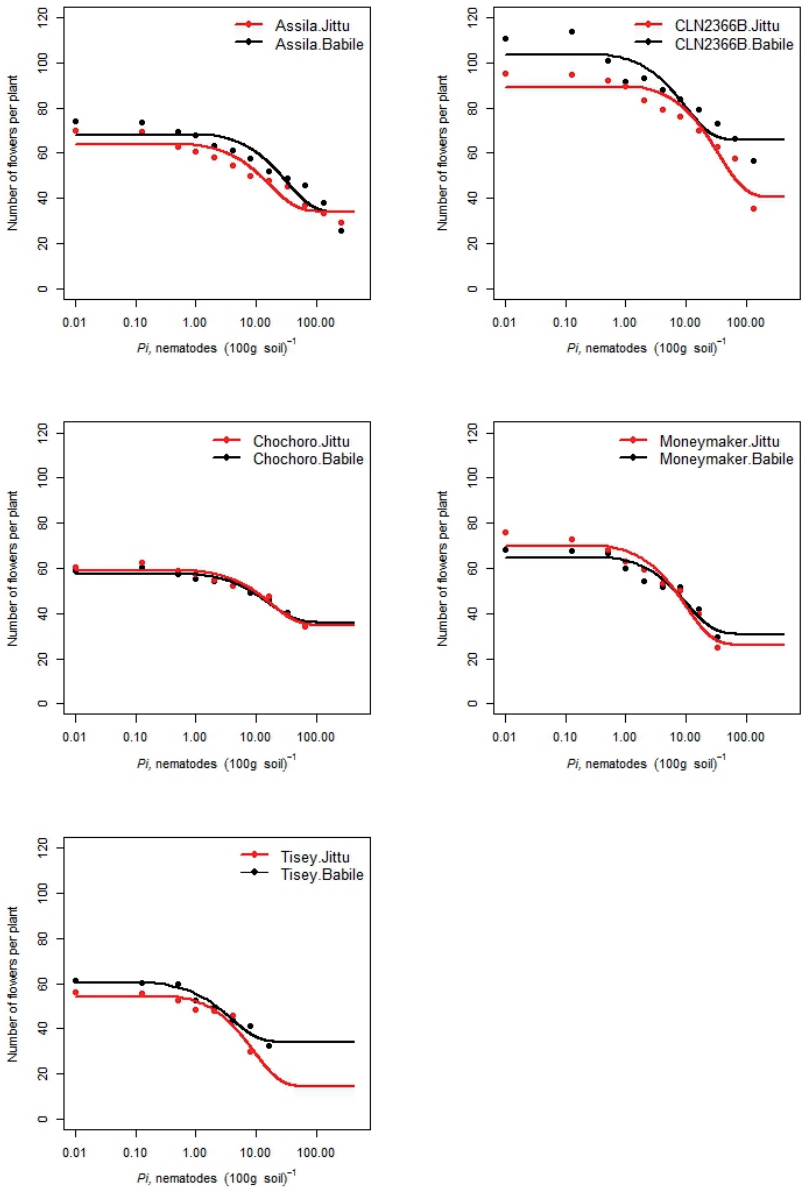


Figure 7.3. The relationship between the initial population density *(Pi)* of *Meloidogyne incognita* populations and tomato cultivars: number of flowers per plant (NFPP). Plants were grown in big pots in the open field experimental station at Tony farm, Dire Dawa. Plants were harvested after 90days and each data point in the graph represents a mean of four plants and the line is the predicted function obtained when the data were fitted to the Seinhorst Model (Y = Ymax*(m + (1 - m)* Z^((*Pi*-T)/T)), where 'Ymax' is the maximum yield at $Pi \leq$ T and 'Y', is yield in terms of any weight, '*m'* the minimum relative yield, '*T'* the tolerance limit and 'Z' a constant <1.

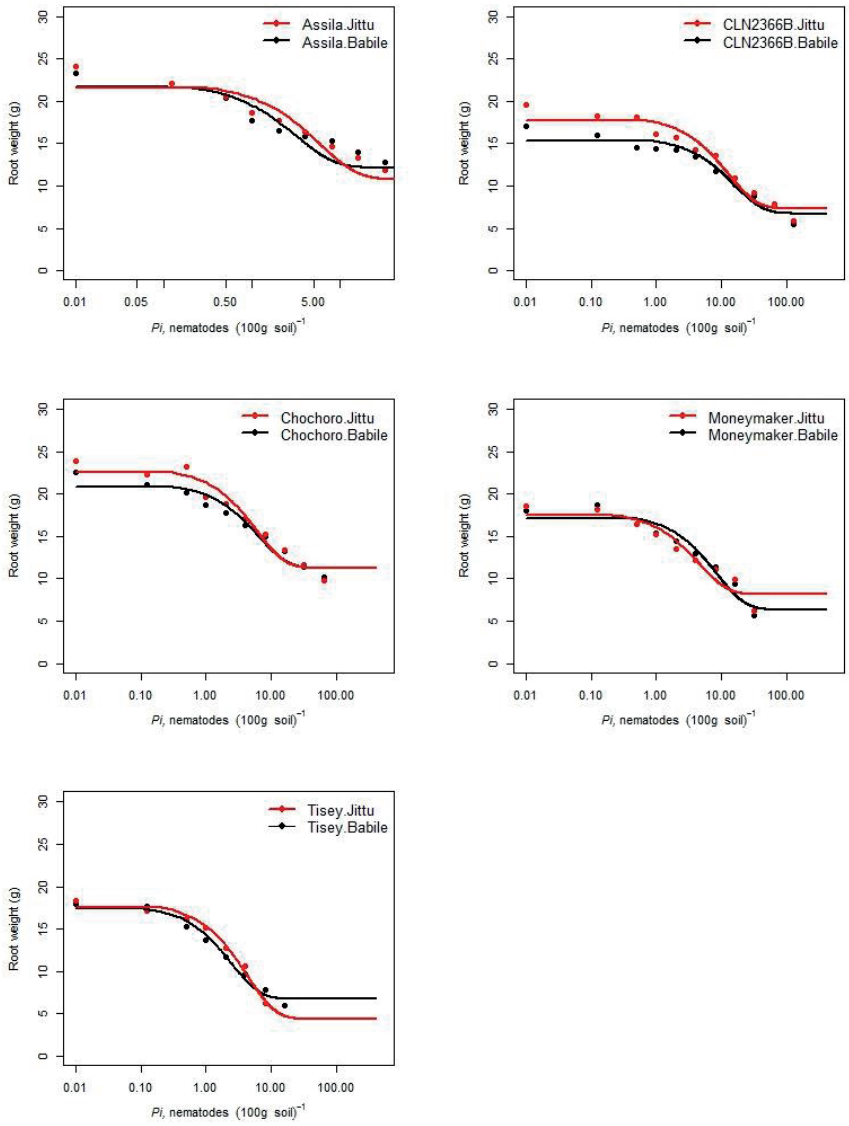


Figure 7.4. The relationship between the initial population density *(Pi)* of *Meloidogyne incognita* populations and tomato cultivars: root weight (RW) in grams (g). Plants were grown in big pots in the open field experimental station at Tony farm, Dire Dawa. Plants were harvested after 90days and each data point in the graph represents a mean of four plants and the line is the predicted function obtained when the data were fitted to the Seinhorst Model (Y = Ymax*(m + (1 - m)* Z^((*Pi*-T)/T)), where 'Ymax' is the maximum yield at $Pi \leq$ T and 'Y', is yield in terms of any weight, '*m'* the minimum relative yield, '*T'* the tolerance limit and 'Z' a constant <1.

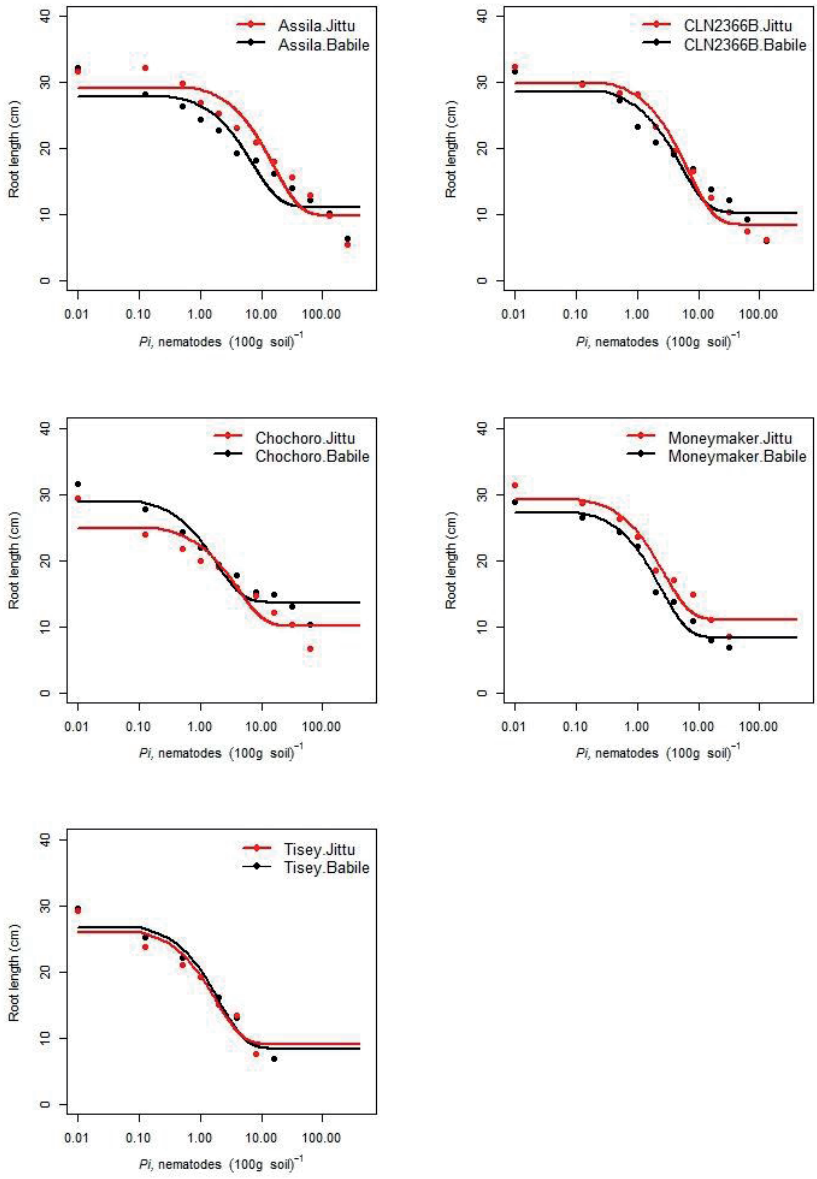


Figure 7.5. The relationship between the initial population density *(Pi)* of *Meloidogyne incognita* populations and tomato cultivars: root length (RL) in centimetres (cm). Plants were grown in big pots in the open field experimental station at Tony farm, Dire Dawa. Plants were harvested after 90days and each data point in the graph represents a mean of four plants and the line is the predicted function obtained when the data were fitted to the Seinhorst Model (Y = Ymax*(m + (1 - m)* Z^((*Pi*-T)/T)), where 'Ymax' is the maximum yield at $Pi \leq$ T and 'Y', is yield in terms of any weight, '*m'* the minimum relative yield, '*T'* the tolerance limit and 'Z' a constant < 1.

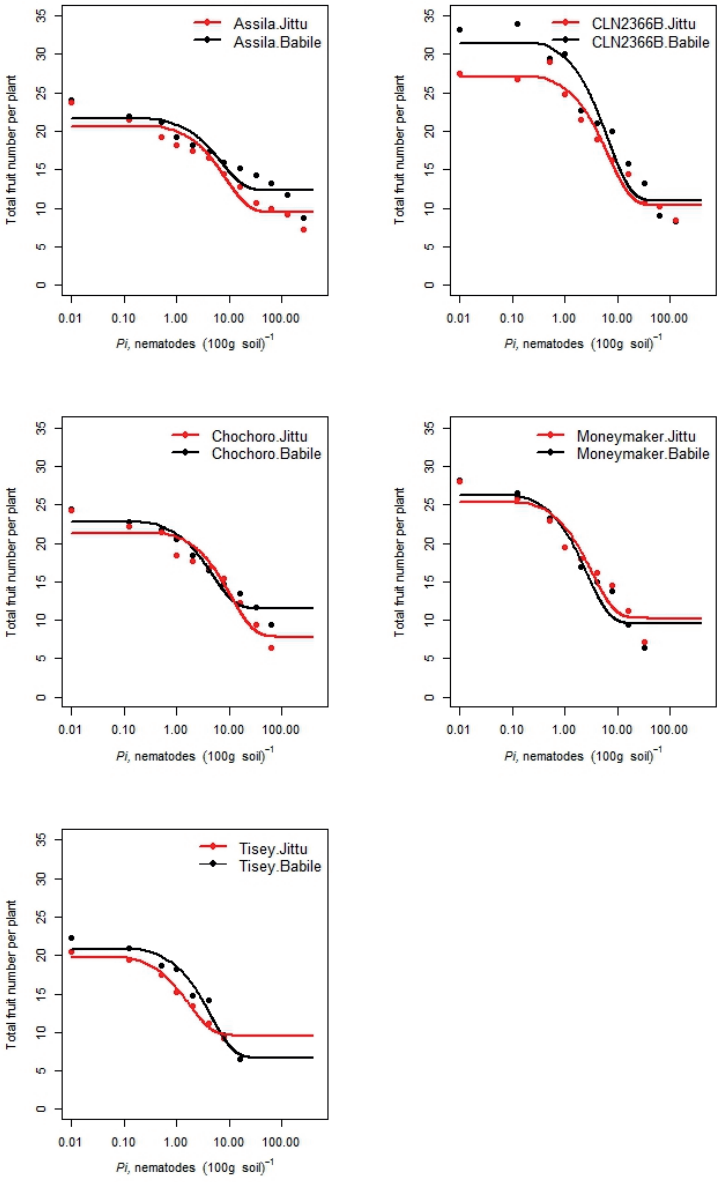


Figure 7.6. The relationship between the initial population density *(Pi)* of *Meloidogyne incognita* populations and tomato cultivars: total number of fruits per plant (TFr). Plants were grown in big pots in the open field experimental station at Tony farm, Dire Dawa. Plants were harvested after 90days and each data point in the graph represents a mean of four plants and the line is the predicted function obtained when the data were fitted to the Seinhorst Model (Y = Ymax*(m + (1 - m)* Z^((*Pi*-T)/T)), where 'Ymax' is the maximum yield at $Pi \leq$ T and 'Y', is yield in terms of any weight, '*m'* the minimum relative yield, '*T'* the tolerance limit and 'Z' a constant <1.

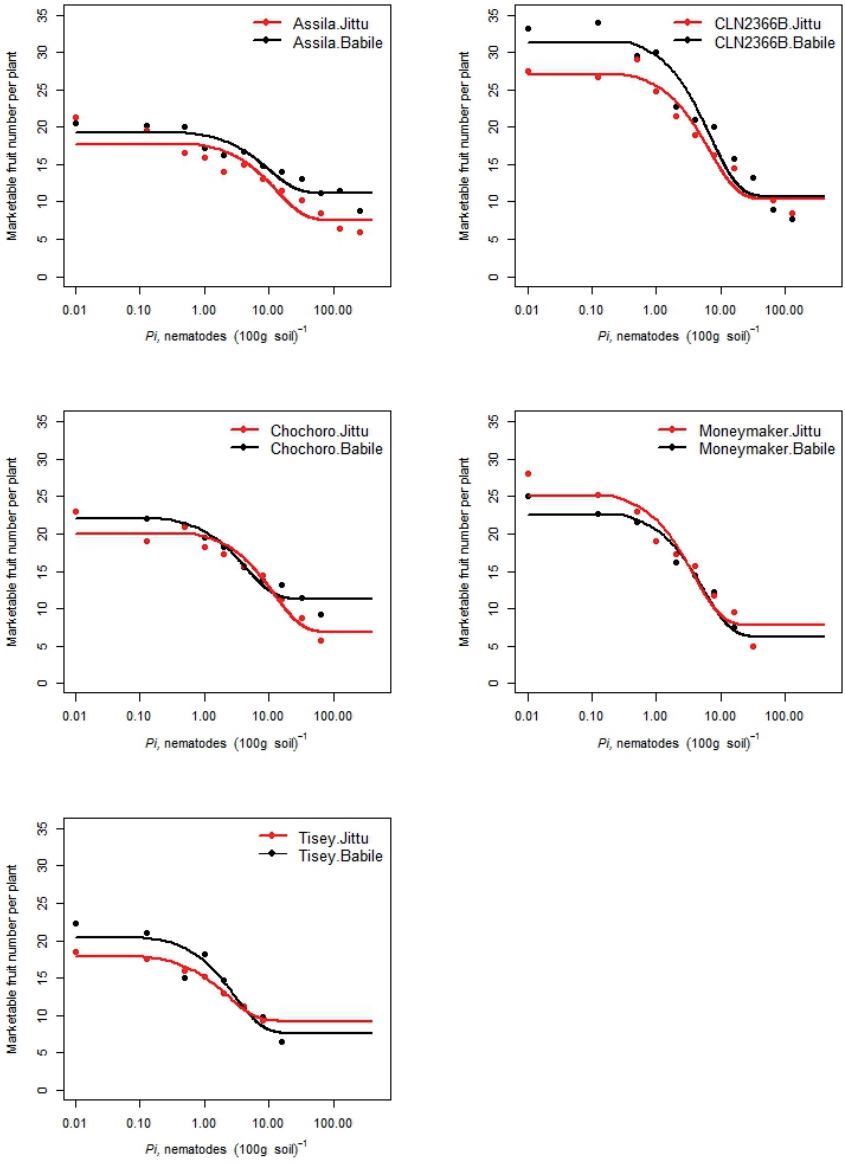


Figure 7.7. The relationship between the initial population density *(Pi)* of *Meloidogyne incognita* populations and tomato cultivars: marketable fruit number per plant (MFr). Plants were grown in big pots in the open field experimental station at Tony farm, Dire Dawa. Plants were harvested after 90days and each data point in the graph represents a mean of four plants and the line is the predicted function obtained when the data were fitted to the Seinhorst Model (Y = Ymax*(m + (1 - m)* Z^((*Pi*-T)/T)), where 'Ymax' is the maximum yield at $Pi \leq T$ and 'Y', is yield in terms of any weight, '*m'* the minimum relative yield, '*T'* the tolerance limit and 'Z' a constant <1.

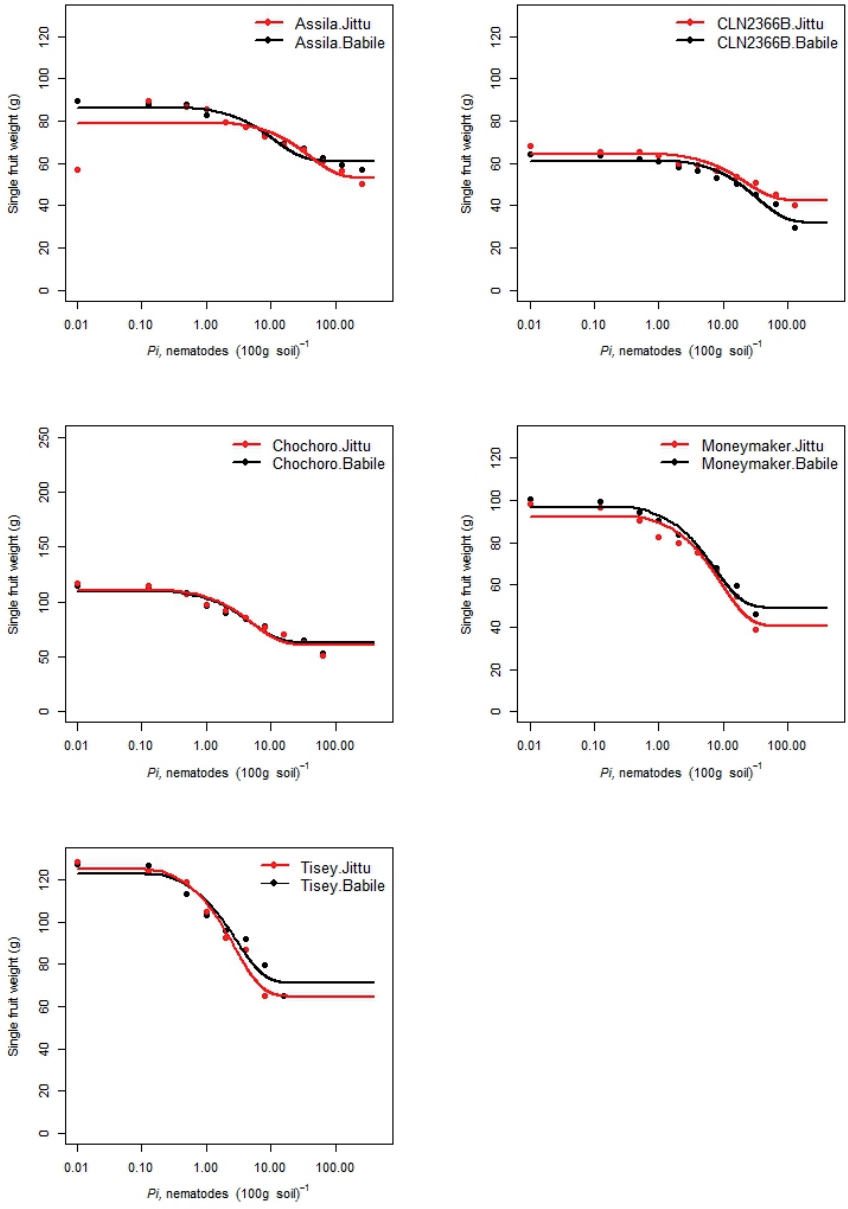


Figure 7.8. The relationship between the initial population density *(Pi)* of *Meloidogyne incognita* populations and tomato cultivars: single fruit weight (SFW) in grams (g). Plants were grown in big pots in the open field experimental station at Tony farm, Dire Dawa. Plants were harvested after 90days and each data point in the graph represents a mean of four plants and the line is the predicted function obtained when the data were fitted to the Seinhorst Model (Y = Ymax*(m + (1 - m)* Z^((*Pi*-T)/T)), where 'Ymax' is the maximum yield at *Pi* ≤ T and 'Y', is yield in terms of any weight, '*m'* the minimum relative yield, '*T'* the tolerance limit and 'Z' a constant <1.

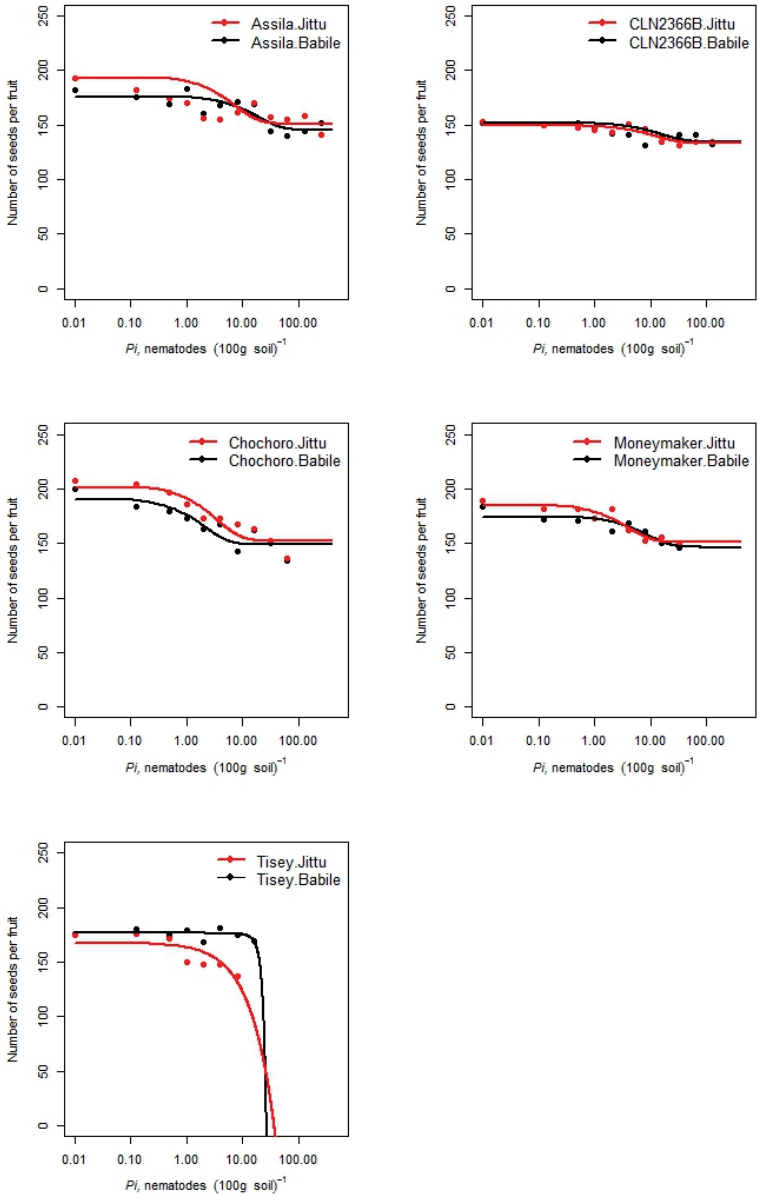


Figure 7.9. The relationship between the initial population density *(Pi)* of *Meloidogyne incognita* populations and tomato cultivars: number of seeds per fruit (NSPF). Plants were grown in big pots in the open field experimental station at Tony farm, Dire Dawa. Plants were harvested after 90days and each data point in the graph represents a mean of four plants and the line is the predicted function obtained when the data were fitted to the Seinhorst Model (Y = Ymax*(m + (1 - m)* Z^((*Pi*-T)/T)), where 'Ymax' is the maximum yield at $Pi \leq$ T and 'Y', is yield in terms of any weight, '*m'* the minimum relative yield, '*T'* the tolerance limit and 'Z' a constant <1.

7.3.3. The Tolerance Limit (*T*) and Minimum Yield (*m*)

For the plant height, the highest *T* was attained by the cultivar *Assila* (9.59 and 7.72J2/100 gram of dry soil) for Babile and Jittu *M. incognita* populations respectively. The lowest *T* was recorded from *Tisey* (0.12 and 0.06J2/100 gram of dry soil) for Babile and Jittu *M. incognita* populations respectively. Similarly for NFPP, the highest *T* was obtained from *Assila* (1.66 and 0.88J2/100 gram) while the lowest was from *Tisey* (0.20 and 0.47J2/100 gram of dry soil) for Babile and Jittu *M. incognita* populations respectively. For the parameter RW, the highest *T* was attained on *CLN-2366B* (0.83 and 0.67J2/100 gram of dry soil) while the lowest was from *Tisey* (0.13 and 0.22/100 gram) for Babile and Jittu *M. incognita* populations respectively. For RL, the highest *T* was obtained on *Assila* (0.38 and 0.83J2/100 gram of dry soil) while the lowest was on *Tisey* (0.11 and 0.09J2/100 gram of dry soil) for Babile and Jittu *M. incognita* populations respectively. The highest *T* for the parameter TFrNPP was obtained from *Chochoro* (0.57J2/100 gram of dry soil) and the lowest *T* was from *Tisey* (0.09J2/100 gram of dry soil) by Jittu *M. incognita* population. The highest *T* for MFrNPP was recorded on *Assila* (0.52 and 0.69J2/100 gram of dry soil) and the lowest on *Tisey* (0.15 and 0.12J2/100 gram of dry soil) for Babile and Jittu *M. incognita* populations respectively. The highest *T* for SFW was from *Assila* (0.60 and 2.12/100 gram) and *CLN-2366B* (1.79 and 1.17/100 gram) while the lowest *T* was on *Tisey* (0.15 and 0.14J2/100 gram of dry soil) for Babile and Jittu *M. incognita* populations respectively. The highest *T* for NSPF was obtained on *Assila* (1.00J2/100 gram of dry soil) while the lowest *T* was obtained from *Chochoro* (0.11J2/100 gram dry soil) for Babile *M. incognita* population. In general, for all the plant parameters studied *Tisey* was found with a lower *T* while *Assila* (except for the parameter RW) was having a higher *T*.

The highest minimum yield (*m*) for the parameter PH was obtained on *CLN-2366B* (0.72) while the lowest *m* was from *Chochoro* (0.31) for Babile *M. incognita* population. For the parameter NFPP, the highest *m* was obtained from *Chochoro* and *CLN-2366B* (0.63) for Babile *M. incognita* population while the lowest *m* was on *Tisey* (0.26) for Jittu *M. incognita* population. For the parameter RW, the highest *m* (0.56) was obtained from cultivar *Assila* for Babile *M. incognita* population while the lowest *m* was on *Tisey* (0.25) for Jittu *M. incognita* population. For the parameter RL, the highest *m* was from *Chochoro* (0.47) while the lowest *m* was from *Moneymaker* (0.30) for Babile *M. incognita* population. The highest *m* for TFrNPP was obtained from *Assila* (0.57) while the lowest *m* was recorded on *Tisey* (0.32) for Babile *M. incognita* population. For MFrNPP, the highest *m* was achieved from *Assila* (0.58) while the lowest *m* was from *Moneymaker* (0.27) for Babile *M. incognita* population. The

highest *m* for SFW was obtained from *Assila* (0.71) for Babile *M. incognita* while the lowest *m* was from *Moneymaker* (0.44) for Jittu *M. incognita* population. The highest *m* for NSPF was from *CLN-2366B* (0.89) for both Babile and Jittu *M. incognita* populations while the lowest *m* was from *Chochoro* (0.76) Jittu *M. incognita* population. A difference was observed for the tested tomato genotypes on their tolerance limit (*T*) and minimum yield (m) for the different plant parameters studied against Babile and Jittu *M. incognita* populations (Table 7.2).

Table 7.2. Parameter estimates of the tolerance limit (*T*) and minimum relative yield (*m*) of the Seinhorst equation for the relationship between plant weight with a range of initial population densities of two (Babile and Jittu) aggressive *M. incognita* populations originated from Ethiopia on five selected tomato cultivars.

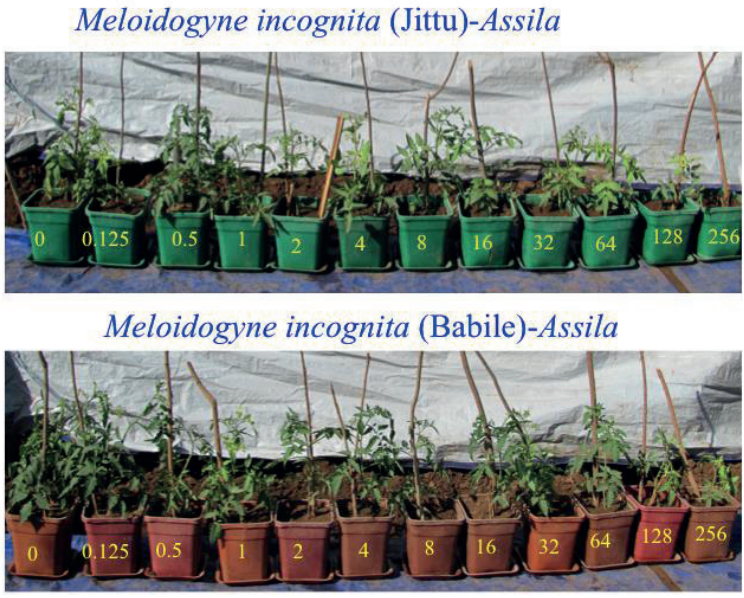
Tomato cultivars	*M. incognita* population	*m*	*T*	Ymax	SE_m	SE_T	SEY_{max}	R^2	df
		Plant Height (cm)							
Assila	Babile	0.51	9.59	66.71	0.18	5.79	1.21	0.89	10
	Jittu	0.52	7.72	67.94	0.10	3.03	0.98	0.94	10
CLN-2366B	Babile	0.72	0.49	70.15	0.03	0.23	1.66	0.93	9
	Jittu	0.65	1.29	62.38	0.06	0.79	1.72	0.89	9
Chochoro	Babile	0.31	4.94	65.67	0.18	1.54	0.47	0.98	8
	Jittu	0.54	2.38	67.21	0.12	1.01	0.93	0.95	8
Moneymaker	Babile	0.65	0.49	78.62	0.02	0.10	0.86	0.98	7
	Jittu	0.67	0.37	78.32	0.05	0.18	2.05	0.93	7
Tisey	Babile	0.59	0.12	76.78	0.03	0.04	2.06	0.97	6
	Jittu	0.67	0.06	69.93	0.06	0.04	4.15	0.85	5
		Number of Flowers Per Plant							
Assila	Babile	0.50	1.66	68.05	0.09	1.35	3.38	0.86	10
	Jittu	0.53	0.88	63.70	0.06	0.59	3.03	0.88	10
CLN2366B	Babile	0.63	0.51	103.49	0.06	0.47	5.47	0.82	9
	Jittu	0.45	1.81	88.94	0.11	1.07	3.48	0.89	9
Chochoro	Babile	0.63	0.85	57.46	0.05	0.29	1.12	0.95	8
	Jittu	0.59	0.92	58.86	0.06	0.35	1.38	0.93	8
Moneymaker	Babile	0.48	0.54	64.82	0.12	0.33	2.83	0.88	7
	Jittu	0.37	0.52	69.65	0.12	0.26	3.09	0.91	7
Tisey	Babile	0.57	0.20	60.29	0.06	0.08	2.00	0.94	6
	Jittu	0.26	0.47	54.30	0.50	0.43	2.47	0.86	5
		Root Weight (g)							
Assila	Babile	0.56	0.16	21.73	0.05	0.13	1.56	0.86	10
	Jittu	0.50	0.30	21.59	0.05	0.17	1.24	0.90	10
CLN2366B	Babile	0.44	0.83	15.31	0.06	0.35	0.60	0.93	9
	Jittu	0.41	0.67	17.76	0.06	0.27	0.74	0.93	9
Chochoro	Babile	0.54	0.34	20.78	0.05	0.15	0.81	0.93	8
	Jittu	0.50	0.31	22.52	0.05	0.13	0.92	0.93	8
Moneymaker	Babile	0.37	0.44	17.16	0.11	0.22	0.84	0.92	7
	Jittu	0.47	0.23	17.50	0.09	0.17	1.16	0.89	7
Tisey	Babile	0.39	0.13	17.41	0.04	0.03	0.59	0.97	6

	Jittu	0.25	0.22	17.49	0.12	0.07	0.52	0.98	5
		Root Length (cm)							
Assila	Babile	0.40	0.38	27.87	0.07	0.29	2.28	0.86	10
	Jittu	0.34	0.83	29.15	0.08	0.49	1.80	0.90	10
CLN2366B	Babile	0.36	0.27	28.68	0.07	0.17	2.28	0.89	9
	Jittu	0.28	0.35	29.88	0.05	0.10	1.32	0.96	9
Chochoro	Babile	0.47	0.09	29.07	0.05	0.05	2.04	0.90	8
	Jittu	0.40	0.21	24.78	0.09	0.17	2.32	0.84	8
Moneymaker	Babile	0.30	0.13	27.31	0.05	0.04	1.30	0.96	7
	Jittu	0.38	0.14	29.22	0.06	0.06	1.75	0.93	7
Tisey	Babile	0.31	0.11	26.77	0.07	0.04	1.76	0.94	6
	Jittu	0.35	0.09	26.22	0.13	0.06	2.36	0.88	5
		Total Fruit Number Per Plant							
Assila	Babile	0.57	0.37	21.59	0.06	0.37	1.57	0.82	10
	Jittu	0.46	0.45	20.64	0.06	0.24	1.16	0.90	10
CLN2366B	Babile	0.35	0.35	31.43	0.07	0.19	2.13	0.91	9
	Jittu	0.38	0.35	27.02	0.04	0.12	1.14	0.95	9
Chochoro	Babile	0.50	0.25	22.83	0.05	0.11	1.00	0.93	8
	Jittu	0.37	0.57	21.34	0.10	0.30	1.18	0.89	8
Moneymaker	Babile	0.36	0.14	26.25	0.08	0.08	2.08	0.90	7
	Jittu	0.40	0.17	25.41	0.09	0.13	2.15	0.87	7
Tisey	Babile	0.32	0.23	20.75	0.10	0.09	0.98	0.94	6
	Jittu	0.48	0.09	19.92	0.04	0.02	0.53	0.98	5
		Marketable Fruit Number per Plant							
Assila	Babile	0.58	0.52	19.23	0.06	0.38	1.00	0.86	10
	Jittu	0.42	0.69	17.70	0.09	0.54	1.31	0.83	10
CLN2366B	Babile	0.34	0.36	31.35	0.07	0.21	2.17	0.91	9
	Jittu	0.38	0.35	27.02	0.04	0.12	1.14	0.95	9
Chochoro	Babile	0.51	0.23	22.02	0.04	0.08	0.87	0.94	8
	Jittu	0.34	0.62	20.04	0.09	0.29	1.01	0.91	8
Moneymaker	Babile	0.27	0.28	22.57	0.08	0.12	1.28	0.94	7
	Jittu	0.31	0.20	25.07	0.09	0.11	1.94	0.90	7
Tisey	Babile	0.37	0.15	20.36	0.12	0.10	1.74	0.84	6
	Jittu	0.52	0.12	17.85	0.04	0.03	0.45	0.98	5
		Single Fruit Weight (g)							
Assila	Babile	0.71	0.60	86.11	0.03	0.28	2.06	0.92	10
	Jittu	0.67	2.12	78.99	0.12	2.64	4.64	0.53	10
CLN2366B	Babile	0.52	1.79	60.99	0.07	0.77	1.61	0.93	9
	Jittu	0.66	1.17	64.22	0.05	0.53	1.48	0.92	9
Chochoro	Babile	0.57	0.26	109.34	0.05	0.15	5.11	0.91	8
	Jittu	0.55	0.27	110.62	0.05	0.14	4.90	0.92	8
Moneymaker	Babile	0.51	0.39	96.74	0.05	0.12	2.49	0.96	7
	Jittu	0.44	0.49	92.14	0.09	0.22	3.57	0.93	7
Tisey	Babile	0.58	0.15	123.08	0.07	0.09	5.70	0.91	6
	Jittu	0.52	0.14	125.33	0.07	0.05	4.06	0.95	5
		Number of Seed per Fruit							
Assila	Babile	0.83	1.00	175.39	0.04	0.55	4.32	0.70	10
	Jittu	0.78	0.39	193.17	0.06	NaN	7.40	-0.41	10

CLN2366B	Babile	0.89	0.84	151.46	0.04	NaN	3.04	-0.03	9
	Jittu	0.89	0.65	149.10	0.02	0.35	2.15	0.75	9
Chochoro	Babile	0.79	0.11	189.77	0.05	0.10	9.21	0.72	8
	Jittu	0.76	0.18	201.54	0.04	0.19	9.15	0.83	8
Moneymaker	Babile	0.84	0.46	174.24	0.05	0.39	3.98	0.73	7
	Jittu	0.82	0.21	184.91	0.03	0.09	3.78	0.87	7
Tisey	Babile	NA	NA	176.45	NA	NA	2.15	0.01	6
	Jittu	NA	NA	344.74	NA	NA	1239.26	0.51	5

Ymax is the yield at densities below T; m, minimum Yield; T, tolerance limit (J2/100gram of dry soil), SEY_{max}, Standard Error for Ymax; $SE_{m.}$ Standard Error for m; SE_T, Standard Error for T; R^2, coefficient of determination, df-degree of freedom

The seedlings of all the tested tomato cultivars were dead at the higher *Pi* value (256J2/100gram soil) except on the cultivar *Assila* for which all the seedlings survived even with the higher *Pi* values of both *M. incognita* populations (Fig 7.10). The seedlings of the local tomato cultivar *Chochoro* were found tolerant till $Pi \leq 64$ for the Jittu population while for the Babile population this was till $Pi \leq 128$. At $Pi = 256$ the seedlings of *CLN-2366B* were not able to survive for both populations of *M. incognita*. Among all the cultivars tested *Tisey* was found highly susceptible to both Babile and Jittu populations and all the seedlings were dead at ($Pi \geq 16$) which was worse than the susceptible control *Moneymaker* where seedlings died at $Pi \geq 64$J2/100 gram of soil.



Meloidogyne incognita (Jittu)-*CLN-2366B*

Meloidogyne incognita (Babile)-*CLN-2366B*

Meloidogyne incognita (Jittu)-*Chochoro*

Meloidogyne incognita (Babile)-*Chochoro*

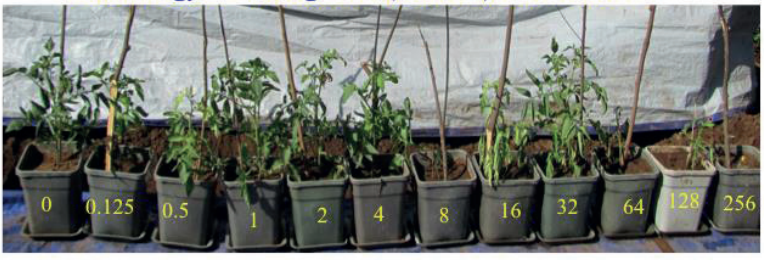


Figure 7.10. The damage of different initial population densities (*Pi*) on five tomato genotypes 10 days after inoculation with *Meloidogyne incognita* Jittu and Babile populations in pot experiments at Dire Dawa (Tony farm).

7.4. Discussion

The Seinhorst model perfectly fitted to all the plant parameter data considered in this damage study. This implied the negative effect of *Pi* on the studied plant data parameters. The population dynamics model showed that the tested tomato cultivars are a host for the studied *M. incognita* populations. As the RF obtained for the tested tomato cultivars were high the tested cultivars are consider as good host for both populations of *M. incognita*. Based on the *T* values generated from this study, *Tisey* is not a preferred tomato cultivar in areas infested with RKN. The tolerance limit (*T*) of *Tisey* was consistently lower for all plant data parameters considered compared to the other genotypes including the susceptible control *(Moneymaker*). While *Assila*, *CLN-2366B* and *Chochoro* showed a higher tolerance limit indicating that these genotypes could be used in nematode infested areas if integrated with other cultural management options such as crop rotation with cereals. The regression analysis performed showed that root galling, *Pf*, Galls/RS, EM/RS, RGI and EMI were positively correlated to an increase in *Pi*. Root galling severity and number of egg masses per root system were found to increase with the increase of inoculum level of both populations of *M. incognita* indicating their aggressiveness on the tested genotypes. This is in agreement with several other studies (Zahid *et al*., 2001; Mekete *et al*., 2003; Charegani *et al*., 2012; Kankam & Adomako, 2014; Dammini Premachandra & Gowen, 2015).

All the four tomato genotypes (*Assila*, *CLN-2366B*, *Chochoro* and *Tisey*) used in the current study to check the relationship between *Pi-Pf* were found having some degree of resistance during the growth chamber resistance screening for the *M. incognita* Jittu populations while for the Babile *M. incognita* population *Assila* and *CLN-2366B* were found susceptible whereas *Chochoro* and *Tisey* were slightly resistant (see chapter 4, Table 4.2). However, in this current study the *Pi-Pf* relation pointed out that all these genotypes are a good host for both populations surprisingly more for the Jittu *M. incognita* populations compared to the Babile *M. incognita* populations. This difference might have been due to the range of inoculum levels used in the current study as compared to the screening performed in the growth chamber using only one inoculum level. Hence, this result suggests that it is very crucial to consider a geometric series of inoculum levels rather than just using one inoculum level while screening for nematode resistance.

The severity of tomato crop damage caused by *Meloidogyne* species may depend on species

and the level of nematode population densities present in the soil at the time of planting (Sasanelli, 1994), populations of RKN, the occurrence of species mixture and the tomato genotypes used (see chapter 2). The *Pi* of Babile and Jittu *M. incognita* populations negatively affected the plant growth, biomass and yield parameters of the tested cultivars. For example, SFW was found negatively affected by increasing *Pi*. This parameter of tomato is important especially when considered for tomato factories (De Vito *et al*., 1991). This is an indication that if tomato growers want to guarantee better tomato production they need reliable information on the initial nematode densities in the soil before establishing their crop (Dammini Premachandra & Gowen, 2015).

It is not recommended to extrapolate *T* and *m* determined elsewhere (see chapter 2). The field where the damage experiment is conducted is one of the main factors that determine the value of *T* and *m*. For example, the *T* and *m* determined in drought prevailing areas should not be extrapolated to areas with ideal growing conditions. Drought was found favoring the damage of crops by nematodes and it may reduce the minimum yield and the tolerance limit compared to damage studies under ideal growing conditions (Wallace, 1973). In ideal conditions nematodes may cause only moderate damage whereas under periods of drought or other related stress factors, they may cause considerable damage reducing the value of *T* and *m*. Moreover, in the face of climate change and tropical agriculture, damage threshold determination studies should be locally studied and extrapolation should be handled with care (if needed). Additionally, the value of *T* and *m* is not equal when they are determined in fields with a history of nematode management practices used and in fields where there were not any management practices. In the former case, the *T* and *m* value may be higher so that to extrapolate and use this value for advisory or predication purpose for fields that did not have any management history will be truly misleading. Therefore, determination of *T* and *m* of a given crop variety for the prevailed *Meloidogyne* species in fields to be planted (local setting) is vital.

Determining the *T* and *m* using naturally infested fields with different nematode population levels is more ideal than determining in artificially inoculated pot studies and it has several advantages. Firstly, growers could easily observe the difference as the experiment is conducted in their local settings that serve as a demonstration plot. Secondly, it will represent the ideal climatic (aerial temperature, relative humidity, temperature, wind) and edaphic (soil texture, soil moisture, soil temperature) conditions for both the crop and the nematode.

Thirdly, the parameter estimates of the yield loss (*T* and *m*) are more close to the true estimate and can be reliably used for any advisory purpose or to predict the population dynamics for the future crop to be planted. Fourthly, the roots of plants grown for damage assessment at field conditions will freely grow, not bounded as in a pot study.

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7.5. References

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Chapter 8: General Discussion and Future Prospects

8.1. Brief Overview

In this PhD research, the biodiversity and distribution of RKN (*Meloidogyne* species) from major tomato growing localities of Ethiopia were investigated. The presence of RKN was earlier reported from vegetable growing localities of Ethiopia (Mandefro & Mekete, 2002). Surveys have mostly been concentrated in the central rift valley areas where most of the commercial vegetable growers, including tomato producers, are found. *Meloidogyne* species were largely identified based solely on morphology and in very limited cases using cytological and biochemical studies (Mandefro & Dagne, 2000; Mekete *et al*., 2003). These studies were mainly focused on identification and distribution of RKN (Abebe *et al*., 2015). A reliable research report on any effort for sustainable management of RKN and easily affordable technology for resource poor farmers of Ethiopia is lacking. The level of tomato growers' awareness about the economic importance or even the existence of PPN including RKN was not properly reported. Hence, this PhD thesis included a survey on 40 major tomato growing localities (Rift Valley, Upper Awash and Eastern Hararghe), used different molecular tools to identify *Meloidogyne* species associated with tomato and developed management strategies that are easily adopted by resource poor farmers of Ethiopia. The tomato growers and extension workers level of awareness towards RKN was assessed using a closed ended questionnaire. This PhD research work was initiated with the null hypothesis that tomato production is threatened by different RKN and different local tomato genotypes are resistant and/or tolerant to the prevailed RKN species. This PhD research output confirmed the fulfilment of the original null hypothesis.

8.2. Awareness about Plant-Parasitic Nematodes in Ethiopia

During my survey work, I have found that 65.5% of the tomato growers participating in the questionnaire survey were not aware of even the existence of PPN in general and RKN in particular (see chapter 3). During the survey, in 'Babile Erer', 'Babile Gende Sudan', 'Erer Gota' and 'Gursum' localities I witnessed a 100% tomato yield loss due to nematode damage. Tomato plants were wilting when they were about to start flowering. When farmers where asked about the possible causes of their tomato plants death they all mentioned unequivocally the Orobanche (broomrape) parasitic higher plant present in their field as the top reason. When I showed them the root damages (a totally rotten root system and roots with big and numerous galls) of their tomato plants due to RKN they all knew the damage symptom but it was hard for them to associate it with any known pathogen or insect. They were all asking me if I could be of help for a nematicide to apply and save their tomatoes and other vegetables.

Plant Nematology is the least studied and recognized science compared to other components (such as plant mycology, plant virology, plant bacteriology, agricultural entomology) of plant protection sciences in Ethiopia. This is equally true both in the eyes of the agricultural policy makers and even within the plant protection professionals (Abebe *et al*., 2015). Plant Nematology related courses were not offered to the plant protection (Plant Pathology, Agricultural Entomology and Integrated Pest Managemnet) students except for a brief overview as a sub-chapter at both undergraduate and postgraduate levels in many local universities. This clearly proved Plant Nematology indeed is an orphan science in Ethiopia. In order to fill such a huge gap, this PhD thesis played a role and created more awareness from the inception of the proposal development. I have been actively participating and presenting a paper on general nematology related topics and more specifically the output of this PhD research on the annual conferences of professional societies such as Plant Protection Society of Ethiopia, Annual Research Review Workshops of local universities and research institutes at national level. My active participation on the publication of the forum article published on the current status and future prospects of EPN and PPN in Ethiopia (Abebe *et al*., 2015) was meant to inform the agricultural policy makers about the economic importance of nematodes in Ethiopia and was part of the awareness creation package.

8.3. Survey of Root-Knot Nematode Problems in Tomato

The highest prevalence (100%) of RKN was found on samples collected from Adami Tullu, Babile (Erer and Gende Sudan), Erer Gota, Hurso, Jittu, Tikur Wuha, Tepo Choronke, Zeway and Koka. The highest incidence (100%) of the RKN was found from Adami Tullu, Jara Weyo, Babile (Erer and Gende Sudan), Erer Gota, Hurso, Jittu, Tikur Wuha, Tony farm, Tepo Choronke, Zeway and Koka locations based on direct observation of galls on collected root samples (see chapter 3). This clearly shows the wider distribution of RKN species in tomato growing areas of Ethiopia. The higher prevalence and incidence of RKN from these localities was hypothesized due to the previous crop used (mostly solanaceous without crop rotation) and the prevailing sandy or sandy loam type soil, which favours nematode infection. The majority of small-scale farmers did not practice weeding (especially once flowering started) on tomato and which may result in the higher RKN incidence or prevalence. In the present study, the presence of *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* on tomato was confirmed using a combination of DNA-based and biochemical identification tools. The two tropical species, *M. incognita* and *M. javanica,* were identified as the most prevalent species. The presence of *M. incognita* and *M. javanica* is not surprising since they

have frequently been reported using morphological studies (Tadele & Mengistu, 2000; Mandefro & Dagne, 2000; Mandefro & Mekete, 2002). Both species were also found co-infesting tomato plants. The occurrence of these *Meloidogyne* species alone, or in mixed populations from samples collected, clearly shows that RKN are widespread in major tomato growing areas of Ethiopia. Investigating the nature of these two major species interaction when present together could be of an interest in the near future.

The absence of RKN from some major tomato growing areas of Ethiopia (Meki, Jeju and Nura era) during my survey work should not mislead the readers, as if the area is free of RKN (see chapter 3). Different reasons could be cited for it. Firstly, the nature of nematode distribution in the soil is not always uniform unless the field is artificially infested or grown with the host crop for several consecutive years without crop rotation. The distribution of RKN in the soil is patchy (Duncan & Phillips, 2013). Secondly, the probability of detecting RKN in one round sampling in areas where nematodes follow a patchy distribution is low (Chen *et al*., 2011). Finally, some natural and cropping system variation may create a passive state for the entire RKN soil populations. The absence of *Meloidogyne* species in Meki (one of the major tomato growing areas) was allocated to the natural flood that stayed for 3 months during 2011/12 growing season (see chapter 3). It supports the significance of flooding as a nematode management option for the resource poor farmers in areas with excess water in the vicinity. However, water is always found to be the most limiting factor for the small-scale farmers to grow crops in Eastern Africa including Ethiopia (Adhikari *et al*., 2015). It should also be noted that flooding might bring a higher risk as it disperse nematodes through the runoff unless it is followed for a reasonable longer dry environmental condition.

Ethiopia is a big country and it is not feasible to include all the tomato-growing areas in a PhD project in one round sampling. I have covered comprehensive major tomato growing areas in the southern and central rift valley, upper Awash and eastern Hararghe. However, a significant amount of tomato production comes from the Northern part of Ethiopia (for example in 2013/14 growing season in the western lowland of Tigray 1655 ha was grown (Chernet & Zibelo, 2014). There is no exact figure on the amount of tomato production from the entire Northern part of Ethiopia reported. There is no report on any PPN related survey work on any crop from this Northern region (Abebe *et al*., 2015). These areas are characterized by different climatic and edaphic factors compared to the central rift valley

(Birhane *et al*., 2011). Therefore, an immediate survey should be initiated which will be of help in mapping the distribution of *Meloidogyne* species at country level. This mapping will serve as a basic reference point for any further management efforts and advisory services to be sought and to avoid redundancy of efforts.

8.4. Biodiversity of Root-Knot Nematode in Tomato

One of the most important prerequisites for any nematode management is correct identification (Powers *et al*., 2005). This crucial information ultimately determines the success of any management effort to reduce RKN populations in the soil. To this end, in this PhD thesis, a comprehensive study that started from identifying the RKN problems in tomato production areas to finding a solution for the prevailed RKN problems through a holistic local tomato varieties resistance screening effort was explored. As far as my knowledge goes, this is the first comprehensive characterization of RKN species from agricultural open farmers fields using molecular tools in the country and the first comprehensive resistance screening that covers greenhouse and field conditions. Different DNA-based molecular tools i.e. SCAR primers, D2-D3 expansion fragments of 28S rDNA sequence homology, NAD 5 gene fragment sequences and biochemical tests (isozyme and malate dehydrogenase) were used to identify 153 RKN isolates collected across the major tomato growing localities (see chapter 3).

Out of the 153 isolates used for molecular or biochemical identification, 48.4% of them were found to be *M. incognita* (74), 41.2% *M. javanica* (63), 6.5% *M. arenaria* (10), 3.3% unidentified *Meloidogyne* spp. (5) and 0.7% *M. hapla* (1). *Meloidogyne fallax*, *M. chitwoodi* and *M. enterolobii*, were not detected despite using their respective SSP. I was able to identify 72.5% of the collected RKN isolates accurately with the SCAR primers developed by Zijlstra *et al*. (2000) and Wishart *et al*. (2002) after rounds of PCR running work. From the unidentified populations with SCAR (due to weak bands), 24.2% was identified using NAD 5 gene fragment sequence based on Janssen *et al*. (2016) and isozyme (Esbenshade & Triantaphyllou, 1987; Carneiro *et al*., 2000) to come up with a total of 96.7% identification of the populations. The remaining 3.3% (5) were unidentified with any of the molecular and/or biochemical tools used (see chapter 3).

The 5 isolates i.e. Fedis-*FED5*, Gursum-*GUR10*, Tepochoronke-*TEP5*, Tibila-*TIB9* and Zeway-*ZEW8* were unidentified despite trying a combination of molecular and biochemical

techniques (see chapter 3). The 28S rDNA of these samples were clearly amplified using the D2D3 primers and the quality of the DNA was considered perfect. However, there was no amplification using SSP, Nad5 gene fragment and the biochemical (isozyme and malate dehydrogenase) tests. The 28S rDNA sequence homology did not give a conclusive identification for these isolates either. These isolates might have been a new species and this is a solid proof that accurate diagnosis requires a combination of molecular and morphological characters (Skantar *et al*., 2008). Unfortunately, while trying all these molecular tools the cultures of these isolate were not found in good conditions and we were not able to proceed to identify them morphologically. Nevertheless, the GPS coordinates and the exact locations from which these isolates were brought had already been registered and known and re-surveying will be considered in the near future.

The efforts made in these species identification indicate huge vacuum and well-suited and reproducible molecular markers to identify the tropical RKNs are needed urgently. From this study, I have learnt that to correctly identify tropical RKN species employing a combination of tools is not a choice when processing large number of samples. Even the routinely used and relatively established molecular tools such as the SSP were not found consistently amplifying the samples in one PCR run. After running several PCR with many samples mostly a few of them were found amplified and in some cases no amplification was done or weak bands were shown including for the positive control. Adjusting the PCR master mix combinations or the amount of DNA or the primer dilutions might be strategies to try to solve encountered problems.

Diagnostic resolution of D2-D3 expansion segments of 28S rDNA is insufficient to discriminate between some of the most closely related, problematic and economically damaging tropical RKN species (Naz *et al*., 2012; McClure *et al*., 2012; Landa *et al*., 2008). The complete 28S rDNA dataset of our isolates only confirmed that this region couldn't be used for identification of tropical RKN species. SSP are simple to perform and can successfully be used to any developmental stage of the nematode (Blok & Powers, 2009). However, there are some challenges associated with this method such as lower sensitivity or specificity of SSP, lack of reproducibility, the need to use large amount of DNA and appearance of weak bands (Adam *et al*., 2007; Blok & Powers, 2009; Onkendi *et al*., 2014).

As confirmed in this study, the recently developed method by Janssen *et al*. (2016) worked well especially for *M. incognita* and *M. javanica* isolates. However, as reported by Janssen *et al* (2016) the relatively uncommon, closely related linages of MIG such as *M. ethiopica* and some populations of *M. arenaria* were found clustered in one Nad5 haplotype indicating lack of identification resolution for these species. Biochemical identification can successfully fill the deficiencies of DNA-based identification techniques and can be used as a confirmation tool when weak bands amplified using SPP. However, this method is female age dependent and as found in this study it is difficult to determine and differentiate band sizes between different species. In this study, the esterase phenotype (EST) was more clearly amplified than the malate dehydrogenase (MDH) phenotype, which was almost always found with additional and unspecified double band.

8.5. Detection of *M. hapla* in Farmers Tomato Field: A surprise

The detection of *M. hapla* in the open tomato production farmer's field at Zeway, Ethiopia, for the first time at 1620 masl elevation came as a surprise. It was believed that *M. hapla* only flourishes at altitudes above 1829 masl in East Africa (Kenya, Tanzania and Uganda), despite the abundance of host plants at lower altitudes (Whitehead, 1969). However, in this study, *M. hapla* is reported at a significantly lower altitude (1620 masl) indicating *M. hapla* can flourish at lower altitudes too. However, it is not clear if this species is distributed in other major tomato growing areas or if it is only restricted to Zeway area. A systematic and timely sampling approach is needed to collect as many information as possible and to find out more about *M. hapla*. There is no adequate information about the *M. hapla* economic importance on tomato crops in Ethiopia. However, of concern is the fact that it was identified in a farm where it has the history of intensive vegetable production including tomato and pepper, thus this is likely to have considerable consequences for tomato production. The economic importance of *M. arenaria* and *M. hapla* on tomato production in Ethiopian agriculture is awaiting investigation.

8.6. Searching for Sustainable and Eco-friendly Management Option

From this study, *M. incognita* and *M. javanica* were found predominantly present in the major tomato growing areas of Ethiopia. This urged the need to look for a sustainable, safe and easily applicable RKN management option for the resource poor farmers of Ethiopia. A management option (searching a resistance source from local tomato varieties and breeding lines) that can easily be adopted by the resource poor farmers of Ethiopia to these major

Meloidogyne species was investigated in this PhD thesis. A total of 26 populations of *M. incognita* and 25 populations of *M. javanica* originating from different tomato growing localities of Ethiopia were checked for their aggressive behaviour on two susceptible tomato cultivars *Moneymaker* and *Marmande* at Haramaya University (Raree) under glasshouse conditions prior to the actual screening experiment. All populations multiplied well on both cultivars indicating their aggressive behaviour. Two populations from each major *Meloidogyne* species 'Babile' and 'Jittu' from *M. incognita* and 'Jittu' and 'Koka' from *M. javanica* that multiplied best on both cultivars were chosen for the subsequent screening work. The most common tomato varieties used by the local farmers were carefully chosen in consultation with tomato breeding unit of MARC in Ethiopia. Additional commercial tomato cultivars were also obtained from MONSANTO (The Netherlands) and breeding lines from AVRDC (Taiwan). A total of 33 tomato cultivars and breeding lines were screened against Babile and Jittu *M. incognita* and Jittu and Koka *M. javanica* populations for their resistance (see chapter 4). In none of the cultivars immunity was found. However, partial resistance was found in several breeding lines and cultivars although not with the same degree of resistance for all tested populations between or within the species. This indicates that population based screening and population based management recommendations are highly needed. The mechanism of resistance in these potential breeding lines and cultivars were also studied and differential penetration was found only on *CLN-2366A* and *CLN-2366B* but delayed development into the adult female was considered as a mechanism of resistance for most of the genotypes studied (see chapter 4). The delay in the adult female development may be important if used as a trap crop though it is not feasible to use tomatoes as a trap crop. Checking the genetic background (Heterozygous or Homozygous) of the genotypes and breeding lines with some potential resistance using a molecular marker is the immediate recommendation from this PhD thesis.

8.7. Heat Stability of Tomato Resistance in Tropical Agriculture

The *Mi-1* gene confers resistance but not immunity to the three most damaging species: *M. incognita*, *M. javanica*, and *M. arenaria* (Milligan *et al*., 1998). It has been the only commercially available source of resistance to RKN in tomato for the last 70 years globally (see chapter 2). The utility of this gene was found limited at higher soil temperature (Devran *et al*., 2010; Verdejo-Lucas *et al*., 2013). Climatic heterogeneity is a general characteristics of Ethiopia and in areas where tomato is largely produced soil temperature at times rises above 28°C (Alemayehu, 2002). The incorporation of heat-stable resistance to *Meloidogyne* spp.

would be a valuable genetic improvement in tomato. In this PhD thesis, I have checked the durability and heat stability of the potential resistance found on some tomato breeding lines at (≤ 27°C) in the growth chamber by exposing further to 28, 32 and 36°C soil temperatures for 24 and 48 hrs period. It was confirmed that local tomato breeding lines with resistance potential can be used when soil temperatures remain below 32°C. The utility of the resistance was also found totally limited at 36°C. The differences observed between breeding lines depending on the RKN population at higher temperatures can help in further optimizing the development of sustainable resistance under local Ethiopian circumstances (see chapter 5).

As confirmed with the tested tomato breeding lines in this PhD study and reported earlier in different crop species such as bean (Mullin *et al*., 1991), pepper (Thies & Fery, 2000), sweet potato (Jatala & Russell, 1972), alfalfa (Griffin, 1969) and cotton (Carter, 1982) temperature sensitivity is a characteristic of several RKN resistance genes. The *Mi-9* gene obtained from the *S. peruvianum* complex and a homologue of *Mi-1* gene was found unaffected by temperature (Bleve-Zacheo *et al*., 2007) but it is not yet commercially available. It is highly advisable to engineer the *Mi-9* heat stable gene to commercially available tomato varieties commonly grown in tropical agriculture including Ethiopia. Any effort to facilitate this commercialization process of tomato seeds with the *Mi-9* gene should be encouraged. The tomato breeding lines (such as *CLN-2366A* and *CLN-2366B*) found to be resistant and somehow stable till 32°C soil temperature lacked the good yield and yield components and agronomic plant parameters (see chapter 5 & 6). There should be some genetic improvement on these breeding lines and the vegetable breeding unit of MARC could take the assignment and accordingly improve the agronomic quality of these breeding lines.

8.8. Performance of Selected Tomato Genotypes in *Meloidogyne* spp. Infested Fields

The practical and applied component of any screening effort is checking the performance of the potential genotypes with resistance in field and farmers growing conditions. Hence, in this PhD work, performance of six tomato genotypes (*Assila*, CLN-2366A, *CLN-2366B*, *Chochoro*, *Eden* and *Tisey*) under local conditions at Tony (Dire Dawa) and Fethiya (Babile) farms were checked. These farms were known from the initial survey to be infested with both *M. incognita* and *M. javanica* populations. The tomato cultivars *Tisey* and *Assila* were new to Ethiopian field conditions although widely grown in the neighbouring east African countries (Kenya, Uganda and Tanzania) (Onduso, 2014). The cultivar *Eden* is being grown for commercial purpose in commercial glasshouses (such as Jittu farm) in Ethiopia. *Chochoro*,

mostly used as a processing tomato, is a widely available local variety and preferred by many tomato growers in Ethiopia (Tegen *et al*., 2015). The cultivar *Assila* and *Eden* were found potentially tolerant tomato cultivars to be promoted for growers of tomato in RKN infested areas of Ethiopia with a reasonable crop rotation interval. These cultivars can easily adapt to the climatic (temperature, relative humidity) and edaphic conditions (soil texture) of Ethiopia as these cultivars have already been successfully used in the neighbouring countries through MONSANTO. In Ethiopia, there is a relatively good extension system and farmers could easily adopt these strategies. However, soil analysis should be integrated with growing *Assila* in a new area as it was found with massive blossom end rot. Calcium deficiency in the soil has long been known to cause blossom end rot. The breeding lines (*CLN-2366A* and *CLN-2366B*) were found partially resistant but were not giving good harvest data. However, these breeding lines might be used as rootstocks on which high yielding varieties are grafted. In general, this work confirmed that tomato varieties that were found SR or HR under the growth chamber screening (see chapter 4) became susceptible under field conditions (see chapter 6) and tolerance level and minimum yield determination experiment using a series of *Pi* (see chapter 7). In this PhD work, resistant screening under growth chamber, pot and field trials were not consistent indicating the importance of screening under field conditions. Resistance screening performed under ideal tropical tomato growing conditions (in the open farmers field) need to be encouraged rather than in artificial conditions using a limited range of inoculum and controlled conditions. In ideal tomato growing conditions, a lot of biotic and abiotic factors may interact and the utility of a cultivar that is found resistant under such condition will have a higher significance compared to the one obtained under artificially controlled conditions.

In Ethiopia, as a means of food security, farmers usually practice intercropping. I recommend that farmers should practice intercropping with great care since the host status of the RKN populations present in Ethiopia is not yet known. In tomato genotypes performance study in the field, the biggest challenge comes from other co-infesting pathogens. Under field conditions, a plant is never found infected with a single pathogen (Johnson & Nusbaum, 1970). The nematode will never be the single factor to be considered. A field with relative uniform infestation or a hot spot area is preferred to check the performance (see chapter 2). This work has gained reasonable attention. Tomato growers in eastern Ethiopia and farmer representatives and officers from agricultural offices visited the tomato varieties performance and damage threshold determination trial at Dire Dawa (Tony farm), which is a step forward

in disseminating the information. Due to time limitation, this field trial was not replicated in time and it was only performed during one season and I will further repeat this field trial provided with enough funding. One major challenge faced during the greenhouse and field resistance screening experiments in Ethiopia was from the devastating insect *Tuta absoluta*.

8.9. Determination of *T* and *m* on Selected Tomato Genotypes under Local Settings

For any advisory purpose or to design control strategies, it is highly recommended to determine the minimum yield (to be expected when higher nematode populations are present in a soil during planting) and a tolerance limit (the level of the nematode population below which there is no economic yield loss) under local conditions for any crop-nematode combinations (Jamali *et al*., 2012). Nematode threshold densities determined in one geographic location is not extrapolated to other locations (Barker *et al*., 1976). In this study, though it was not in my original PhD proposal, the tolerance limit (*T)* and minimum yield (*m)* of selected tomato cultivars (*Assila*, CLN2366B, *Chochoro*, *Tisey* and *Moneymaker* (susceptible control) was determined against Babile and Jittu *M. incognita* populations (see chapter 7). The result perfectly supplemented the tomato varieties performance studied under the two field conditions (see chapter 6). I have also noticed that all penetrated J2 may not form feeding sites and thereby form root galls, as many J2 were recovered during root extraction without visible root galls in their root surfaces from the damage pot test. It may imply that J2 might not find suitable nourishment from the plant tissues to establish feeding sites. It may also be possible that 2nd generation J2 just entered the root (though it is unlikely since it was found from roots with less root galls from the 1st generation). This indicates that root gall is a poor indicator of crop resistance or susceptibility.

Based on the damage model fitted to the data all the tested plant parameters were found negatively affected by both populations of *M. incognita.* As the reproduction factor (RF) obtained for the tested tomato cultivars were high we can consider the tested cultivars as a good host for both populations of *M. incognita* and monocropping should be avoided. In general, for all the plant parameters studied *Tisey* was found with a lower *T* (as lower as 0.09J2/ 100 gram dry soil) while *Assila* (except for the parameter RW) was having a higher *T* (as high as 9.59J2/100 gram dry soil). The difference observed for the tested tomato genotypes to their minimum yield (m) for the different plant parameters studied against Babile and Jittu *M. incognita* populations indicate that this critical parameter should preferable be determined at population levels and in local settings. The history of the field

(management or prevailed species), climatic conditions of the field (drought or ideal conditions), temperate or tropical agriculture and the type of soil on which the damage experiment is being conducted are very important factors to consider while determining *T* and *m*.

8.10. Conclusions

In general, in this PhD thesis, I confirmed a consistent population difference in aggressiveness through all the screening steps involved i.e. in greenhouse screening, mechanism study, heat stability determination, field performance study and damage and population dynamics investigation. The Jittu *M. incognita* population was the most aggressive compared to Babile *M. incognita* and Jittu and Koka *M. javanica* populations. This is a clear indication that there should always be extra care while treating RKN populations. Moreover, in this PhD work, screening for nematode resistant under growth chamber, pot and field trials were not consistent indicating the importance of screening under ideal tomato growing field conditions. The tomato genotypes *Assila*, *Eden*, *CLN-2366A* and *CLN-2366B* (after genetic improvement or could be used as a rootstock for high yielding tomato varieties) and the tolerant local genotype (*Chochoro*) are recommended as a management option in tropical agriculture with some non-host (mostly cereals) rotation schemes. These resistance sources (*Assila*, *CLN-2366A* and *CLN-2366B*) could also be used in protected agriculture where the *M. incognita* and *M. javanica* problem is prevalent (Kiewnick *et al*., 2011) such as in the glasshouses and protected tomato production in Europe where tropical RKN are also found to be present. A general schematic procedure for resistance screening against PPN more importantly RKN is presented in Fig 8.1.

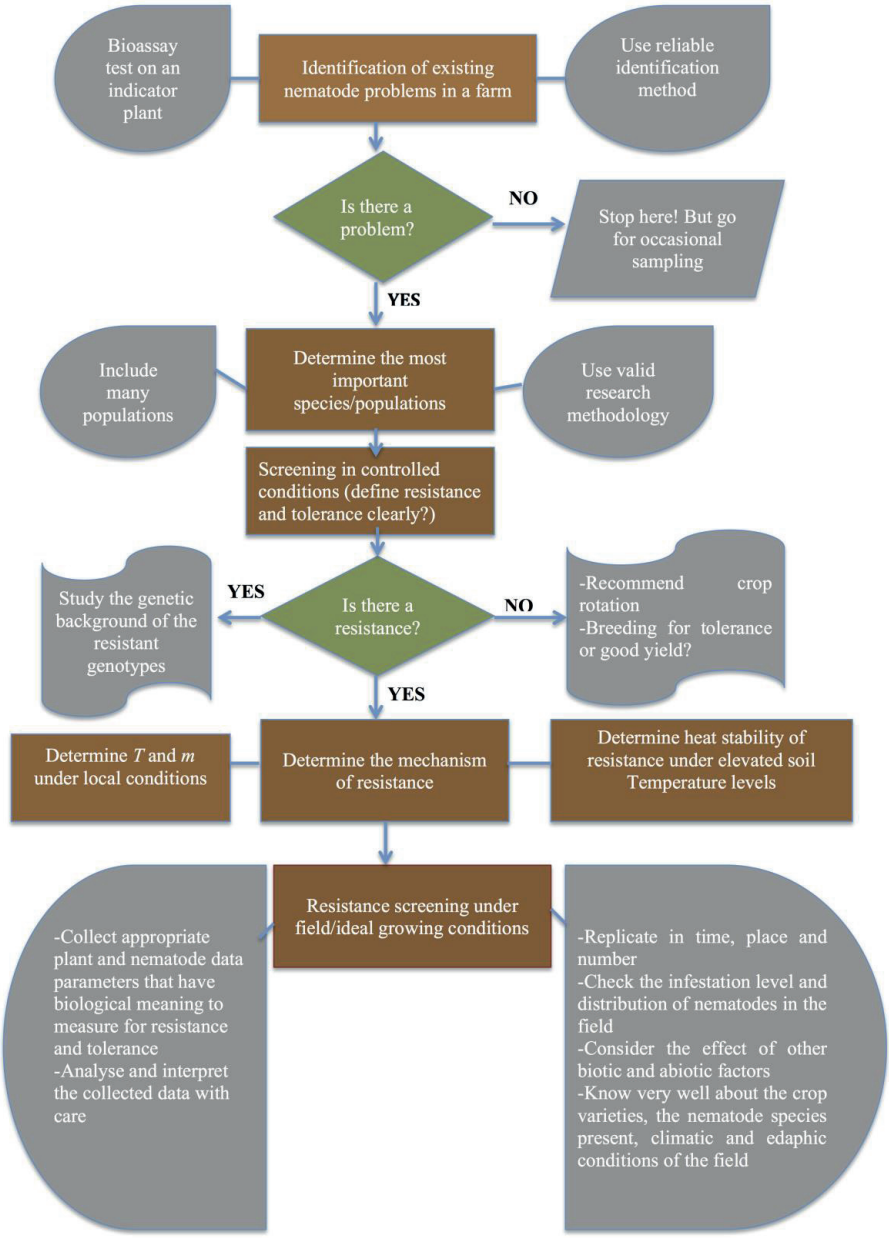

Figure 8.1. Schematic presentation of resistance screening procedures for plant-parasitic nematodes case example RKN.

8.11. Future Prospects: What is for the Future?

The future prospects and recommendations from this study are:

- Launching postgraduate Plant Nematology program at least at two universities known for their agricultural research expertise could help using the limited human and resource facilities at hand. Training technically equipped nematologists that can work at universities, research institutes and as agricultural extension agents may be helpful. There should be a workable national plan to launch, strengthen and improve Nematology education, research and extension services in Ethiopia.
- Agricultural universities and research institutes should at least be well equipped with a controlled greenhouse and growth chambers to be used for research purposes. The occurrence and problem of *Tuta absoluta* during my Ethiopian screening effort in the glasshouses of Haramaya University proved the lack of such infrastructures.
- Create linkages between local, east African and foreign universities and local research institutes so that the output of the basic research performed at the universities will be forwarded to the research institutes for the applied component and extension services.
- Writing projects on nematode problems on economically important crops and avail these projects for postgraduate Crop Protection, Plant Pathology, Agricultural Entomology and IPM students across different universities in the country which will generate a reliable research information about RKN and create more awareness will help a lot.
- Nematologists should focus on the damage potentials of economically important PPNs identified from economically important crops of Ethiopia and thereby focus on management practices that are affordable by the resource poor farmers.
- Ethiopia is known as centre of diversity for many food crops and management practices aiming breeding for resistance and/or tolerance cropping system could be rewarding.
- Awareness creating projects can be designed to reach SSF, investors, extension workers and policymakers about the economic importance of PPN and more specifically RKN on vegetables including tomato.
- A survey on RKN problems of tomato from the Northern part of Ethiopia will help in mapping the distribution of *Meloidogyne* species at country level.
- Tomato growers can be advised on efficient and wise use of resistant and tolerant tomato varieties. Growers could be advised to practice well-designed and appropriate crop rotation schemes. The agricultural research extension scientists in Ethiopia can specifically address and train the resource poor farmers to use a solarized soil to raise

their tomato seedlings for transplanting. This is at least affordable to obtain healthy planting material.

- Heat stability should be considered as an integral part of any nematode resistance screening effort for tropical crops including tomato in the future.
- Easy access should be facilitated for the tomato growers to get *Meloidogyne* species resistant tomato seeds or rootstocks.
- Priority should be given for locally feasible and economical management options such as resistant cultivars.
- Farmers can be trained to check the presence of nematodes in their fields by using soil from their fields and growing a susceptible plant in it in a pot.
- It will be also helpful if the government opens nematode diagnostic services for checking samples of nematode problems.
- Morphological identification should also be useful if combined with the DNA-based and isozyme profiles.

8.10. References

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Summary

The biodiversity, distribution and associated problems of root-knot nematodes (*Meloidogyne* spp.) in major tomato growing areas of Ethiopia were studied. A total of 212-rhizosphere soil and 123 root samples were sampled from 40 localities in tomato growing areas of Ethiopia (Rift valley, Upper Awash and Eastern Hararghe) during the 2012/13 growing season (see chapter 3). This is the first comprehensive survey (in terms of area coverage on the basis of a single vegetable crop-tomato) conducted in Ethiopia. The presence of *Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla* on tomato was confirmed using a combination of SCAR primers and biochemical identification tools. *Meloidogyne incognita* and *M. javanica* were the predominant *Meloidogyne* species across the sampled areas. Both species were also found co-infesting tomato plants. *Meloidogyne hapla* was detected for the first time in an open tomato production farmer's field at 'Zeway' location with 1620 masl elevation. The occurrence of these *Meloidogyne* species alone, or in mixed populations, clearly showed that root-knot nematodes are widespread in major tomato growing areas of Ethiopia.

A bioassay test was conducted in the greenhouse on the 212 soil samples collected from different tomato growing localities (see chapter 3). Out of the 212 composite soil samples collected, 100 samples (47.2%) were found infested by various *Meloidogyne* species eight weeks after the start of the bioassay test. Out of the 123 root samples collected directly from the fields, 80 of them (65%) had root galls. There was significant variability in the prevalence and incidence of root-knot nematodes between sampled localities. The highest incidence (100%) of RKN was found in Adami Tullu, Jara Weyo, Babile (Erer and Gende Sudan), Erer Gota, Hurso, Jittu, Tikur Wuha, Tony farm, Tepo Choronke, Zeway and Koka locations based on direct observation of root galls on collected root samples. The highest prevalence (100%) of RKN was found on samples collected from Adami Tullu, Babile Erer, Babile Gende Sudan, Erer Gota, Hurso, Jittu, Tikur Wuha, Tepo Choronke, Zeway and Koka.

For the molecular and biochemical identification of *Meloidogyne* species a total of 153 isolates were used. Out of the 153 isolates studied, 48.4% of them were found to be *M. incognita* (74), 41.2% *M. javanica* (63), 6.5% *M. arenaria* (10), 3.3% unidentified *Meloidogyne* spp. (5) and 0.7% *M. hapla* (1). Five isolates i.e. Fedis (FED5), Gursum

(GUR10), Tepo Choronke (TEP5), Tibila (TIB9) and Zeway (ZEW8) were unidentified despite trying a combination of molecular and biochemical techniques (see chapter 3).

A questionnaire with a total of 646 respondents was conducted to assess the knowledge and practice of farmers and factors associated with RKN damage on tomato. Out of the 646 respondents, 280 (43.3%) of them reported to have RKN damage symptoms when shown the symptoms of RKN while 366 (56.7%) of them did not report damage. The highest effect on RKN damage on tomato roots was found to be the previous crop, soil texture, awareness about RKN and source of irrigation water used (see chapter 3).

The two predominant RKN (*M. incognita* and *M. javanica*) were used to screen for resistance using local tomato varieties and breeding lines (see chapter 4). The aggressiveness of 26 populations of *M. incognita* and 25 populations of *M. javanica* was assessed on tomato cultivars *Marmande* and *Moneymaker* prior to the resistance screening experiment. On both cultivars *Marmande* and *Moneymake*r all *M. incognita* and *M. javanica* populations formed a high number of egg masses indicating highly aggressive behaviour. The two most aggressive populations of *M. incognita* ('Babile' and 'Jittu') and *M. javanica* ('Jittu' and 'Koka') were selected and used for further testing on 33 tomato genotypes. The resistance screening and mechanism of resistance was performed after inoculation with 100 freshly hatched (<24 hr.) second-stage juveniles (J2). Eight weeks after inoculation, the number of egg masses produced on each cultivar was assessed. For the resistance mechanism study, J2 penetration and their subsequent development inside the tomato roots were examined at 1, 2, 4 and 6 weeks after inoculation. None of the 33 tomato genotypes were immune for *M. incognita* and *M. javanica* populations used. However, several tomato genotypes were found to have a significant effect ($p < 0.05$) on the number of egg masses produced indicating possible resistance. For *M. javanica* populations more plants from cultivars or breeding lines on which no egg masses were found compared to *M. incognita* populations. The lowest number of egg masses for both populations of *M. incognita* was produced on cultivars *Bridget40, Galilea,* and *Irma* while for *M. javanica* it was on *Assila, Eden, Galilea, Tisey, CLN-2366A, CLN-2366B* and *CLN-2366C*. Cultivar, time (weeks after inoculation) and their interaction were significant sources of variation ($p < 0.05$) for J2 penetration and their subsequent development inside the tomato roots. Differential penetration was found in breeding lines such as *CLN-2366A, CLN-2366B* and *CLN-2366C*. However, many of the selected tomato genotypes resistance for the tested *M. incognita* and *M. javanica* populations was expressed

by delayed nematode development. Developing a simple screening technique to be used by local farmers or extension workers was found crucial to facilitate selection of a suitable cultivar. In chapter 2 an overview is given of the damage potential of RKN on tomato (*Solanum lycopersicum*) and the different management strategies of *Meloidogyne* spp. on tomato with particular emphasis on the *Mi* resistance gene. The effectiveness of the only commercially available RKN resistance gene (the *Mi*-gene) was reported to be limited in higher soil temperatures. In chapter 5, a study was initiated with the objective to check the durability of the potential resistance genes found in seven (*CLN-2366A, CLN-2366B, CLN-2366C, CLN-2037H, CLN-2037A, CLN-2037B* and *CL5915-206-D4-2-2*) tomato breeding lines after screening in controlled greenhouse conditions ≤ 27°C (see chapter 4) by exposing them to higher soil temperatures at 28, 32 and 36°C for 24 and 48hrs period. The aggressive Babile and Jittu *M. incognita* and Jittu and Koka *M. javanica* populations originally collected from Ethiopia were used. Temperature, cultivar and time had a significant ($P < 0.01$) effect on the numbers of second-stage juveniles of Babile and Jittu *M. incognita* and Jittu and Koka *M. javanica* populations that penetrated the plant roots. The utility of the potential resistance found in our breeding lines during the controlled growth chamber resistance screening experiment was found limited at the higher soil temperatures of 32 and 36°C. For both Babile and Jittu *M. incognita* populations a significantly higher number of J2 was found in the roots of all the tested tomato breeding lines after 48hrs heat exposure compared to the 24hrs heat exposure for each soil temperature level studied. For both Jittu and Koka *M. javanica* populations after 48hrs exposure to 36°C the mean number of J2 that penetrated the roots of all tested breeding lines was significantly higher compared to the 24hrs exposure time. From our observations it was found clear that local tomato breeding lines with resistance potential can be used when soil temperatures remain below 32°C. Differences were observed between breeding lines depending on the RKN population at higher temperatures and this knowledge can help in further optimizing the development of sustainable resistance under local Ethiopian circumstances.

Tomato resistance screening against *Meloidogyne* spp. has often been performed in controlled greenhouses, which does not represent the actual tomato production conditions. A study was initiated to assess the tolerance and resistance of selected tomato cultivars under local conditions on two *Meloidogyne* spp. infested fields (Dire Dawa-Tony farm and Babile-Fethiya Farm) in eastern Ethiopia (see chapter 6). A total of seven tomato varieties (*Assila*, *CLN-2366A*, *CLN-2366B*, *Chochoro*, *Eden*, *Moneymaker* and *Tisey*) were grown in both locations. A randomized complete block design was used with four replications for each

cultivar at both locations. The plant growth, biomass, yield and yield component data that were considered as a measure of varieties tolerance were found significantly ($p < 0.01$) different among the tomato varieties. Except the initial population densities (*Pi*) at planting, all nematode related parameters (final population density, root galls per root system, egg mass per root system, mean number of eggs per egg mass, root gall index, egg mass index and reproduction factor) that were used as a measure of varieties resistance were found statistically ($p < 0.01$) different among the tested tomato genotypes. Positive correlation of plant data parameters indicated that total number of fruits per plant, fruit set percentage, number of fruits per cluster, number of fruit clusters per plant and shape index were the most important fruit yield components contributing to fruit yield per hectare. A similar performance trend was obtained among the tested tomato genotypes across both locations though the level of damage was found dependant on the *Pi* present in the soil. A cultivar potentially tolerant or resistant at Dire Dawa (Tony farm) was also found performing nearly similar at Babile (Fethiya farm). The tomato varieties *Assila* and *Eden* are recommended in nematode infested areas with strict crop rotation. The tomato cultivar *Tisey* was found to be the most susceptible while the local cultivar *Chochoro* was found to be tolerant. The tomato breeding lines *CLN-2366A* and *CLN-2366B* were found good in reducing the nematode populations but they need some critical genetic improvement (preferably they can be used as a rootstock).

Reliable data are required to relate the effect of a range of *Pi* on plant growth, biomass and yield for specific crop-nematode associations under local conditions. In chapter 7, the effect of a series of *Pi* of Babile and Jittu *Meloidogyne incognita* populations on four tomato cultivars (*Assila*, *Chochoro*, *Moneymaker* and *Tisey*) and one clone (*CLN-2366B*) for growth and yield and the relationship with final population densities (*Pf*) were studied. Each tomato cultivar was inoculated with a geometric series of *Pi* (0, 0.125, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 and 256 J2/ 100 gram of dry soil) and was allowed to grow till the crop reaches senescing. The relationship between *Pi* and *Pf* was fitted to the Seinhorst population dynamics model ($Pf = (M * Pi) / (Pi + M/a)$ while the effect of *Pi* on different plant parameters considered was fitted to the Seinhorst yield model ($Y = Ymax*(m + (1 - m)* 0.95^((Pi-T)/T))$. Based on the damage model fitted to the data all the tested plant parameters were found negatively affected by both populations of *M. incognita.* The Jittu *M. incognita* population was found having more effect on the majority of parameters compared to Babile *M. incognita* population. As the reproduction factors (RF) obtained for the tested tomato cultivars were

high we can consider the tested cultivars as good host for both populations of *M. incognita*. The highest RF for *M. incognita* populations was obtained at lower *Pi* (0.125 J2/100 gram of dry soil) and reduced with increasing *Pi* on all the tested cultivars. Severity of root galling and number of egg masses per root system increased with increasing inoculum levels of both populations of *M. incognita*. The cultivar, *M. incognita* population and initial population density (*Pi*) had a highly significant ($P < 0.001$) effect on all the plant and nematode data parameters considered. The seedlings of all the tested tomato genotypes were dead at the higher *Pi* value (256 J2/100 gram dry soil) except for the cultivar *Assila* of which seedlings survived even with the higher *Pi* values for both *M. incognita* populations. Among all the cultivars tested *Tisey* was found highly susceptible to both Babile and Jittu populations and all the seedlings were dead at ($Pi \geq 16$) which was worse than the susceptible control *Moneymaker* where seedlings died at $Pi \geq 64$J2/100 gram of soil. For all the plant parameters studied *Tisey* was found to have a lower damage threshold *T* while *Assila* (except for the parameter root weight) was having a higher *T*. A difference was observed for the tested tomato genotypes on their minimum yield (m) for the different plant parameters studied against Babile and Jittu *M. incognita* populations. Determination of *T* and *m* of a given crop variety for the prevailing *Meloidogyne* species in fields to be planted (local setting) is vital.

In conclusion, this PhD thesis presented for the first time the biodiversity of *Meloidogyne* species associated with tomato using a combination of molecular and biochemical tools. The experiments on aggressiveness, resistance screening, mechanism of resistance, heat stability, field performance and damage potential showed a consistent *Meloidogyne* spp. population difference in aggressiveness. Promising cultivars or breeding lines should be carefully tested with local populations and under local circumstances to provide clear-cut solutions for Ethiopian tomato farmers.

Samenvatting

De biodiversiteit, de verspreiding en de problemen met wortelknobbelnematoden (*Meloidogyne* spp.) in belangrijke tomaatproducerende gebieden van Ethiopië werden bestudeerd. Een totaal van 212 bodemmonsters en 123 wortelmonsters werden verzameld op 40 locaties in de Rift-vallei, Upper Awash en Oost Hararghe tijdens het groeiseizoen 2012-2013 (zie hoofdstuk 3). Dit is het eerste uitgebreide onderzoek in termen van dekkingsgraad op basis van één enkele teelt (tomaten) uitgevoerd in Ethiopië. De aanwezigheid van *Meloidogyne incognita*, *M. javanica*, *M. arenaria* en *M. hapla* op tomatenplanten werd bevestigd met een combinatie van SCAR primers en biochemische identificatiemiddelen. *Meloidogyne incognita* en *M. javanica* waren de overheersende *Meloidogyne* soorten in de bemonsterde gebieden. Beide soorten werden ook samen aangetroffen op tomatenplanten. *Meloidogyne hapla* werd voor het eerst ontdekt in het open veld in Ethiopië op tomaat (*Solanum lycopersicum*). Dit was op de locatie 'Zeway' die zich op 1620 meter boven zeeniveau situeert. De uitgevoerde bemonstering toonde duidelijk aan dat wortelknobbelnematoden wijdverspreid zijn in belangrijke tomaatproducerende gebieden van Ethiopië en opbrengstderving veroorzaken. Een werd een biotoets uitgevoerd in de kas op de 212 bodemmonsters die werden verzameld (zie hoofdstuk 3) en in 100 monsters (47,2%) werden acht weken na de start van de biotoets wortelknobbelnematoden gevonden op de tomatenplanten. Van de 123 wortelmonsters die werden verzameld op de velden bleek 80 van hen (65%) knobbels te vertonen. Er was aanzienlijke variatie in de aanwezigheid van besmetting alsook in de grootte van de besmetting tussen de bemonsterde plaatsen. De hoogste incidentie (100%) van *Meloidogyne* spp. werd gevonden in Adami Tullu, Jara Weyo, Babile (Erer en Gende Sudan), Erer Gota, Hurso, Jittu, Tikur Wuha, Tony farm, Tepo Choronke, Zeway en Koka locaties op basis van directe observatie van knobbels op de wortels.

Voor de moleculaire en biochemische identificatie van *Meloidogyne* soorten werden in totaal 153 isolaten gebruikt. Daarvan bleken er 48,4% *M. incognita* (74) te zijn, 41,2% *M. javanica* (63), 6,5% *M. arenaria* (10), 0,7% *M. hapla* (1) en 3,3% (5) kon niet geïdentificeerd worden ondanks het combineren van moleculaire en biochemische technieken (zie hoofdstuk 3).

Er werd een enquête uitgevoerd bij de tomatenboeren om te achterhalen in welke mate ze kennis hebben over wortelknobbelnematoden en de schade die ze kunnen veroorzaken aan hun gewas. Van de 646 respondenten gaf 280 (43,3%) van hen aan schade te hebben nadat

hen de symptomen (knobbels) werden getoond, 57,7% gaf aan geen schade te hebben. Er bleek een sterke link te zijn tussen het voorkomen van schade en het voorgaande gewas, de bodemtextuur, de gebruikte bron voor irrigatie en hun kennis over wortelknobbelnematoden. (zie hoofdstuk 3).

De twee meest aangetroffen wortelknobbelnematoden, *M. incognita* en *M. javanica*, werden gebruikt voor het screenen van lokale tomatenrassen en klonen op resistentie (zie hoofdstuk 4). Daarvoor werd eerst de agressiviteit van 26 populaties van *M incognita* en 25 populaties van *M. javanica* beoordeeld op tomatencultivars Marmande en Moneymaker. Op beide cultivars vormden alle *M. incognita* en *M. javanica* populaties een groot aantal eipakketjes. De twee meest agressieve populaties van *M. incognita* ('Babile' en 'Jittu) en *M. javanica* (' Jittu 'en' Koka ') werden geselecteerd voor de resistentiescreening van 33 tomaat genotypes. Acht weken na inoculatie met 100 vers uitgekomen (<24 uur) tweedestadiumjuvenielen (J2) werd het aantal eipakketjes dat gevormd werd op elke cultivar beoordeeld. Om het resistentiemechanisme te onderzoeken werd penetratie en de verdere ontwikkeling in de tomatenwortels onderzocht op 1, 2, 4 en 6 weken na inoculatie. Geen van de 33 tomaat genotypes waren immuun voor *M. incognita* en *M. javanica*. Er werden echter wel bij verschillende genotypen een significant ($p < 0,05$) lager aantal eipakketjes vastgesteld wat wijst op mogelijke resistentie. Er waren meer planten bij de verschillende cultivars en klonen waarbij voor *M. javanica* geen eipakketjes werden aangetroffen in vergelijking met *M. incognita*. Het laagste aantal eipakketjes voor beide populaties van *M. incognita* werd geteld op cultivars Bridget40, Galilea, en Irma terwijl voor *M. javanica* dit was op *Assila*, *Eden*, *Galilea*, *Tisey*, *CLN-2366A*, *CLN-2366B* en *CLN-2366C*. Cultivar, tijd (weken na inoculatie) en hun interactie waren belangrijke bronnen van variatie ($p < 0,05$) voor J2 penetratie en de verdere ontwikkeling in de tomatenwortels. Lagere penetratie werd gevonden in klonen *CLN-2366A*, *CLN-2366B* en *CLN-2366C* maar het was voornamelijk een vertraagde ontwikkeling van de *M. incognita* en *M. javanica* populaties die werd geobserveerd in de geselecteerde genotypes. Het ontwikkelen van een eenvoudige screening techniek die kan gebruikt worden door boeren of voorlichters is essentieel om de keuze van een geschikte cultivar te vereenvoudigen.

In hoofdstuk 2 wordt een overzicht gegeven van het schadepotentieel van *Meloidogyne* spp. op tomaat en de verschillende strategieën voor controle met bijzondere nadruk op de *Mi*-resistentie. Het *Mi*-gen is het enige commercieel beschikbare resistentiegen en is niet hittebestendig bij hogere bodemtemperatuur. In hoofdstuk 5 werd onderzocht in welke mate de potentiële resistentiegenen gevonden in zeven klonen (*CLN-2366A*, *CLN-2366B*, *CLN-*

2366C, *CLN-2037H*, *CLN-2037A*, *CLN-2037B* en *CL5915-206-D4-2-2*) hitte bestendig zijn door ze gedurende 24 en 48 uur bloot te stellen aan hogere bodemtemperatuur (28, 32 en 36 °C). Temperatuur, cultivar en tijd hadden een significant ($p < 0,01$) effect op het aantal J2 van 'Babile' en 'Jittu' *M. incognita* en 'Jittu' en 'Koka' *M. javanica* populaties die de plantenwortels penetreerden. Bij de hogere bodemtemperaturen (32 en 36°C) bleek de potentiële resistentie verloren te zijn gegaan. Voor beide *M. incognita* populaties werd een aanzienlijk hoger aantal J2 gevonden in de wortels van alle geteste tomatenklonen na 48u blootstelling aan hitte (28, 32 en 36°C) in vergelijking met de 24 uur hitteblootstelling. Voor de beide *M. javanica* populaties was dit enkel het geval bij 36°C. Er werden verschillen waargenomen tussen de klonen afhankelijk van de *Meloidogyne* populatie. Op basis van de resultaten kan worden gesteld dat lokale klonen met resistentiepotentieel kunnen gebruikt worden bij bodemtemperaturen beneden de 32°C. Deze kennis kan helpen bij de ontwikkeling en optimalisatie van duurzame resistentie onder lokale Ethiopische omstandigheden.

Resitentieonderzoek en screening met *Meloidogyne* spp. wordt vaak uitgevoerd in gecontroleerde omstandigheden in incubatoren of kassen en dit geeft geen getrouwe weergave van de lokale omstandigheden waaronder tomaten in Ethiopië worden geteeld. Daarom werden resistentie en tolerantie van selecteerde tomatencultivars onderzocht onder de plaatselijke omstandigheden op twee *Meloidogyne* spp. besmette percelen (Dire Dawa-Tony farm en Babile-Fethiya Farm) in het oosten van Ethiopië (zie hoofdstuk 6). Een totaal van zeven tomatenrassen (*Assila*, *CLN-2366A*, *CLN-2366B*, *Chochoro*, *Eden*, *Moneymaker* en *Tisey*) werden gekweekt op beide locaties. De plantengroei, biomassa, opbrengst en opbrengstfactoren die als maat voor tolerantie werden beschouwd waren significant ($p < 0,01$) verschillend tussen de tomatenrassen. Met uitzondering van de initiële populatiedichtheid (*Pi*) bij de aanplant waren alle nematodenparameters (eindpopulatie *Pf*, aantal knobbels per wortelstelsel, eipakketjes per wortelstelsel, gemiddeld aantal eitjes per eipakketje, wortelknobbelindex, eipakketjesindex en reproductiefactor) die werden gebruikt als maat voor resistentie statistisch ($p < 0,01$) verschillend tussen de geteste genotypes tomaat. Een gelijkaardige trend werd gezien op beide locaties voor de verschillende geteste tomaten genotypes. De ernst van de schade was wel afhankelijk van de *Pi*. Een cultivar met potentiële tolerantie of resistentie in Dire Dawa (Tony farm) gaf dezelfde resultaten in Babile (Fethiya farm). De tomatenrassen *Assila* en *Eden* worden aanbevolen in wortelknobbelnematoden besmette gebieden op voorwaarde dat aan vruchtwisseling wordt gedaan. De tomaten cultivar *Tisey* bleek het meest gevoelig te zijn terwijl de lokale cultivar *Chochoro* tolerant was. De

tomaatklonen *CLN-2366A* en *CLN-2366B* presteerden goed in het verminderen van de nematodenpopulatie maar ze moeten een aantal kritische genetische verbetering ondergaan. Bij voorkeur kunnen ze nu worden gebruikt als onderstam.

In hoofdstuk 7 werd het effect van een oplopende reeks van *Pi* van 'Babile' en 'Jittu' *M. incognita* populaties op de groei en de opbrengst en de relatie met de uiteindelijke eindpopulatie (*Pf*) op vier tomatencultivars (*Assila*, *Chochoro*, *Moneymaker* en *Tisey*) en een kloon (*CLN-2366B*) onderzocht. Elke cultivar werd geïnoculeerd met een geometrische reeks *Pi* (0, 0,125, 0,5, 1, 2, 4, 8, 16, 32, 64, 128 en 256 J2/100 gram droge grond). De planten werden onderhouden tot ze afstierven onder lokale omstandigheden in Dire Dawa (Tony farm). De relatie tussen *Pi* en *Pf* werd bepaald met het Seinhorst populatiedynamiekmodel ($Pf = (M * Pi)/(Pi + M/a)$, terwijl het effect van *Pi* op verschillende plantparameters werd berekend met het Seinhorst opbrengstmodel ($Y = Ymax * (m + (1 - m) * 0,95 ^ ((Pi-T)/T))$. Alle geteste plantenparameters werden negatief beïnvloed door beide populaties van *M. incognita* op basis van het opbrengstmodel. De 'Jittu' *M. incognita* populatie had een grotere negatieve invloed op de meeste parameters in vergelijking met de 'Babile' *M. incognita* populatie. De reproductiefactoren (*Rf*) voor de geteste tomatencultivars waren hoog wat betekent dat de geteste cultivars als goede gastheer voor beide populaties *M. incognita* kunnen worden beschouwd. De hoogste *Rf* voor de *M. incognita* populaties werd verkregen bij lagere *Pi* (0,125 J2/100 gram grond) en verminderde met toenemende *Pi* voor alle geteste cultivars. Wortelknobbelvorming en het aantal eipakketjes per wortelstelsel namen toe met toenemende *Pi* voor beide populaties van *M. incognita*. De cultivar, *M. incognita* populatie en initiële populatie (*Pi*) hadden een sterk significant ($P < 0,001$) effect op alle gemeten nematodenparameters. De zaailingen van alle geteste tomaat genotypes waren dood bij de hogere *Pi* waarde (256 J2/100 gram grond) met uitzondering van de cultivar Assila waarvan zaailingen overleefden, zelfs met de hogere *Pi*-waarden voor beide *M. incognita* populaties. Van alle geteste cultivars bleek *Tisey* zeer gevoelig voor zowel 'Babile' en 'Jittu' *M. incognita* populatie en de zaailingen stierven af bij $Pi \geq 16$ terwijl bij de controlecultivar *Moneymaker* pas bij $Pi \geq 64$ J2/100 gram grond was. Voor alle plantenparameters bleek *Tisey* een lagere schadedrempel *T* te hebben terwijl *Assila* (behalve voor de parameter wortelgewicht) de hoogste *T* liet optekenen. Voor de geteste tomaat genotypes werd een verschil waargenomen in hun minimale opbrengst (*m*) voor de verschillende plantenparameters die onderzocht werden bij besmetting met 'Babile' en 'Jittu' *M. incognita* populaties. Bepaling van de *T* en m van een cultivar van een gewas voor de lokale

Meloidogyne soorten in van essentieel belang om aangepast advies te kunnen geven over gewasopbrengst.

In dit proefschrift werd voor de eerste keer de biodiversiteit van *Meloidogyne* soorten geassocieerd met tomaat in Ethiopië onderzocht met een combinatie van moleculaire en biochemische technieken. De experimenten op agressiviteit, resistentiescreening, resistentiemechanisme, hittebestendigheid, veldprestaties en potentiële schade toonden aan dat er belangrijke verschillen zijn tussen populaties van *Meloidogyne* spp. Veelbelovende cultivars, lijnen of klonen moeten zorgvuldig worden getest met de lokale *Meloidogyne* populaties onder plaatselijke omstandigheden om onderbouwd advies te kunnen geven aan Ethiopische tomatenboeren.

Curriculum vitae

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- **PhD Student in (Plant Nematology)**
  - 1st October 2012 - present Ghent University, Belgium. Dissertation "Biodiversity of Root-Knot Nematodes (*Meloidogyn*e spp.) Associated with Tomato and Characterization and Screening of Tomato Genotypes against *Meloidogyne* spp. from Ethiopia."

- **Postgraduate Diploma in Higher Education Teaching (PGDHET):**
  - September 2013–August 2014, Haramaya University, Ethiopia.

- **M.Sc. in Postgraduate International Nematology Course**
  - September 2008- September 2010, Ghent University, Belgium. Great Distinction (with best student award 2010) Thesis "Variation in a secreted protein Ubiquitin-like from *Globodera pallida* populations in relation to virulence."

- **B.Sc. in Crop Production and Protection**
  - September 2004–July 2006, Haramaya University, Ethiopia. Greatest Distinction 3.92/4.00 (for 20 different courses I took I have scored A^+ grade). Thesis "Assessment of cooking quality of ten common bean (*Phaseolus vulgaries*) genotypes under laboratory conditions"

- Since 17 July 2006 to present employed at Haramaya University as a lecturer

Publications in SCI-indexed journals

- **Seid, A.,** Fininsa, C., Mekete, T., Decraemer, W. & Wesemael, W.M.L. (2016). Resistance screening of breeding lines and commercial tomato cultivars against *Meloidogyne incognita* and *M. javanica* populations (Nematoda) from Ethiopia. *Submitted to Euphytica*
- **Seid, A.,** Fininsa, C., Mekete, T., Decraemer, W. & Wesemael, W.M.L. (2015) Tomato (*Lycopersicon esculentum* L.) and root- knot nematodes (*Meloidogyne* spp.)-A Century Old Battle. *Nematology* 17(9): 995-1009.
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- **Seid**, **A.,** Fininsa, C., Mekete, T., Decraemer, W. & Wesemael, W.M. L. (2015). Resistance screening of tomato cultivars to *Meloidogyne incognita* and *M.*

javanica populations from Ethiopia. Abstracts of the 67th International Symposium on Crop Protection.

- **Seid**, **A.,** Fininsa, C., Mekete, T., Decraemer, W. & Wesemael, W.M. L. (2014). Biodiversity of Root-Knot Nematodes (*Meloidogyne* spp.) from Major Tomato Growing Areas of Ethiopia**.** Proceedings of the 6th International Congress of Nematology. *Journal of Nematology* (Abstract), 46 (2): 231.
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Active Contributions to International Conferences

- **Seid, A.,** Fininsa, C., Mekete, T., Decraemer, W. & Wesemael, W.M.L. (2016). "Resistance screening of breeding lines and commercial tomato cultivars against *Meloidogyne incognita* and *M. javanica* populations from Ethiopia" is accepted for **poster presentation** in the 32nd annual symposium of European Society of Nematology from 28 August to 1 September 2016, Braga, Portugal.
- **Seid, A.,** Fininsa, C., Mekete, T., Decraemer, W. & Wesemael, W.M.L. (2016). "Resistance screening of breeding lines and commercial tomato cultivars against *Meloidogyne incognita* and *M. javanica* populations from Ethiopia" **Oral presentation** in the 22nd annual conference of Plant Protection Society of Ethiopia, from 10 to 11 March 2016, EIAR, Hiruy Hall, Addis Ababa.
- **Seid, A.,** Fininsa, C., Mekete, T., Decraemer, W. & Wesemael, W.M.L. (2014). "Tomato (*Lycopersicon esculentum*) and root- knot nematodes (*Meloidogyne* spp.)-A Century Old Battle" **poster presentation** at the GAP international symposium, 27 November 2014, Ghent University, Ghent, Belgium.
- **Seid, A.,** Fininsa, C., Mekete, T., Decraemer, W. & Wesemael, W.M.L. (2015). Resistance screening of local tomato cultivars against *M. incognita* and *M. javanica* populations from Ethiopia. **Oral presentation** at the 67th International Symposium on Crop Protection at Ghent University, on May 2009, 2010, 2013 and 2015 Belgium.
- **Seid, A.,** Fininsa, C., Mekete, T., Decraemer, W. & Wesemael, W.M.L. (2014). Biodiversity of Root-Knot Nematodes in Major tomato growing areas of Ethiopia. **Poster presentation** in the 6th International Nematology Congress, Cape Town, South Africa, May 3-10, 2014.

- **Seid, A.,** Fininsa, C., Mekete, T., Decraemer, W. & Wesemael, W.M.L. (2013). Biodiversity of Root-Knot Nematodes in Major tomato growing areas of Ethiopia. **Oral presentation** in the 20th annual conference of Plant Protection Society of Ethiopia, from 25 to 26 December 2013, EIAR, Hiruy Hall, Addis Ababa.

Professional Society Membership

- Plant Protection Society of Ethiopia
- European Society of Nematologists
- Organization of Nematologists of Tropical America (ONTA)

Appendix

Table A1. Plant parameters (recorded from the pre-tagged plants per row in a consistent manner at both experimental locations) used as a measure of nematode tolerance

Plant Parameters	Description
Plant height (PH)	It was measured from the soil level to the main apex of the plant and mean values were expressed in centimetres.
Number of leaves (NL)	The number of leaves per plant was counted and thereby its mean calculated.
Number of flowers per plant (NFPP)	The total number of flowers per plant was counted and their mean was calculated.
Total fruit number per plant (TFr)	The total number of fruits (including marketable and unmarketable fruits) per plant was counted and their mean was calculated.
Marketable fruit number per plant (MFr)	The number of fruits that were free from any visible damage symptoms per plant was counted and later their mean was computed.
Unmarketable fruit number per plant (UnFr)	Includes fruits with cracks, damaged by insects, diseases, birds, too small and sunburn were counted (Lemma, 2002).
Single fruit weight (SFW)	Three tomato fruits per plant were randomly picked, their weight was measured separately using a sensitive balance and their mean was expressed in grams.
Marketable yield (MY) (ton/ha)	This is determined based on the total number of marketable fruit weight and single fruit weight and then converted into ton per hectare.
Fruit set percentage (FSP)	Calculated by dividing the total number of fruits per plant (TFrNPP) by the total number of flowers per plant (NFPP) and multiplied by 100 to express as a percentage (%).
Root size (RS)	The total weight of roots per plant was taken after removing the adhered soils and after the final fruit harvesting time and their mean expressed in grams. The difference between RS and FRW is that the latter was taken early flowering (when about to start) while the earlier was from senescence plants.

Root length (RL)	The adhered soil from the roots was gently washed away using tap water and excess water was removed through blotting with tissue paper. The root length per plant was measured from the soil level to the tip of roots and their mean was expressed in centimetres.
Days to 50% flowering (50FL)	Number of days from the date of transplanting to the appearance of 50% flowering.
Frequency of fruit picking (FFP)	The total frequency of fruit harvest per cultivar was recorded.
Fresh shoot weight (FSW):	Taken from the pre-tagged plants per row and taken with destructive sampling when plants were about to start flowering and their mean was expressed in grams.
Dry shoot weight (DSW)	After taking the FSW, the same shoots were chopped into smaller fragments and oven dried at 72°C for 72hrs to measure their dry weight and their mean was expressed in grams.
Fresh root weight (FRW)	FRW was taken per plant using a destructive sampling. The roots were washed gently with tap water and all the adhering soil was carefully removed. The roots were then blotted using tissue papers to remove excess water from the roots and their mean was expressed in grams.
Stem diameter (SD)	Measured per plant (from the point where the first primary branch arises) from the pre-tagged plants per row and their mean was expressed in centimetres (cm).
Number of primary branches (NPB)	The number of primary branches per plant from the pre-tagged plants was counted at the maturity stage and later means were computed.
Days to first harvest (DFrH):	Number of days from the date of transplanting to the first fruit harvest date from the pre-tagged plants.
Number of flowers per cluster (NFPc)	Counted from the bottom, middle and upper parts per plant from the pre tagged plants and then their mean was computed.
Number of flower clusters per plant (NFcPP)	The number of flower clusters per plant was counted from the pre-tagged plants per row and later their mean was computed.
Number of fruits per cluster (NFrPc)	Counted from the bottom, middle and upper parts per plant from the pre-tagged plants and then their mean was computed.

Number of fruit clusters per plant (NFrcPP)	the number of fruit clusters per plant was counted from the pre-tagged plants per row and later their mean was computed.
Shelf life (SL)	In order to check the tomato breeding lines or cultivars' shelf life, the days taken by fruits from the breaker stage until the firmness of the fruits was totally lost were recorded. The fruits were harvested at the breaker stage and kept under room temperature at Haramaya University laboratory. The temperature was recorded in the morning, mid-day and later in the evening to make the daily average temperature.
Fruit polar diameter (FPD)	Three randomly picked fruits per plant from the pre-tagged plants were used to determine the polar (from the stem to the blossom end) diameter of the fruits using a Vernier Caliper. Mean values were expressed in millimetres (mm).
Fruit equatorial diameter (FED):	The same fruits that were used to determine the FPD were measured for their equatorial (transverse diameter) and mean values were expressed in millimetres (mm).
Shape index (SI)	It was calculated by dividing the mean of FPD by the mean of FED.
Number of seeds per fruit (NSPF)	Three randomly chosen fruits were picked per plant from the pre-tagged plants and their seed was extracted and counted separately and later their mean number was calculated.
Mean ten fruit weight (M10FrW)	The mean weight of ten fruits per plant from the pre-tagged plants per row were taken and expressed in kilograms (Kg).
Total soluble solids (TSS)	It was determined from fruits used for measuring FPD and FED. The juice of these fruits was extracted using a juice-extracting machine and their total soluble solid was measured with a digital refractometer (ATAGO PR-101, Tokoyo, Japan) and expressed in °Brix.

Table A2. Yield and yield component parameters of tomato varieties grown in Dire Dawa (Tony farm) and Babile Gende Sudan (Fethiya farm) locations during 2015/16 growing season

Location	Tomato Varieties	SD	NPB	NFPc	NFcPP	NFrPc	NFrcPP	FPD	FED	SI	NSPF	M10FW	TSS
Dire Dawa (Tony farm)	Assila	4.0 a	5.3 c	4.0 a	17.3 b	3.0 a	16.3 c	67.5 b	51.0 b	1.3 d	190.0 b	0.81 b	4.7 a
	CLN-2366A	4.0 a	3.8 a	5.8 b	20.3 c	4.5 b	10.8 a	43.8 a	53.3b	0.8 b	152.8 a	0.59a	5.0 b
	CLN-2366B	3.8 a	3.8 a	6.0 b	19.0 c	4.8 b	9.3 a	41.8 a	47.3a	0.9 b	142.8 a	0.49 a	5.2 b
	Chochoro	4.3 a	5.5 c	4.0a	17.5 b	3.0 a	18.3 c	62.3 b	57.0c	1.1 c	211.0 c	0.97 c	5.6 c
	Eden	4.0 a	3.5 a	4.3 a	13.8 a	3.8 a	10.3 a	70.3 c	57.3c	1.1 c	220.5 c	1.05 c	5.1 b
	Moneymaker	3.8 a	4.3 ab	4.5 a	15.8 a	3.8 a	10.8 a	43.8 a	64.8d	0.7 a	181.8 b	0.73 b	6.0 d
	Tisey	3.8 a	3.5 a	4.5 a	16.0 ab	3.3 a	13.0 b	71.3 c	59.3c	1.2 c	182.5 b	1.17 d	5.0 b
Babile (Fethiya Farm)	Assila	4.0 a	5.3c	4.0 a	17.3 b	3.0 a	16.3 c	67.5 b	51.0 b	1.3 d	187.5 b	0.81 b	5.0 a
	CLN-2366A	4.0a	3.8 a	5.8 b	20.3 c	4.5 b	10.8 a	43.8 a	53.3 b	0.8 b	149.8 a	0.58 a	5.0 a
	CLN-2366B	3.8 a	3.8 a	6.0 b	19.0 c	4.8 b	9.3 a	41.8 a	47.3 a	0.9 b	137.0 a	0.49 a	5.5 ab
	Chochoro	4.3 a	5.5 c	4.0 a	17.5 b	3.0 a	18.3 c	62.3 b	57.0 c	1.1 c	210.0 c	0.95 c	6.0 b
	Eden	4.0 a	3.5 a	4.3 a	13.8 a	3.8 a	10.3 a	70.3 c	57.3 c	1.1 c	216.0 c	1.05 c	5.5 ab
	Moneymaker	3.8 a	4.3 ab	4.5 a	15.8 a	3.8 a	10.8 a	43.8 a	64.8 d	0.7 a	176.5 b	0.71 b	6.8 c
	Tisey	3.8 a	3.5 a	4.5 a	16.0 a	3.3 a	13.0 b	71.3 c	59.3 c	1.2 c	181.5 b	1.14 d	5.3 ab

SD-stem diameter (cm), NPB-number of primary branches, NFPc-number of flower per cluster, NFcPP-number of flower cluster per plant, NFrPc-number of fruit per cluster, NFrcPP-number of fruit cluster per plant, FPD-fruit polar diameter (mm), FED-fruit equatorial diameter (mm), SI-shape index, NSPF-number of seeds per fruit, M10FW-mean of ten fruit weight (kg) and TSS-total soluble solids Means within the same column that shared the same letter are not significant at $p \leq 0.05$ based on Tukey HSD.

Table A3. Pearson's correlation between selected data parameters from tomatoes grown in Dire Dawa (Tony farm) and Babile (Fethiya farm) during 2015/16 growing season.

	PH	NL	NFPP	NFcPP	TFr	NFrcPP	FSP	SFW	DFrH	NSPF	TSS	SI	RS	RL	FSW	FRW	Pi	Pf	G/RS	EM/RS	MY	Meggs /EM
PH	-	.86**	.58**	.40*	-.20	-.49**	-.76**	-.36	.24	-.69**	.28	-.65**	-.40*	-.41*	-.49**	-.61**	-.15	.33	.42*	.43*	-.66**	.10
NL	.89**	-	.43*	.31	-.24	-.49**	-.63**	-.29	.50**	-.45*	.45*	-.71**	-.27	-.28	-.27	-.41*	-.15	.43*	.54**	.54**	-.60**	.33
NFPP	.76**	.50**	-	.81**	.33	-.41*	-.77**	-.81**	-.43*	-.83**	-.15	-.49**	-.36	-.07	-.21	-.16	.05	-.25	-.20	-.17	-.62**	-.43*
NFcPP	.47*	.31	.75**	-	.56**	-.01	-.43*	-.60**	-.40*	-.62**	-.14	-.21	-.36	.20	-.15	.16	-.09	-.37	-.32	-.32	-.25	-.41*
TFr	-.16	-.34	.31	.53**	-	.63**	.34	-.25	-.38*	-.06	-.10	.12	.26	.32	-.06	.51**	.31	-.38*	-.37	-.38*	.30	-.53**
NFrcPP	-.58**	-.55**	-.45*	-.02	.58**	-	.82**	.33	-.03	.46*	-.03	.50**	.48**	.21	.05	.51**	.24	-.06	-.10	-.13	.67**	-.06
FSP	-.86**	-.74**	-.74**	-.36	.40*	.81**	-	.63**	.16	.81**	.09	.56**	.53**	.29	.17	.50**	.16	-.02	-.07	-.10	.78**	-.01
SFW	-.62**	-.43*	-.85**	-.59**	-.28	.34	.59**	-	.39*	.76**	.17	.46*	.13	-.10	-.12	-.12	-.08	.22	.19	.16	.80**	.56**
DFrH	.11	.45*	-.44*	0.4*	-.44*	-.03	.09	.38*	-	.29	.83**	-.44*	.12	-.33	-.22	-.28	-.15	.76**	.82**	.77**	.17	.74**
NSPF	-.80**	-.61**	-.84**	-.60**	.012	.49**	.82**	.78**	.27	-	.12	.47*	.35	.17	.22	.29	.09	.04	.04	-.002	.73**	.24
TSS	.34	.51**	-.09	-.21	-.27	-.23	-.12	.12	.84**	.06	-	-.62**	.02	-.34	-.40	-.26	-.04	.60**	.71**	.64**	.07	.56**
SI	-.78**	-.79**	-.52**	-.21	.09	.5**	.56**	.49**	-.44*	.48*	-.72**	-	.22	.41*	.38*	.45*	.004	-.45*	-.55**	-.53**	.46*	-.18
RS	-.24	-.17	-.37	-.43	.06	.28	.39*	.29	.34	.42*	.28	.01	-	-.13	.06	.23	.72**	.31	.26	.27	.31	.16
RL	-.27	-.23	.004	.21	.18	.07	.16	-.14	-.34	.01	-.46*	.29	-.62**	-	.53**	.68**	-.38*	-.6**	-.61**	-.57**	-.09	-.38*
FSW	-.42*	-.24	-.26	-.14	-.10	-.002	.21	-.02	-.11	.20	-.32	.33	-.45*	.69**	-	.70**	-.21	-.36	-.43*	-.37	-.17	-.20
FRW	-.48*	-.39*	-.10	.09	.29	.26	.35	-.17	-.31	.20	-.40*	.34	-.38*	.70**	.85**	-	-.04	-.5**	-.54**	-.51**	-.04	-.38*
Pi	.04	-.14	.12	-.04	.28	.14	.09	-.13	-.12	.09	.06	-.08	.74**	-.6**	-.62**	-.43*	-	.08	.14	.10	.05	-.20
Pf	.33	.52**	-.23	-.33	-.45*	-.08	-.14	.30	.78**	.06	.69**	-.42*	.51**	-.75**	-.53**	-.69**	.23	-	.96**	.98**	.08	.69**
G/RS	.35	.56**	-.23	-.35	-.50**	-.17	-.17	.29	.82**	.04	.75**	-.48**	.46*	-.71**	-.48**	-.67**	.15	.99**	-	.98**	.05	.71**
EM/RS	.32	.51**	-.22	-.32	-.43*	-.08	-.13	.30	.77**	.06	.69**	-.42*	.54**	-.77**	-.56**	-.71**	.26	1.0**	.98**	-	.10	.68**
MY	-.73**	-.66**	-.63**	-.24	.38*	.71**	.85**	.78**	.07	.78**	-.04	.53**	.31	-.001	-.08	.06	.05	-.02	-.06	-.01	-	.18
Meggs/ EM	-.07	.09	-.55**	-.38*	-.36	.27	.22	.71**	.71**	.36	.48*	.05	.31	-.55**	-.40*	-.53**	-.01	.77**	.77**	.77**	.45*	-

*PH-plant height, NL-number of leaves, NFPP-number of flower per plant, NFcPP-number of flower cluster per plant, TFr-total fruit number per plant, NFrcPP-number of fruit cluster per plant, FSP-fruit set percentage, SFW-single fruit weight, DFrH-days to first fruit harvest, NSPF-number of seeds per fruit, TSS-total soluble solids, SI-shape index, RS-root size, RL- root length, FSW-fresh shoot weight, FRW-fresh root weight, Pi- initial population density in the soil, Pf-final population density at harvest, G/RS-number of galls per root system, EM/RS- number of egg masses per root system, MY-marketable yield per hectare and Meggs/EM-mean number of eggs per egg mass. Correlation values with * and ** is significant at $p < 0.05$ and $p < 0.01$ respectively.*

Figure A1. A) Plant growth, yield and yield component data collection from the pre-tagged plants, B) Fresh shoot weight (g), C & F) Shelf life of tomato genotypes under room temperature at Haramaya University Plant Pathology laboratory D) Fresh root weight (g) and E) Fruit weight in gram.

Figure A2. Fruit equatorial and polar diameter being measured; A) Chochoro, B) Moneymaker, C) A collection of the tested tomato fruits from field to home and D, E, & F) The juice of the fruit on preparation for TSS and NSPF.

Figure A3. A) Stalking on preparation from locally available wood, B) Weeding, loosening the soil and fertilizer application to follow-Tony farm, C) Unhealthy tomato plants (Babile-Fethiya farm), D) A closer look to check the presence of Orobanche or broomrape parasitic higher plants, E) Weed free field and with stalking-Tony farm, F) Aerial view of the Tony farm experimental field, G) Tony farm at flowering stage and H) Babile-Fethiya farm tomato plants don't look normal compared to Tony farm.

Figure A4. The Tony farm experimental field as visited by Prof. dr Chemeda Fininsa the local co-promoter and president of Haramaya University (left), the Belgian co-promoter Prof. dr ir Wim Wesemael (Middle) and The researcher (Mr. Awol Seid). (A) A photo taken with the logo of the participating institutions for this research and VLIR-UOS the funding organizations, (B & C) The research is accompanied by his local and Belgian promoters both in the middle and edge of the Tony farm research field and D) The researcher following the damage and population dynamics experiments on bigger pots in outdoor.