EDUARDO MONDLANE UNIVERSITY

FACULTY OF AGRONOMY AND FORESTRY ENGINEERING

DISTRIBUTION AND CHARACTERIZATION OF COWPEA GENOTYPES FOR RESISTANCE TO ROOTKNOT NEMATODES (*Meloidogyne* spp.) IN MOZAMBIQUE

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DECLARATION

I, Joseph Kisitu, do hereby declare that this dissertation is my own initiative and has never been submitted to Eduardo Mondlane University or any other institution of learning for any academic purpose.

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THESIS ABSTRACT

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important food crop in Mozambique. The crop is cultivated almost exclusively by smallholder farmers in warm marginal environments of the country. One of the key field hindrances to the success of this crop are rootknot nematodes (*Meloidogyne* Spp.), that reduce cowpea yield. A study was conducted to establish rootknot nematode distribution, damage intensity (measured by incidence and severity), species identification and cowpea genotypes' resistance to *Meloidogyne javanica*. To assess rootknot nematode distribution and extent of damage, main cowpea growing areas including eight districts in three provinces (Gaza, Inhambane and Nampula) of Mozambique were selected. Rootknot nematode incidence in cowpea was recorded as the percentage of cowpea plants infested with rootknot nematodes. Severity of rootknot damage was achieved by scoring for the extent of rootknot nematode galling on roots of cowpea plants. Out of the 72 cowpea fields surveyed, 56.9% were infested with rootknot nematodes, with cowpea fields from Inhambane province registering the highest cases of rootknot nematode infestation. *M. incognita*, *M. javanica*, and *M. enterolobii* were identified to be associated with cowpea rootknot. The highest frequency of *M. incognita* and *M. javanica* was observed in cowpea fields from Inhambane and Gaza provinces, respectively. The 3 species generally occurred more frequently in Inhambane than the rest of the provinces. Generally, rootknot nematode galling score across provinces was low, with 1.9 as the highest gall index score obtained at province level. The highest mean rootknot galling score was observed in Homoine district. To characterize cowpea for resistance to rootknot nematodes (*M. javanica*.), twenty five cowpea genotypes from a Cowpea Breeding Program of the Faculty of Agronomy and Forestry Engineering at Eduardo Mondlane University, Maputo Mozambique, were used in an experiment conducted at Mozambique Agricultural Research Institute (IIAM). A randomized complete block design was used, where each genotype was replicated three times. Out of the twenty five cowpea genotypes assessed, four were found to be resistant, sixteen were tolerant and five were susceptible.

Keywords: cowpea, rootknot nematodes, distribution, Mozambique, damage intensity, resistance.

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CHAPTER ONE: INTRODUCTION

1.1 General background

Cowpea (*Vigna unguiculata* (L.) Walp.) is broadly adapted to tropical and subtropical regions, and an important food crop globally. It is cultivated in many African, Asian and South American tropical countries (Karikari & Molatakgosi, 1999, Kitch *et al.*, 1998). It is one of the crops dominating legume production in sub-Saharan Africa, besides groundnut (*Arachis hypogaea* L.) and soybean (*Glycine max* (L.) Merr.) in terms of cultivated area and production (Cork *et al.,* nd). The crop is important as source of food and protein, and a major component of cropping systems (Ehlers and Hall, 1997). World cowpea production is estimated to occur on more than 11 million hectares, with Africa as the leading producer at 97% (Turner, 2012). According to FAOSTAT (2014), world cowpea production is estimated at 4.5 million tonnes, dominated by West African countries such as Nigeria, Niger and Burkina Faso. Nigeria is the leading global and regional producer at about 55.4% of world production. Cowpea is also an important crop in southern Africa, in countries such as, the United Republic of Tanzania, Zimbabwe and Mozambique are important producers (Chiulele *et al.*, 2011; FAOSTAT, 2014)

In Mozambique, cowpea is an important food crop. It is the fourth most cultivated crop after maize, cassava, and groundnuts. It is cultivated almost exclusively by smallholder farmers primarily for household consumption in form of grain, fresh leaves and fresh pods, besides being increasingly used as a source of family income through sell of its various products in open markets (Chiulele *et al.*, 2011). The crop is mostly produced in various provinces of the country, such as Nampula, Inhambane, Zambezia, Gaza and Maputo (INE, 2006; Chiulele *et al.*, 2011). In addition to the household uses, the crop is high in quality-protein (about 26.61%), lipids (3.99%), carbohydrates (56.24%), minerals (3.84%), crude fiber (1.38%), gross energy (1.51%) and vitamins. The crop thus provides excellent supplements to the lower nutritional content and quality of cereals, roots and or tubers often consumed in most resource-poor households (Bressani, 1985; Chiulele *et al.*, 2011). Cowpea is also an important source of green manure which helps in improving soil structure (Infonet-biovision, 2016). Cowpea plants also have a symbiotic relationship with soil bacteria (Rhizobium spp.) in root nodules for fixing atmospheric nitrogen. This enhances soil fertility, reduces fertilizer costs especially to low input smallholder farming households (INIA, 2000). However, average cowpea yields in Mozambique have

persisted below 300 kg ha-^{1} (INIA, 2000), which calls for research intervention. Some of the constraints responsible for low cowpea yields include abiotic stresses (drought, low soil fertility amongst others) and biotic stresses such as pests (thrips, pod borers and pod suckers), diseases (fungal, viral and bacterial diseases) and rootknot nematodes (Emechebe & Lagoke, 2002; INIA, 2003; Adati *et al.*, 2007; Chiulele *et al*., 2011).

1.2 Problem statement

In Mozambique, the agriculture sector plays a very important role in her economic development, despite the various challenges the sector faces. Besides contributing to meeting the United Nations' Sustainable Development Goals (SDG 2), the agriculture sector contributes more than 20% to the national GDP, with legumes such, as cowpea contributing 10% to total crop value (Nhlengethwa *et al*., 2014). Apart from being a food and income security crop, cowpea contributes to the sustainability of cropping systems by checking weeds and improving soil fertility, through atmospheric nitrogen fixation (Chiulele *et al*., 2011; Badiane *et al*., 2004; and Bressani, 1985). However, cowpea production is faced with numerous constrains that leave farmers with little or no returns to investment. Among the major constraints include biotic stresses such as pests, diseases and rootknot nematodes (Adati *et al.*, 2007; Chiulele *et al*., 2011).

Rootknot nematodes (*Meloidogyne* spp.) have been reported to be one of the main biotic constraints to cowpea production in Mozambique (INIA, 2003). Additionally, *Meloidogyne* spp. have been reported to be the leading plant damaging parasites, considering all plant parasitic nematode genera (Trudgill & Blok, 2001; Abad *et al.* 2003; Jones *et al.*, 2013). Rootknot nematodes are not only one of the major diseases of cowpea but also pre-dispose this crop to secondary infections such as those due to *Fusarium* spp. (Powers *et al.*, 2005; Fery *et al*., 1994). Rootknot nematodes have also been sighted by Chiulele (2010) as one of the major biotic constraints facing cowpea production in Mozambique. However, published information on the distribution of *Meloidogyne* species in cowpea growing areas of the country is very limited. Elsewhere, reduction of cowpea yield as a result of rootknot nematodes (*Meloidogyne* spp.) infection has been reported. In West Africa, up to 89% reduction in cowpea yield has been reported to occur as a consequence of rootknot nematode infestation (Adesiyan *et al*., 1990). Crozzoli *et al*. (1999) reported cowpea yield loss of 72% occurring as a result of rootknot nematode infestation. Such losses may be escalated by insufficient availability of nematode

resistant cowpea cultivars among locally cultivated cowpea, and inadequate information on the distribution of rootknot nematodes in cowpea growing areas of the country. Therefore, knowledge on the resistance status of locally cultivated cowpea cultivars and information on the distribution of *Meloidogyne* species is needed to make informed decisions, during rootknot nematode management and control.

Effective control of nematodes has of recent been remarkably achieved through heavy use of synthetic nematicides. However, the negative environmental and health effects associated with the use of synthetic nematicides have made majority of them to be withdrawn from the market. In addition, rootknot nematodes have not only adapted and or developed resistance to some nematicides, but have also evolved to break host resistance (Onkendi *et al.*, 2014; Kepenekci *et al*., 2016). These events therefore, pose a need of identifying and investigating other possible control alternatives, such as the use of host resistance, which can possibly be achieved through screening locally available crop germplasm for possible resistance to nematodes.

1.3 Research justification

Reduction in cowpea yield poses a big threat to the food, income and nutritional security of a large portion of Mozambique's smallholder farmer population, which heavily relies on this crop to meet their food, income and nutritional needs. This reduction in yield is manifested through reduced foliar biomass, number of fresh pods and grain, emanating from rootknot nematode infestation of cowpea fields (INIA 2003, Chiulele, 2011). Unfortunately, published information on the identity and distribution of rootknot nematode species affecting cowpea farmers in Mozambique is very limited. In addition, limited research effort has been directed towards characterizing local cowpea germplasm for resistance against rootknot nematodes. This therefore poses a crucial need for identifying rootknot nematode species affecting smallholder farmer fields and their distribution in country. There is also a need to screen locally available cowpea germplasm for resistance against local populations of *Meloidogyne* species. Identification of local populations of *Meloidogyne* species was seen as a way to facilitate the use of host (cowpea) resistance as a nematode management and control approach. This was to be achieved through using information obtained from this study, in the making of informed

decisions on a combination of techniques, such as variety recommendation, and other approaches to manage, and control rootknot nematodes especially in the identified hotspot areas.

1.4 Research Objectives

The current study was aimed at contributing to the improvement of cowpea production in Mozambique through establishing the distribution and extent of the cowpea rootknot problem caused by nematodes (*Meloidogyne* spp.) in major cowpea growing areas of Mozambique, and through characterization of local cowpea genotypes for resistance to (*Meloidogyne* spp.).

The specific objectives of the study were:

1. To establish the distribution of rootknot nematodes and evaluate the incidence and intensity of the rootknot problem in cowpea growing areas in Mozambique;
2. To identify the rootknot nematode species (*Meloidogyne* spp.*)* affecting cowpea;
3. To identify cowpea genotypes resistant to rootknot caused by *Meloidogyne javanica*.

1.5 Research Hypotheses

The research will be conducted to verify the following hypotheses:

1. The intensity of rootknot problem due to rootknot nematodes varies significantly across and within selected cowpea growing localities.
2. Selected cowpea growing localities are infested with at least one rootknot nematode species.
3. At least one cowpea genotype is resistant to rootknot caused by rootknot nematodes.

1.6 Thesis outline

The study is made up of different specific objectives which were addressed in different chapters that make up this thesis. The thesis chapters are discrete and independent papers and therefore, overlaps are most likely to occur in terms of content and references among the different chapters. Below is how the chapters are organized:

1. Chapter one: Introduction.
2. Chapter two: Literature review.
3. Chapter three: Determination of the intensity and distribution of cowpea rootknot due to *Meloidogyne* spp. within and across selected cowpea growing areas in Mozambique.
4. Chapter four: Characterization of cowpea genotypes for resistance to rootknot caused by rootknot nematodes (*Meloidogyne javanica*) in Mozambique.
5. Chapter five: General overview

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CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

This chapter provides a context to the study by reviewing relevant literature on rootknot nematode research, with emphasis on management, control and various factors involved in host plant-rootknot nematode interactions. This chapter also provides information on resistance of plants to diseases but more the resistance of plants to rootknot nematodes, sighting the most studied rootknot nematode resistance conferring genes in cowpea and a few other plants. The chapter therefore reviewed information on (1) cowpea production in Mozambique and associated production constraints, (2) taxonomy of rootknot nematodes, (3) species and symptoms of rootknot nematodes, (4) epidemiology of *Meloidogyne* rootknot and the rootknot nematode life cycle, (5) plant-nematode interactions in the rhizosphere that lead to parasitism by *Meloidogyne* species, (6) involvement of host plant genes in rootknot nematode parasitism, (7) rootknot nematode secretions and their role in plant parasitism, (8) plant damage by rootknot nematodes, (9) management of rootknot nematodes, (10) resistance against diseases in plants, (11) resistance and tolerance to rootknot nematodes among host plants and mechanisms involved, (12) genetics of cowpea resistance against rootknot nematodes, and (13) assaying plants for rootknot nematode resistance.

2.2 Cowpea production in Mozambique and associated production constraints

Cowpea is widely grown in Mozambique, mainly in the coastal regions of the country, for grain fresh leaves and fresh pods (Chiulele *et al*., 2011; Bulletin of tropical legumes, 2013). It is an important crop where the grain and leaves are major sources of food and family income, particularly for the resource-poor households (Chiulele *et al*., 2011). In many parts of the country, a higher importance is afforded to the leaves than the grain. For this reason, farmers grow spreading varieties that are characterized by high biomass. This biomass serves for a long period as vegetable produce usually referred to as "meat for the poor", due to its low cost and high protein content (Bulletin of tropical legumes, 2013).

Average cowpea yields in Mozambique have persisted below 300 kg ha^{-1} (INIA, 2000). With average yield estimated at 275kg ha^{-1} the country's cowpea yield is low compared to the largest producer, Nigeria at about 609kgha^{-1} (FAOSTAT, 2014). This has been attributed to production constraints especially disease (viral, bacterial, fungal) and plant parasitic nematodes,

exacerbated by limited availability of resistant cowpea cultivars due to limited breeding work (Emechebe & Lagoke, 2002; INIA, 2003; Chiulele *et al*., 2011). These constraints present a great challenge to successful field performance of cowpea.

Cowpea is affected by a number of fungal diseases such as, Alternaria leafspot caused by *Alternaria* spp. (Grange & Aveling, 1998), Choanephora pod rot caused by *Choanephora cucurbitarum* (Munoz and Tamayo, 1994), Colletotrichum stem disease caused by *Colletotrichum dematium* (Smith *et al.,* 1999a), latent anthracnose (Latunde-Dada *et al*.,1999), Pre- and post-emergence damping-off induced by *Pythium ultimum* (Aveling & Adandonon, 2000), macrophomina blight (Ratnoo *et al.*,1997; Afouda,1999), Sphaceloma scab caused by *Sphaceloma* spp. (Emechebe 1980; Nakawuka & Adipala 1997), Cercospora and Pseudocercospora leaf spots induced by *Cercospora canescens* and *Mycosphaerella cruenta*, respectively (Emechebe & Shoyinka, 1985), brown rust induced by *Uromyces appendiculatus* (Emechebe and Shoyinka 1985; Konate & Ouedraogo, 1988; Stofella *et al.,* 1990) amongst others.

Viral diseases of cowpea include: blackeye cowpea mosaic potyvirus (BlCMV), cowpea aphid-borne mosaic potyvirus (CABMV), cowpea mosaic comovirus (CPMV), cucumber mosaic cucumovirus (CMV), cowpea chlorotic mottle bromovirus (CCMV), cowpea mottle carmovirus (CPMoV), southern bean mosaic sobemovirus (SBMV), cowpea severe mosaic comovirus (CPSMV), (Hampton *et al.,* 1997). While bacterial diseases include: cowpea bacterial blight and cowpea bacterial pustule (Emechebe & Lagote, 2002).

Damage caused by nematodes on cowpea includes rootknot due to *Meloidogyne* spp., root lesion disease due to *Pratylenchus* spp., dagger nematode disease due to *Xiphinema* spp. amongst others (Oyewale & Bamaiyi, 2013). However, amongst all plant parasitic nematode species, *Meloidogyne* species have been sighted as nematode species with the highest economic importance. This has been mainly due to the high virulence levels observed in studies involving *Meloidogyne incognita* and *M. javanica* (Sarmah & Sinha 1995; Puruthi *et al.,* 1995; Khan *et al.,* 1996; Sikora *et al.*, 2005; Pham *et al.*, 2013; Onkendi, 2014).

2.3 Rootknot nematodes

Plant parasitic nematodes are small, microscopic round worms, which live in the soil and attack the roots of plants (Noling, 1999), the first report of nematodes associated with plant disease was made in England by Needham in 1743 (Agrios, 2005), however, rootknot nematodes were first named in 1879 by Cornu as *Anguillula marioni* in France (Esfahani, 2009) Rootknot nematodes are plant parasitic nematodes from the genus *Meloidogyne*, parasitism by this species was first reported in cassava (Neal, 1889), they exist in soil in areas with hot climate (typical of the tropics). About 2000 plants are susceptible to the infections of this genus and they have been reported to cause approximately 5% global crop loss (Sasser et al, 1984).

2.3.1 Taxonomy of rootknot nematodes

Rootknot nematodes belong to kingdom metazoa, phylum nematoda, class secernentea, order tylenchida, family meloidogynidae, and genus *Meloidogyne*. Main plant parasitc species if rootknot nematodes include: *Meloidogyne javanica, M. arenaria, M. incognita* and *M. hapla* (Eisenback & Triantaphyllou, 1991; CABI, 2016).

2.3.2 Identification of rootknot nematode species

A number of diagnostic techniques have been developed and used to tell apart the several lineages to which the various rootknot nematodes belong. These diagnostic techniques include morphological methods such as the perineal pattern analysis of female rootknot nematodes (Hunt & Handoo 2009), host range tests (Hartman& Sasser, 1985), biochemical (Esbenshade & Triantaphyllou, 1985) and molecular techniques (Castagnone-Sereno *et al*.1995). Over time, these have under gone rootknot nematode diagnosis has under gone several changes aimed at improving accuracy of diagnosis. The methods mentioned above have several short coming such as development stage dependence in the case of morphological methods, ambiguity of results, poor band visibility, and low sensitivity for the case of earlier molecular techniques. The latest diagnostic break through has identified polymorphism in the mitochondrial coding genome of tropical rootknot nematodes. This has circumvented the short comings encountered in the earlier techniques. PCR primers for the identified polymorphisms have been developed and used in successful identification tropical rootknot nematodes. (Janssen *et al*., 2016)

2.3.3 Symptoms of rootknot nematode infection on plants

On the attacked plants, rootknot nematodes cause symptoms such as, reduced growth and fewer, small, pale green and or yellowish leaves (Figure 1A) that tend to wilt in warm weather, fewer and poor quality blossoms and fruits. Characteristic symptoms of the disease appear on the underground parts of the plants (Figure 1B). Infected roots develop the typical rootknot galls that are two to several times as large in diameter as the healthy root. Several infections along the root give the root a rough, clubbed appearance (Mitkowski & Abawi, 2003). Necrosis and rotting of infected roots particularly late in the season also occurs. There is presence of small swellings over the surface of infected underground food storage organs in plants such as carrots, potatoes, peanuts, and yam. These swellings eventually become quite prominent and cause distortion or cracking. Infected tree roots develop galls roughly proportional in size to the length of time since infection (Agrios, 2005)

2.3.4 Damage caused on host plants by rootknot nematodes

Rootknot nematodes are particularly damaging crops in tropical and sub-tropical countries (Sikora *et al*., 2005). Crop damage is worsened by the presence of a mixed community of plant parasitic nematodes in the field rather than a single species occurring alone (Noling, 2009). Rootknot nematode damaged roots do not use water and fertilizers effectively (Gapasin, 1980). Such damage is exhibited as patches of stunted and yellowed plants, presence of root galls, excessive branching of roots, reduced root system, poor germination and plant death may be observed in cases of heavy infestations (Adegbite, 2011).

Rootknot nematode infection also predisposes growing crops to secondary infections especially soil borne pathogens for example; soil born fungi such as *Fusarium spp*. (Powers *et al.*, 2005, Siddiqui *et al*., 2012; Mongae *et al.*, 2013). Damage of plants by rootknot nematodes has been observed to be severe during early plant growth stages. Therefore, plants should be given ample protection during early growth stages, to avert devastating effects of rootknot nematodes (Makumbi *et al*. 2000). However, nematode damage is usually associated and or confused with symptoms of other problems (Theberge, 1985) and this usually makes above ground symptomatic diagnosis of nematode da mage a difficult process.

Figure 1: Symptoms of rootknot nematode infection on cowpea leaves. (A) Stunted growth of cowpea plant due to rootknot nematode infection. (B) Galls on cowpea roots as a result of rootknot nematode infection.

2.3.5 The rootknot nematode life cycle and epidemiology of rootknot

As shown in Figure 1, the rootknot nematode cycle is composed of several developmental stages starting with eggs through a series of juvenile molts to adulthood. The life cycle begins when an adult nematode female lays eggs into gelatinous masses, which are made up of a glycoprotein matrix to keep the eggs together and protect them against environmental extremes and predation. Eggs are usually laid on the surface of galled roots, but can also be found within the galled tissue. The egg masses become firmer and brown with age. Embryogenesis proceeds in the egg and development to the first juvenile stage (J1) takes place inside the egg to become a second stage juvenile (J2). Hatching of the J2 is dependent on sufficient moisture and adequate temperatures. The J2 stage is attracted to roots, as it is very vulnerable in the soil (Perry *et al.*, 2009).

The J2 then penetrate the root in the zone of elongation, migrate through the apoplast, first to the root apex, and then to the developing vascular cylinder where it develops into J3, J4 and adult stage (Wyss & Munch, 1992). The nematode then stimulates root cells around its head to go through repeated rounds of mitosis (karyokinesis) uncoupled from cytokinesis, leading to formation of multinucleated giant cells and syncytia (Gheysen & Fenoll, 2002). Giant cells serve

as the obligate nutritive source for the developing nematode and exhibit extensive remodeling of their cell walls. Giant cell formation, coupled with expansion and proliferation of nearby pericycle and cortical cells, results in the characteristic rootknot gall. The nematodes ingest the cytoplasm of the plant-derived giant cells through their stylets. After three molts, the nematode develops into pear-shaped, egg laying females. Both giant cells and syncytia serve as metabolic sinks that funnel plant resources to the parasitic nematode (Curtis, 2008). Giant cell formation may be impeded by the presence, in some plants of specific resistance (R) genes which enable the plant to recognize and block invading avirulent nematodes (Williamson & Hussey, 1996; Abad, 2003; Bird, 2009). This improves resistance of the plant against these pathogens.

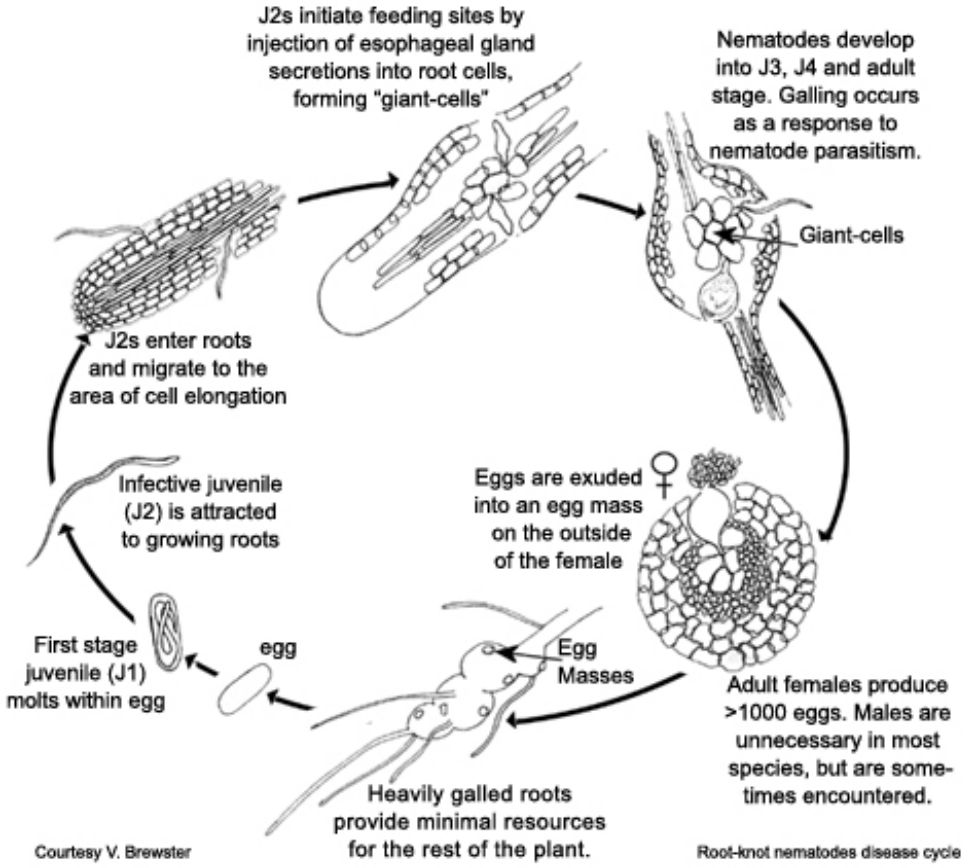

Figure 2: The rootknot nematode life cycle. (Source: Mitkowski and Abawi , 2003).

2.3.6 Plant-nematode interactions in the rhizosphere that lead to parasitism by *Meloidogyne* species

Some endoparasitic plant nematodes do not feed during their migrations in soil and roots. They depend on stored lipid reserves for energy during this migration. After depletion of these lipid reserves, their continued survival depends on finding a food source. The infective stages of rootknot nematodes recognize their hosts via complex sequences of behavioral patterns in response to various environmental cues (Haas *et al*., 1997; Haas, 2003; Curtis, 2008). Additionally, plant signals are essential for nematodes to locate hosts and feeding sites (Robinson *et al.,* 1987).

Plant roots exude a range of compounds into the rhizosphere which mediate below ground interactions with rootknot nematodes (Bertin *et al.,* 2003). According to Zhao *et al*. (2000), Robinson, (2002) and Wuyts *et al*. (2006), plant exudates can repel or attract nematodes to roots, inhibit their motility or even cause their death. For example, plant signals, particularly, plant hormones such as Indole - Acetic Acid (IAA), present in root exudates induce production of nematode secretions, increase nematode mobility and trigger a rapid alteration of the surface cuticle of *Meloidogyne incognita* (Lopez de Mendoza *et al*., 2000; Akhkha *et al*., 2002, 2004; Curtis, 2007). Indol- Acetic Acid has also been reported to shift the response of resistant plants towards susceptibility (Dropkin *et al*., 1969; Sawhney & Webster, 1975; Duncan *et al*., 1996; Curtis, 2007, 2008).

Besides reports on nematodes synchronizing their life cycle with IAA concentrations, tips of root elongation zones, which are preferred sites for nematode invasion and root cells surrounding the nematode head have been reported to have high levels of IAA concentration (Karczmark *et al*., 2004; Mancuso *et al*., 2005). This suggests that this hormone plays a vital role in the host-recognition processes for sedentary plant parasitic nematodes, such as *Meloidogyne* species.

The high levels of concentration of IAA in the root cells surrounding the nematode head can be attributed to the fact that nematodes possess in their heads two bilaterally symmetrical amphids. These are chemo-sensory organs to which IAA binds (Curtis, 2008). This use of IAA as host recognition cue by rootknot nematodes has been observed in *Meloidogyne incognita*

(Curtis, 2007). Curtis (2008) argues that, understanding of these signaling and perception processes occurring in plant-nematode interactions will reveal targets for chemical or genetic interventions, which will probably lead to successful control of these menaces.

2.3.7 Interaction between host plant genes and rootknot nematodes

Through host plant transcription pattern comparisons, infection of plants with rootknot nematodes has been reported to initiate complex changes in host plant gene expression (Gheysen, 2002). Amongst these complex changes include induction of genes that are likely to contribute to establishing a parasitic interaction (Gheysen, 2002; Puthoff *et al.,* 2003). In regard to this, extensive changes in cell wall architecture have been reported to occur during syncytia and giant cell development. This is an indication of the upward regulation of host plant genes that encode host plant cell-wall degrading enzymes. For example, up regulation of host polygalacturonase and endoglucanase genes has been reported to occur after infection with rootknot nematodes (Wang *et al.,* 1999; Mahalingam *et al.,* 1999; Goellner *et al.,* 2000; Goellner *et al.,* 2001).

Expression patterns of endoglucanase genes have been reported to be consistent with their having a role in syncytium formation and giant cell development (Goellner *et al.,* 2000; and Goellner *et al.,* 2001). Additionally, as transcription regulators required for the formation and maintenance of meristems, Phan and Knox genes have also been co-localized in the feeding sites of rootknotnematodes (Koltai *et al.,* 2001). This suggests a possibility of host plant genes involvement in maintenance of metabolic sink activities of giant cells.

2.3.8 Rootknot nematode secretions and their role in plant parasitism

Through a stylet located at their anterior, nematodes have been reported to secrete substances from two sub-ventral and one dorsal esophageal gland cells. These secretions are thought to play a vital role in the infection and formation of host feeding cells. Secretions from the sub-ventral glands play important functions in early stages of parasitism while those from the dorsal gland take part in development and maintenance of feeding sites (Davis *et al.,* 2000; Williamson & Gleason, 2003). Genes and gene homologs encoding plant cell wall degrading enzymes like β-1,4-endoglucanase or cellulose, pectate lyase and polygalacturonase have also been identified in plant-parasitic nematodes and quite often, transcripts of these enzymes have been localized to the sub-ventral gland parasitism (Smant *et al.,* 1998; Goellner *et al.,* 2000;

Popeijus *et al*., 2000; Dautova *et al*., 2001; Muetter *et al*., 2001; De Boer *et al*., 2002; Doyle & Lambert, 2002) confirming the role of sub-ventral gland secretions in parasitism.

Another gene family expressed in rootknot nematode esophageal glands is the chorismate mutase. Transgenic expression of the chorismate mutase gene has been reported to result into suppression of lateral root formation, suppression of vascular system development and reduction of plant auxin levels (Lambert *et al*., 1999; Doyle & Lambert, 2003). These effects further indicate that esophageal gland secretions have a strong bearing on rootknot nematode parasitism in hosts.

2.4 Management of rootknot nematodes

Control of rootknot nematodes has been primarily accomplished through chemical nematicides and remarkable reduction of nematode populations has been achieved (Akhtar *et al.*, 2000; Onkendi *et al.*, 2014). However, because most nematicides have been withdrawn from use due to their high toxicity and damage to the environment, other environmentally benign nematode control alternatives have been sought and some experimented, amongst which include cultural, biological and integrated approaches (Adegbite, 2011).

2.4.1 Use of organic amendments to control rootknot nematodes

Singh *et al.* (1994) reported that application of oil cake of pangonia (*Pangonia glabra*) and margosa (*Azadiracta indica*) each at a rate of 2.5 mt/ha was very effective in reducing rootknot In addition, Nazli *et al.* (2008) reported that leaf extract of *Gliricidia sepium* has some insecticidal, and antibacterial activity and it causes 60% mortality of J2 of *M. incognita.*

In vitro (Petri- dish) studies of extracts of *Hyoscyamus niger* (Solanaceae), *Melia azedarach* (Meliaceae), and *Xanthium strumarium* have been reported to be associated with higher death rates of rootknot nematode juveniles at concentrations at 6 and 12% concentration. Similar concentrations as well as 3% of the same extracts were found to be 100% effective against nematode egg hatching (Kepenekci, 2016). Used singly, cowdung was effective in reducing galling and nematode multiplication on lentil (Rizvi *et al.* 2015). Singh and Sitaramaiah, (1994) reported that application of oil cake of pangonia (*Pangonia glabra*) and margosa (*Azadiracta indica*) each at a rate of 2.5 mt/ha was very effective in reducing rootknot

on okra and tomato. In addition, in their in vitro assays (Petri-dish), Nazli *et al.* (2008) found out that leaf extract of *Gliricidia sepium* has some some nematicidal activity and can causes up to 60% mortality of J2 of *M. incognita.*

2.4.2 Biological control of rootknot nematodes

This concept has eco-friendly pest management strategy that utilizes deliberate introduction of living natural enemies, to lower the population level of a target pest. Besides the use organic amendments, biological control has become one of the most promising nematode control alternatives (Stirling, 1991; Brand *et al*., 2010).). In the past, chemical agents such as methyl bromide against rootknot nematodes have been widely used to control rootknot nematodes but due to associated environmental hazards, research has been directed towards developing environment friendly nematode control alternatives such as biological control (Brand *et al*.., 2010; Kiewnick, 2010; Moosavi *et al*., 2010). An array of biological control agents including bacterial and fungal nematode antagonists have been reported to control nematodes. Fungal antagonists of nematodes include nematode-trapping fungi, predacious fungi, endoparasitic fungi, egg parasitic fungi, and cyst parasitic fungi (Casas & Estrella, 2007). Additionally, Kiewnick (2010) suggested that these biological control organisms may also be plant growth promoters or nematode parasites or predators.

2.4.3 Use of antagonistic fungi to control rootknot nematodes

Hashem and Abo-Elyousr (2011) stress the causing of lethal effect on nematodes and enhancement of plant growth, as the two mechanisms biological control agents minimize crop yield losses by nematodes. The same authors add that application of biological control agents such as *Pseudomonas fluorences*, *Purpureocillium lilacinus* and *Pichiagu illiermondii* enhances the growth of the plant through supplying nutrient elements, thereby inducing the systematic resistance of the plant. Additionally, antagonistic fungi such as *Purpureocillium lilacinus* have been reported to be parasitic on nematodes and their eggs (Atkins *et al.*, 2003).

Antagonistic fungi have been reported to control the development of nematodes by attacking the egg or larvae of the nematodes and using the nematodes as a food source for further growth and reproduction (Ayalew, 2014). Fungi also attack nematodes by producing toxic compounds, which affect nematode survival, reproduction, development and ability to infest and

attack crops or by parasitizing them directly (Singh and Mathur, 2010). Fungi also increase the systemic resistance of the plant by inducing plant defense mechanisms (Sehebani and Hadavi, 2008). Fungi, as a biological control agent against rootknot nematodes, are utilized through application in the soil. Fungal spores applied to the soil in the rhizosphere of the plant to fasten the effect of the fungi on the nematodes, since nematodes are largely available in the rhizosphere of the plants. Maximum nematicidal efficacies of the fungus against rootknot nematodes can be achieved by synchronizing the active phase of fungus with the sedentary phase of female nematodes and eggs (Brand *et al.*, 2010)., without or with minimal negative side effects as compared with chemical control methods.

Various fungal species which are antagonistic, pathogenic and parasitic on nematodes, such as *Trichoderma harzianum, Purpureocillium lilacinus, Dactylella oviparasitica* and *Pochonia chlamydosporia* amongst others have been used in biological control of nematodes. For example, Sehebani and Hadavi, (2008) have shown that the use of a fungus; *Trichoderma harzianum* to control *Meloidogne javanica* decreases nematode infection and egg hatching level of the nematodes. Lytic enzymes; serine protease and chitinase produced by *Purpureocillium lilacinus* have been reported to penetrate and attack eggs and cuticles of nematodes (Atkins *et al.*, 2003; Hashem and Abo-Elyousr, 2011). Research by Kepenekci and Oksal (2015) established that numbers of nematodes were decreased by increasing the inoculum level of the entomopatogenic fungus *Purpureocillium lilacinum*. Furthermore, treating of nematode infested soils with spores of the fungus *Dactylella oviparasitica* has been reported to result into a parasitic effect of this fungus on the eggs of rootknot nematodes (Stirling *et al.* (1979). Nematode control studies involving *Pochonia chlamydosporia* a soil fungus have shown that this fungal species can result into *Meloidogyne* egg parasitism levels as high as 50-97% (Giné *et al.*, 2016). In addition to the above fungi, other fungal species such as *Verticillium chlamydosporium, V. catenulatum, Fusarium oxysporum, F. solani, Fusarium* spp., *Acremonium strictum, Gliocladium roseum, Cylindrocarpon* spp. have also been reported to be parasitic on *Meloidogyne* spp. (Verdejo-Lucas *et al*., 2002).

2.4.4 Use of bacteria to control rootknot nematodes

As an endospore forming bacteria, *Pasteuria penetrans* has been reported to be an obligate parasite of rootknot nematodes in which nematode parasitism by endosporulation has

been found to increase with an increase in the number of nematode females initially pre-inoculated with the bacterial spores (Darban *et al.*, 2016). Besides *Pasteuria penetrans*, *Pseudomonas* spp. has also been reported provide considerable control of rootknot nematodes (Ayalew, 2014).

2.4.5 Other approaches used in rootknot nematode management

In addition to the above mentioned rootknot nematode management and control approaches, a number of other measures have been used either singly or in combination. These have involved field practices, such as crop rotation, fallowing, weed control, use of nematode resistant plant material, practicing field sanitation, use soil amendments amongst other practices (Hill, 1988).

2.5 Resistance against diseases in plants

Parasites are ubiquitous and have detrimental effects on their hosts (Price 1980), resulting in strong selection for defensive mechanisms that prevent or mitigate the harm caused by infection. These defenses can be divided into two broad classes: resistance and tolerance (Price 1980; Horns & Hood, 2012). Resistance is defined here as the ability of the host to reduce the growth of the pathogen, thus reducing both the loss of host fecundity due to infection and the pathogen transmission from the infected host, but not the probability of becoming infected (avoidance). Tolerance is defined as the host's ability to offset the negative effects of infection on host fecundity, but without limiting pathogen growth or reproduction and thus without effects on pathogen transmission (Horns and Hood, 2012),

According to Agrios (2005), disease tolerant plants are obviously susceptible and this tolerance is suggested to result from specific, heritable characteristics of the host plant that allow the pathogen to develop and multiply in the host while the host, either by lacking receptor sites for or by inactivating or compensating for the irritant excretions of the pathogen, still manages to produce a good crop. Despite the many various ways in which the term has been used, tolerance describes a host plant's response to infection. The concept of tolerance therefore suggests endurance and implies that "plant A" undergoes the same stress as "plant B" but "plant A" withstands this stress better.

On the other hand, Vanderplank (1963) divided disease resistance into two classes: one, vertical resistance, which is controlled by a few "major" resistance genes and is strong but is effective only against one or a few specific races of the pathogen and two, horizontal resistance, which is determined by many "minor" resistance genes and is weaker but effective against all races of a pathogen species. These two types of resistance are also termed as partial, also called quantitative, polygenic resistance, and R gene resistance, also called race specific, monogenic, or vertical resistance respectively and together confer true resistance against plant diseases (Agrios, 2005). It has been proposed in Agrios (2005) that each major or minor gene for resistance represents one or several steps in a series of biochemical reactions and that it usually operates in conjunction with several other genes. Together, these genes enable the plant to produce certain types of plant cell substances and structures that interfere with, or inhibit, the growth, multiplication, or survival of the attacking pathogen, and in that way they inhibit, or stop, the development of disease.

2.6 Resistance, tolerance to rootknot nematodes among host plants, and mechanisms involved

Resistance against rootknot nematodes among host plants has been commonly defined as the ability of the host plant to support low or no nematodes reproduction (Roberts, 2002). This resistance is often as a result of the presence in a host plant of specific resistance genes that can segregate within that particular species. Once the host plant has been attacked by rootknot nematodes, resistance genes may block or suppress one of the critical steps in nematode parasitism. On the other hand, host plant tolerance has been defined as hosts that show little or no plant damage or crop loss in response to nematode infection (Williamson & Roberts, 2009).

A host plant may be resistant to one nematode species but intolerant to the other. For example, some resistant sweet potato cultivars have been found to be intolerant to *Meloidogyne incognita*. This is because they put up a strong hypersensitive reaction that damages root growth and function (Roberts & Sheuerman, 1984; Williamson & Roberts, 2009). In some compatible nematode-host interactions, some susceptible hosts have been observed to be tolerant. This tolerance has been observed to occur in some maize cultivars and has been attributed to the rapid regeneration of roots to maintain adequate root function (Roberts, 1992).

Rootknot nematode resistance traits comprise several features. Resistance can be dominant, recessive or additive in expression and can be conferred by single major genes or by combinations of two or more genes or quantitative trait loci. The resistance phenotype can be characterized as strong or partial, depending on the extent to which nematode reproduction and root galling are suppressed (Williamson & Roberts, 2009). *Meloidogyne* resistance genes differ in their mechanisms of action, strength, durability and location of effect.

Activation of resistance (R) genes triggers a set of responses including an oxidative burst generally regulated by salicylic acid dependent defense pathway (Glazebrook, 2005). For some rootknot nematode resistance genes such as *Mi-1* in tomato, resistance has been mediated through rapid hypersensitive reactions (HR) observed as early as 12 hours post inoculation. This HR does not occur throughout the course of migration of the second stage juvenile (J2) but rather when the J2 attempts to establish a feeding site (Ho *et al*., 1992; Williamson & Roberts, 2009). Additionally, increased expression of glycosyltransferase has been observed to occur after infection of the plant with nematodes and it has been suggested to have a role in *Mi* mediated resistance (Schaff *et al*., 2007). Furthermore, an enhanced production of reactive oxygen species associated with nematode infection has been observed to be associated with nematode infection of resistant compared with susceptible tomato roots (Williamson & Roberts, 2009).

Apart tomato, where a hypersensitive response occurs only when the nematode attempts to initiate a feeding site, hypersensitive responses characterized by immediate or rapid necrosis in the root epidermis or cortex within 1-2 days post nematode inoculation, have been observed in Me_7 (a gene that is responsible for resistance against rootknot nematodes in pepper) mediated rootknot nematode resistance in pepper. These responses were also associated with the formation of phenolic compounds such as chlorogenic acid. These observations are evident for the deployment of various mechanisms by resistance genes, with variable timing and location, to evade rootknot nematode infection (Ho *et al*., 1992; Pegard *et al*., 2005).

There is limited knowledge about how the *Mi-1* gene recognizes the presence of nematodes. However, it has been suggested that the gene may be capable of recognizing avirulence products or pathogen effectors (Williamson & Roberts, 2009). It is further suggested that the gene may be capable of recognizing modifications to the host plant products altered by

the pathogen. Resistance mediated by the *Mi-1* gene is not a single gene role. It has been observed that plant genes *Hsp90* and *Sgt1*, required for resistance mediated by other R genes are also required for *Mi-1* mediated resistance (Bhattarai *et al*., 2007; Williamson & Roberts, 2009). Furthermore, mutations in the locus *Rme1*, unlinked to *Mi-1*, have rendered previously resistant host plants susceptible (Martinez de Ilarduya, *et al*., 2001), indicating a possible inter-dependence of resistance genes.

2.7 Genetics of cowpea resistance against rootknot nematodes

Host resistance remains the most cost-effective and environmentally benign approach for rootknot nematode control, however, the sad note is that resistance is not available in many crops and not always durable where available (Bird, 2009; Abad *et al.* 2003; Williamson & Hussey, 1996). Studies on cowpea genetics have provided evidence that resistance to rootknot nematodes in cowpea involves a cascade of additive genes. In their genetic studies on resistance against rootknot nematodes in cowpea, Fery and Dukes in (1980) identified a rootknot nematode resistance gene which they designated *Rk*. Roberts *et al.* (1996) argues that all resistance against rootknot nematodes in cowpea is conferred by the *Rk* gene, with alleles such as *rk*, rk^i, *Rk*, and *Rk*2. In addition to these alleles, another allele *rk3* has been observed to contribute to additive resistance in some cowpea breeding lines and cultivars (Ehlers *et al*, 2002).

The *Rk* gene confers a delayed but strong resistance mechanism without a hypersensitive reaction-mediated cell death process. This resistance process allows nematode development but blocks reproduction (Das *et al.,* 2010). In resistant cowpea cultivars, the resistance conferred by the *Rk* gene does not decrease the rate of root penetration or J2 migration through the epidermis to the vascular cylinder. However, the *Rk* gene allows giant cell formation and nematode development to the third and fourth stage juveniles up to 9 days post inoculation, just like it happens in susceptible cowpea cultivars. Subsequently, an increased vacuolation of giant cells in roots of resistant cultivars is observed by 14 days post inoculation (Das *et al*., 2008). Complete vacuolation and collapse of giant cells is observed at 19 to 21 days post inoculation. A few juveniles may reach a young female stage but rarely produce eggs and or egg masses. These incompatible responses are associated with deterioration and death of immature nematodes (Das *et al*., 2008; Williamson & Roberts, 2009).

Despite the fact that the *Rk* gene is effective in reducing reproduction in most *Meloidogyne* populations, studies have shown that some *Meloidogyne* populations are capable of reproducing on, and causing yield loss in cowpea cultivars with this gene (Roberts & Matthews, 1995; Roberts *et al.*, 1995). The existence of such virulence dynamics has prompted research into new sources of resistance. This has eventually led to the discovery of a stronger broader-based resistance mediated by another resistance gene *Rk2*. The *Rk2* gene confers a higher level of resistance to *Rk*-virulent *M. incognita* and *M. javanica* isolates compared to the *Rk* gene (Roberts *et al*., 1996, 1997). The gene *Rk2* came to light when Roberts *et al*. (1996) identified cowpea line IT84S-2049, to be completely resistant to diverse populations of rootknot nematodes such as *M. incognita,* and *M. javanica*. Some populations of *M. incognita,* and *M. javanica* had earlier been established to be virulent against the *Rk* present in commercial cowpea varieties such as CB5 and CB46. Eventually systematic studies indicated that the superior resistance in line IT84S-2049 was conferred by a single dominant gene which was either allelic to the *Rk* gene or a different gene closely linked to *Rk*. The emergence of aggressive *Meloidogyne* populations damaging cowpea cultivars with resistance genes makes regular nematode resistance bio-assaying a necessity. This will therefore lead to continued use of resistance as nematode management tool (Ehlers *et al.,* 2002).

2.8 Assaying plants for rootknot nematode resistance

A number of approaches have been used to assay plants for resistance to rootknot nematodes. Traditionally, assessment of different plants for resistance to rootknot nematodes has been achieved through use of pot experiments (Figure 3B). However, high through put screening protocols have been developed for both large and small seeded plants (Atamian *et al*., 2012).

"High throughput technologies" is the generic name for an expanding range of advanced experimental and computational tools and techniques that enable rapid, intelligent, parallel acquisition of experimental data, increasing the productivity of research and development by orders of magnitude over traditional approaches (https://connect.innovateuk.org/web/high-throughput-technologies-htt). During high through put screening using pouches (Figure 3A) and trays, each plant (cowpea and common beans) is inoculated with 1500 and 500J2 respectively while as 3000J2 have been deemed sufficient for individual plant inoculation in pot screenings (low throughput experiments). The experiment is then left to stand for about 28-30 days

(pouches) and 6-8weeks (pots). Evaluation of plants for resistance then follows; it is achieved through assessing the extent of nematode reproduction on plants. Development of high through-put screening has enabled large scale resistance assays within a small growth area (Atamian *et al*., 2012).

Figure 3: High and low through put screens. (A) Roots stained with erioglaucine in a pouch (high throughput screening). (B) Tomato plant in traditional experimental plastic pot. (*Source*: Atamian *et al*. (2012)

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CHAPTER THREE:

INTENSITY AND DISTRIBUTION OF *Meloidogyne* Spp. IN SELECTED COWPEA GROWING AREAS OF MOZAMBIQUE

Abstract

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important food crop in Mozambique. The crop is cultivated almost exclusively by smallholder farmers in warm marginal environments of the country. Rootknot nematodes are among the major constraints limiting cowpea yield in the country. Therefore, understanding their distribution and damage intensity within and across cowpea growing areas is crucial in making control decisions. A study was conducted to determine rootknot nematode distribution, damage intensity (incidence and severity), and species identification in 8 districts belonging to 3 provinces namely: Gaza, Inhambane, and Nampula, of Mozambique. Rootknot nematode incidence in cowpea plants was recorded as the percentage of cowpea plants infested with rootknot nematodes in relation to the total number of plants sampled. Severity of rootknot damage was achieved by scoring for the extent of rootknot nematode galling on cowpea roots to obtain a rootknot gall index. A scale of 1-5 was used to determine the total of this index. Out of the 72 cowpea fields surveyed, 56.9% were infested with rootknot nematodes, majority were from Inhambane province. *M. incognita*, *M. javanica*, and *M. enterolobii* were identified to be associated with cowpea rootknot. The highest frequency of *M. incognita* and *M. javanica* was observed in cowpea fields from Inhambane and Gaza provinces, respectively. *M. enterolobii* was also observed from a cowpea in Inhambane province. The 3 species generally occurred more frequently in Inhambane than the rest of the provinces. Inhambane province registered the highest (39.8%) mean rootknot nematode incidences respectively. Homoine district (in Inhambane province) had the highest mean rootknot nematode incidence (55.8%) which did not differ significantly from other districts except Moma with 4.7% mean rootknot nematode incidence. Generally, rootknot nematode galling score across provinces was low, with highest mean galling score being observed in Inhambane province (1.9). Gaza and Nampula provinces recorded a galling score of 1.2 and 1.3 respectively. Homoine district recorded higher mean rootknot galling severity (2.1) than other districts.

Keywords: cowpea, *Meloidogyne* Spp., distribution, damage intensity

3.1 Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is a broadly adapted and an important food crop in Mozambique. The crop is cultivated almost exclusively by smallholder farmers in coastal regions of the country. The major producing provinces are: Nampula, Inhambane, Zambezia, Gaza and Maputo (INE 2008, Chiulele *et al.* 2011, Tropical Legumes project, 2013) in Mozambique. Cowpea ranks fourth most cultivated crop after maize, cassava and groundnut (INE, 2008). The crop is produced for household consumption, mainly for its grain, young leaves and fresh pods that are used as food or vegetables and as a source of income (Chiulele *et al.*, 2011, Bulletin of tropical legumes, 2013). The crop is an excellent source of protein estimated at 25% on dry weight basis but it also contains considerable quantities of carbohydrates, vitamins and minerals (Bressani, 1985; Davis *et al*., 1991). It is an important component of cereal and starchy tuber cropping systems in which it has important ecological roles such as improving soil fertility, reducing erosion, suppressing weeds and fixing atmospheric nitrogen when in a symbiotic relationship with rhizobium bacteria and hence improving the performance of crops grown with or after it (Tarawali *et al*., 2002; infonet biovision.com, 2015).

However, average cowpea yields in Mozambique have for long remained below 300 kg ha^{-1}. The major constraints contributing to this are biotic constraints namely: insect pests (aphids, flower thrips, pod sucking bugs and pod borers diseases (Colletotrichum stem disease, cowpea bacterial pustule, cowpea aphid borne mosaic virus, cowpea golden mosaic virus), nematodes, parasitic weeds (*Alletra vogeli* and *Striga* spp.) and abiotic constraints such as drought and low soil fertility amongst others (Munoz and Tamayo, 1994; Hampton *et al*., 1997; Emechebe & Lagote, 2002; INIA, 2000, 2003; Chiulele, 2010). Within nematodes, rootknot nematodes of the genera *Meloidogyne* have been reported to be the most important in damaging cowpea (Fery *et al*., 1994). Attack of cowpea from rootknot nematodes causes galling damage on roots, affecting normal translocation, and when severe, may result into chlorosis, necrosis of roots and rotting (Gapasin, 1980, Agrios, 2005). Studies conducted elsewhere have reported yield losses of up to 72% in susceptible varieties and 22% in resistant cultivars (Crozzoli, *et al*., 1997, 1999). In Mozambique, information regarding the impact of rootknot nematodes on cowpea production and yield is still scarce. In addition, information on rootknot nematode species causing major damage on cowpea, there distribution and intensity is also lacking. Such information would

contribute to efficient rootknot nematode management through facilitating timely and precise decision making during implementation of nematode control measures.

In order to make suitable and effective management recommendations against *Meloidogyne* spp., the identification of species, their distribution and incidence is crucial to cowpea production in Mozambique. The current study was aimed at establishing the level of rootknot nematode intensity, identification of *Meloidogyne* species in cowpea growing areas of Mozambique and to locate hotspot areas.

The specific objectives of the study were:

1. To determine the distribution of rootknot nematodes and evaluate rootknot damage intensity in cowpea growing areas.
2. To identify rootknot nematode species (*Meloidogyne* spp.*)* affecting cowpea

The study was conducted to test hypothesis that rootknot nematodes are widely distributed in cowpea growing areas, and that multiple species are present.

3.2 Materials and methods

3.2.1 Description of the study area

The study was conducted in three of the main cowpea producing provinces of Mozambique, namely: Nampula, Gaza and Inhambane (INE 2008, Chiulele *et al.* 2011). Nampula province is located 15.250°S and 39.500°E in northeastern Mozambique with an area of 79,010 km² (Geohive, 2014, Macauhub.com). The Province borders Niassa Province to the northwest and west, Zambezia Province to the southwest, and the Indian Ocean to the east. Two districts (Moma and Meconta) were selected from this province for this study, based on their importance in cowpea production. Moma is one of the coastal districts of the province and experiences average annual temperatures higher than 25^{o}C and an annual rainfall range between 800 and 1200mm with an alfisol soil type. Meconta is one of the inland districts of the province with an annual rainfall of between 1000 to 1400mm with predominant soil types of ultisols and oxisols (Ricardo & Russell, 2006).

Gaza province is located -23.0222° S, 32.7181° E,in the south of Mozambique with an area of 75,334 km2 and Xai-Xai is its capital. The province is bordered by Inhambane province to the east, Manica Province to the north, Maputo Province to the south, South Africa to the west, and Zimbabwe to the northwest. The province is characterized by arid conditions with an annual rainfall of only 400-600mm with soils of sandy texture (INE, 2006). Three districts were sampled in this province, namely: Bilene, Chibuto and Mandlakazi, based on their importance in cowpea production.

Inhambane province is located -23.0° S, 34.3° E, on the Indian Ocean coast in the southern part of the country with an area of 68,615 km2 and Inhambane being its capital. The province is bordered by Manica and Sofala provinces to the north, Gaza province to the west and the Indian Ocean coast to the south and east. The province is characterized by a warm rainy season between November and March and sandy textured soils (INE, 2006; visit mozambique.net, 2016). In this province, the study took place in 3 districts, namely: Inharrime, Jangamo and Homoine, based on their importance in cowpea production.

3.2.2 Sampling of cowpea fields for rootknot nematode infestation

Figure 4 shows the geographical location of all sampled areas. The study was conducted in a total of three provinces and 8 districts. In each district, 3 localities were selected based on concentration of cowpea farmer fields. In each locality, 3 farmer fields were selected, thus 9 farmer fields were sampled per district. For each of the provinces (Gaza and Inhambane), 27 cowpea fields were sampled, while 18 were sampled for Nampula province. For each field, a maximum of 0.5 acres occupied by cowpea plants were considered for sampling. A zigzag sampling pattern was used in each of the fields selecting between 5-10 plants to constitute a bulk sample per field. Along the zigzag transect, 5 -10 plants were selected uprooted using a shovel and combined together to form a bulk sample. All the root samples from each field were placed in polythene bags with the sample code, location name, sampling date, district name, and location coordinates and placed in a cooler box. The root samples were then taken to the laboratory for rootknot nematode evaluation.

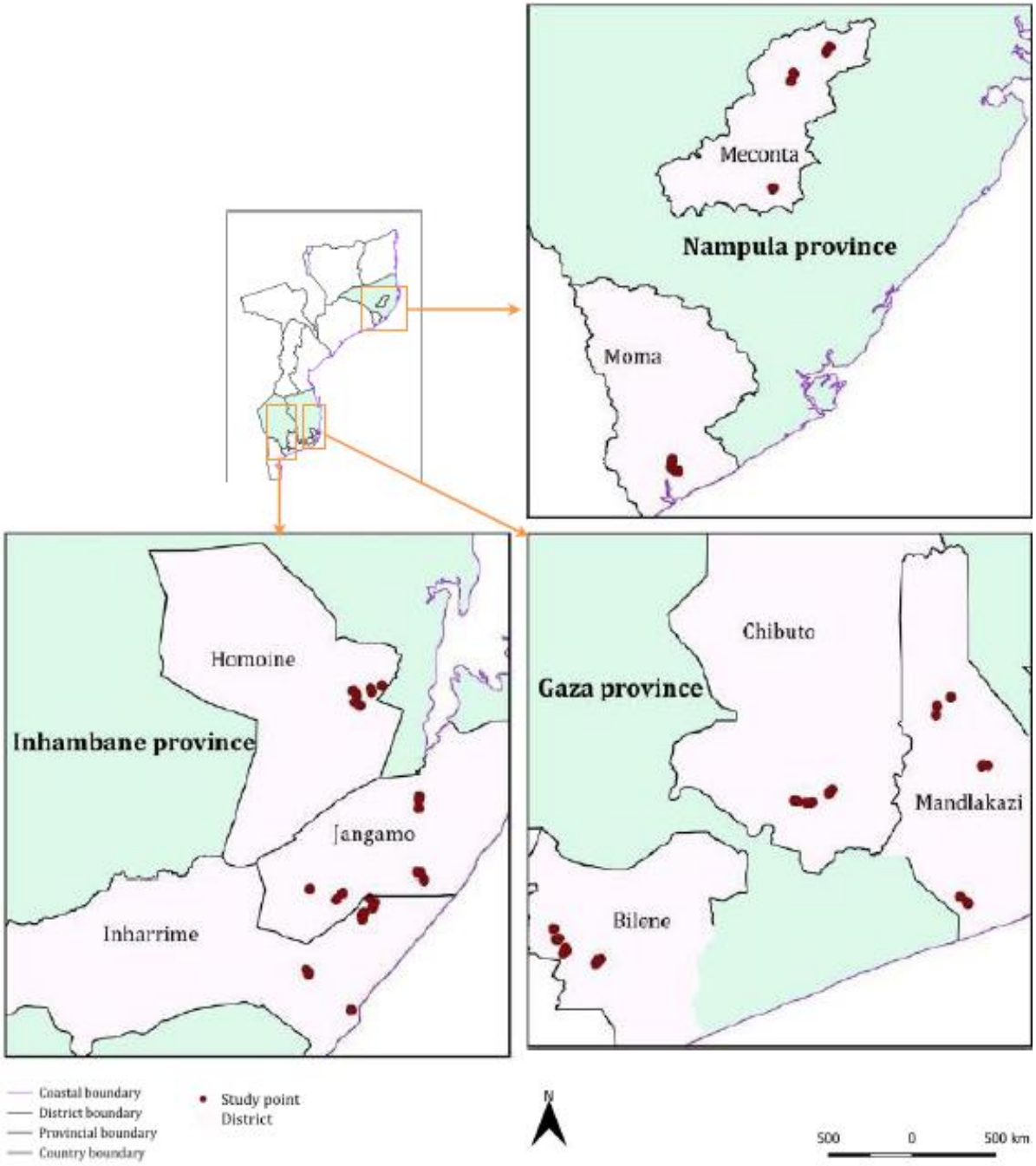


Figure 4: Map of Mozambique showing sampled cowpea fields.

3.2.3 Evaluation of incidence, prevalence and distribution of rootknot nematodes

Table 1 summarizes the amount plant samples collected. Overall, 511 cowpea plant samples were obtained from the three provinces. Other data collected included observations like soil type, previous crops, plant age, cropping system, weeds present and the use of fertilizers and pesticides and other relevant data. Some of the limitations faced during the survey involved some cowpea field owners restricting access of the survey team to their fields, while majority limited the number of plants to be obtained from their fields for the study.

Table 1: Number of cowpea plant samples collected from surveyed areas

Province	District	Number of cowpea plant samples
	Bilene	66
	Chibuto	59
Gaza	Mandlakazi	59
	Inharrime	63
	Jangamo	67
Inhambane	Homoine	72
	Meconta	66
Nampula	Moma	59
Total		511

Form the fields, cowpea root samples were taken to the phytopathology laboratory at the Faculty of Agronomy and Forestry Engineering, Eduardo Mondlane University, Maputo, Mozambique. In the laboratory, root samples were cleaned of soil and other debris under a gentle stream of tap water before blotting them dry with blotting paper. Samples were then dipped in a 1 liter solution containing 15mg of phloxine B for 5 minutes to stain egg masses (Coyne *et al.*, 2007). In addition to nematode symptoms observed from the field, further confirmation of incidence was carried out in the laboratory as some plant roots would not clearly show symptoms of nematode infection until after staining with phloxine B to improve symptom visibility (Figure 5). Incidence was evaluated as presence (P) or absence (A) of rootknot nematodes based on the presence of rootknot galls or egg masses (stained pink by phloxine B) or both. Incidence was thereafter calculated as a ratio of rootknot infected plants to the total number of plants sampled in

each respective farmer field and expressed as a percentage (Equation 1.) and recorded in Microsoft excel. Determination of distribution of rootknot nematodes was accomplished by processing of survey coordinates using software, Quantum GIS, version 2.16 (QGIS Development Team, 2009. QGIS Geographic Information System. Open Source Geospatial Foundation). A map showing rootknot nematode and hence disease distribution and intensity was produced.

$$Incidence\ of\ root\ knot\ nematode\ infestation = \left(\frac{Number\ of\ root\ knot\ nematode\ infected\ plants}{Total\ number\ of\ sampled\ plants\ per\ field}\right) * 100 \qquad (1)$$

Prevalence (percentage of cowpea fields infested with rootknot nematodes) was calculated from equation 2.

$$prevalence\ of\ rootknot\ nematodes = \left(\frac{Number\ of\ cowpea\ fields\ infested\ with\ rootknot\ nematodes}{Total\ number\ of cowpea\ fields\ sampled}\right) * 100 \qquad (2)$$

Figure 5: Rootknot nematode egg masses stained with phloxine B

3.2.4 Determination of rootknot nematode disease severity (rootknot nematode gall index) in cowpea

This was achieved by scoring individual cowpea plants (roots) for rootknot gall index. A scale ranging from 1-5 (Figure 6) modified from Bridge and Page (1980) was used where 1: no galling damage, 2: root shows only small knots but clearly visible and main root is free of knots 3: larger knots predominant but main root is clean, 4: knotting observed on main roots and 5: majority of main roots knotted.

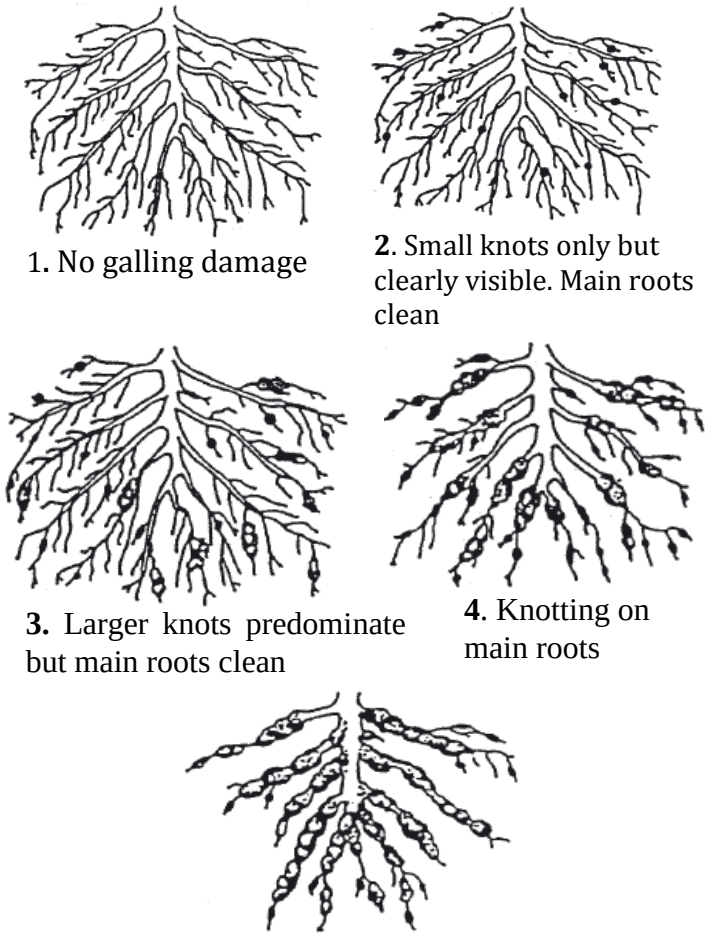


Figure 6: Root gall rating chart. *Modified from* Bridge and Page (1980)

3.2.5 Identification of rootknot nematode species

This was accomplished by extracting triplicates (per sample) of engorged female rootknot nematodes from root samples using a pin, surgical blade, and a pair of scalpels under a dissecting microscope. By selecting one plant from each cowpea field, heavily galled plants from the 41 rootknot nematode infested cowpea fields were used for the extraction of female nematodes used in the identification process. However, not all the root sample where heavily infested to provide sufficient numbers of rootknot nematode females. Due to this, females were extracted from only 36 individual plant root samples. These therefore provided a total of 36 female rootknot nematode samples. From each plant, females were extracted in triplicates to provide enough analyzable DNA (Deoxyribose Nucleic Acid). Samples of these female rootknot nematodes were preserved in absolute alcohol, in labeled, tight sealing Eppendorf tubes. These samples were then sent to Nematology Research Unit, Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium for identification, using a method described by Janssen *et al.* (2016). Out of the 36 samples, only 22 provided enough DNA material for PCR (Polymerase Chain Reaction) analysis.

The identification method was based on the exploitation of nucleotide polymorphisms occurring in mitochondrial coding genes of tropical rootknot nematodes. The choice for the exploitation of the mitochondrial coding genome was based on the fact that nucleotide polymorphisms occurring in this genome harbor enough variation to distinguish these closely related lineages. In particular, the barcode region Nad5 existing in mitochondrial coding genome of clade I rootknot nematodes was used to tell apart lineages of these parthenogenetic organisms, using PCR primers specific for Nad5 (Janssen *et al.* 2016).

3.2.6 Determination of *Meloidogyne* species frequency index

A total of 72 cowpea fields were surveyed for rootknot nematode infestation. Of the 72 cowpea fields, 41 (56.9) fields were found to be infested with rootknot nematodes. Frequency index of identified *Meloidogyne* species was calculated at province and district level, but also, an overall index was calculated taking into account a total of the 72 cowpea fields sampled. Rootknot nematode species frequency index was calculated and expressed as a percentage (Equation 3.).

$$Meloidogyne\ species\ frequency\ index = \left(\frac{Number\ of\ cowpea\ fields\ infested\ with\ a\ particular\ Meloidogyne\ species}{Total\ number\ of\ cowpea\ fields\ sampled}\right) * 100 \quad (3)$$

3.2.7 Data analysis

Data analaysis was conducted using the statistical program Stata (Version 12, StataCorp, College Station, Texas), Data did not conform to parametric ANOVA models and were analyzed following a Kruskal Wallis non parametric model. Post hoc multiple comparisons of means was conducted using Dunn's method (method of adjustment was none). Data was presented as tables of means and standard error, graphs were applicable and a map.

3.3 Results

3.3.1 Distribution of rootknot nematodes among provinces and districts

Rootknot nematodes were found to be widely distributed among all surveyed areas. Out of the 72 cowpea fields surveyed, 41 (56.9%) were infested with rootknot nematodes. Twenty, 16 and 5 cowpea fields were infested with rootknot nematodes in Inhambane, Gaza and Nampula provinces, respectively, resulting into 88.8%, 59.2% and 27.7% prevalence, respectively. The highest (8) number of rootknot nematode infested cowpea fields was observed from Homoine district while the least (2) was from Moma district (Table 2.).

Table 2: Prevalence of rootknot nematode infestation

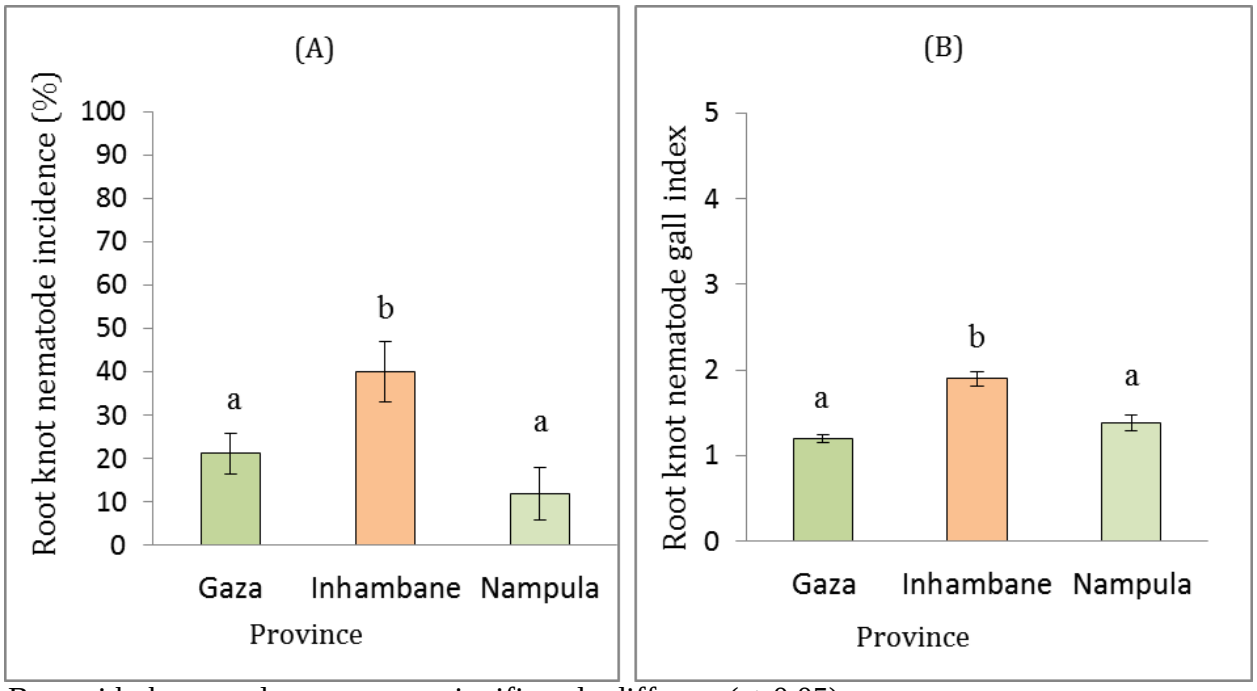
		Number of Cowpea fields infested with rootknot nematodes	Prevalence (%)	
Province	District		District	Province
Gaza	Mandlakazi	6	66.6	59.2
	Bilene	5	55.5	
	Chibuto	5	55.5	
Inhambane	Inharrime	6	66.6	74.0
	Jangamo	6	66.6	
	Homoine	8	88.8	
Nampula	Meconta	3	33.3	27.7
	Moma	2	22.2	

3.3.2 Rootknot nematode incidence and severity among provinces and districts

The highest mean incidence of rootknot nematodes was recorded in Inhambane province (39.88%) followed by Gaza (21.14%) and Nampula (11.83%) having the least incidence. Significant differences among the three provinces in terms of mean incidence of rootknot nematode damage were observed (*P=0.0053, X^2=10.465, n=72 and df = 2*). Mean incidence registered from Inhambane province was higher than that observed from Gaza and Nampula provinces (Figure 7A, annexes 2, 3 and 4).

There was a significant difference (*P=0.0347, X^2=15.105, n=72* and *df = 7*) in mean incidence of rootknot nematode damage across districts of the different provinces. Homoine and Moma districts registered the highest (55.88%) and lowest (4.77%) mean incidence, respectively. Mean incidence of rootknot nematode damage registered by Homoine district was significantly superior to those observed in Moma, Meconta, Inharrime, Chibuto, Bilene and Mandlakazi districts unlike Jangamo district (Table 3, annexes 6, 7 and 8). Moma, Meconta, Inharrime, Chibuto, Bilene and Mandlakazi were not significantly different with regard to mean incidence of rootknot nematode damage.

Differences in provinces in terms of mean root gall index scores amongst the 3 provinces were observed (observed (*P=0.0001, X^2=56.729, n=511 and df = 2*). Inhambane province recorded the highest mean root gall index score, followed by Nampula and Gaza, at 1.9, 1.4 and 1.2, respectively. Inhambane was significanly superior to Nampula and Gaza with regard to gall index score, while Nampula and Gaza were not significantly different (Figure 7B, annexes 8, 9 and 10).



Bars with the same letters are not significantly different (p>0.05)

Figure 7: Incidence (A) and gall index (B) of rootknot nematodes among provinces

Across the eight districts, significant differences were observed in mean rootknot nematode gall index scores (*P = 0.0001, X^2 = 81.384, n=511* and *df = 7*). Moma district registered the least mean rootknot nematode gall index but this did not significantly differ from scores registered by Chibuto and Bilene districts. Homoine district registered significantly the highest mean rootknot nematode gall index score but this did not differ significantly from that registered by Jangamo district. Moma district recorded the lowest rootknot nematode gall index score, while Jangamo and Homoine recorded the highest followed by Inharrime and Meconta (Table 3, annexes 11, 12 and 13).

Table 3: Mean variation of rootknot nematode incidence and galling damage in cowpea across districts

Province	District	Rootknot nematode Incidence (%)	Rootknot nematode gall index (1-5)
Gaza	Mandlakazi	21.77 ± 7.62ab	1.28 ± 0.08bc
	Bilene	24.33 ± 9.93ab	1.12 ± 0.05a
	Chibuto	17.33 ± 6.82a	1.20 ± 0.07ab
Inhambane	Inharrime	24.66 ± 11.26ab	1.60 ±0.13c
	Jangamo	39.11 ±12.18bc	1.95 ± 0.14d
	Homoine	55.88 ± 11.55c	2.11 ± 0.14d
Nampula	Meconta	18.88 ± 11.33a	1.69 ± 0.17c
	Moma	04.77 ± 3.39a	1.03 ± 0.02a
	P. value (α = 0.05)	*0.0347*	*0.0001*
	Number of observations	*72*	*511*
	Degrees of Freedom (df)	*7*	*7*
	Chi-squared value (X^2)	*15.105*	*81.384*

Means with the same letters in a column are not significantly different (P>0.05)

3.3.3 *Meloidogyne* species identified on cowpea plants from sampled areas

From the current study, 3 species of rootknot nematode, namely: *M. incognita*, *M. javanica*, and *M. enterolobii* were identified to be associated with rootknot of cowpea roots. Of 27 cowpea fields sampled from each province, *M. javanica* was observed from 4 and 6 cowpea fields of Inhambane and Gaza provinces, respectively. Of 18 cowpea fields sampled from Nampula province, *M. javanica* was observed only from one field. *M. incognita* was from 7 and 3 cowpea fields of Inhambane and Gaza provinces, respectively. *M. enterolobii* was observed only in 1 cowpea field from Inhambane province (Figure 8, annex 17).

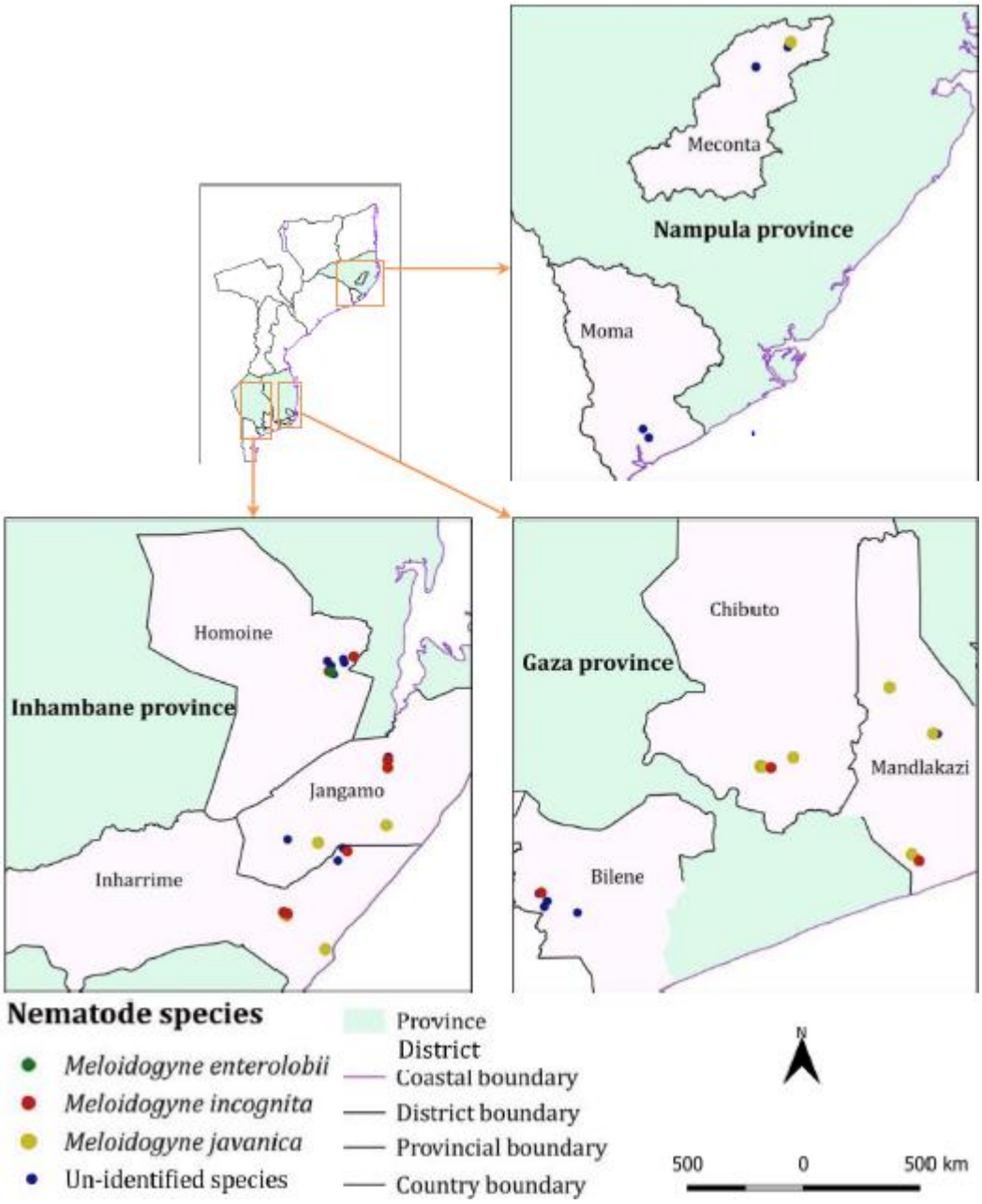


Figure 8: A map showing identified *Meloidogyne* species and where they occur

Of the 72 cowpea fields sampled, *M. javanica*, *M. incognita* and *M. enterolobii* were observed in 15.3%, 13.8% and 1.4%, respectively, of the cowpea fields surveyed (Table 4). *M. javanica* was observed from 22.2%, 14.8% and 5.5% of the cowpea fields surveyed in Gaza, Inhambane and Nampula provinces, respectively. *M. incognita* was observed only in 25.9% and

11.1% of the cowpea fields surveyed in Inhambane and Gaza provinces, respectively. *M. enterolobii* was observed only in 3.7% of cowpea fields surveyed from Inhambane province. Inhambane province registered the highest diversity with regard to *Meloidogyne* species, followed by Gaza and Nampula provinces. Table 4 summarizes *Meloidogyne* species frequency indices observed from the surveyed provinces.

Table 4: Rootknot nematode species frequency indices in surveyed provinces (%)

	Meloidogyne spp. frequency index (%)		
Province	*M. javanica*	*M. incognita*	*M. enterolobii*
Gaza	22.2	11.1	0
Inhambane	14.8	25.9	3.7
Nampula	5.5	0	0
Overall	15.3	13.8	1.4

Within districts, the highest occurrence of *M. javanica* was observed from 33% of cowpea fields surveyed from Chibuto and Mandlakazi districts, respectively. Cowpea fields from Jangamo district registered the highest occurrence of *M. incognita* at 33%. Only 11% of cowpea fields from homoine district were infested with *M.enterolobii.* Table 5 provides a summary of the frequency index of rootknot nematode species observed from cowpea fields of districts

Table 5: Rootknot nematode species frequency indices in surveyed districts (%)

		Meloidogyne spp. frequency index (%)		
Province	District	*M. javanica*	*M. incognita*	*M. enterolobii*
Gaza	Bilene	0.00	11.00	0.00
	Chibuto	33.00	11.00	0.00
	Mandlakazi	33.00	11.00	0.00
Inhambane	Inharrime	22.00	22.00	0.00
	Jangamo	22.00	33.00	0.00
	Homoine	0.00	22.00	11.00
Nampula	Meconta	11.00	0.00	0.00
	Moma	0.00	0.00	0.00

3.3.4 Other field observations made during the survey

During the field survey, it was observed that the majority of the fields were infested with weeds ranging from a few to many weeds including both herbaceous and grass types of weeds. Weeds such as *Amarantus* spp. were observed infested with rootknot nematodes. Only one farmer applied fertilizer to cowpea crops while all other farmers applied neither fertilizer nor pesticides. Farmers, especially in Inhambane and Gaza provinces practiced mixed cropping, with groundnuts, cassava, and maize as common crops across all sampled areas, while pigeon pea, water melons and Bambara ground nut were generally less frequent. Sandy soils appeared to be dominant across all sampled areas. It was also observed that cowpea fields were generally cultivated on sandy soils.

Generally, the rootknot damage appeared to be more severe on erect cowpea varieties than prostrate ones. Apart from farmers in Nampula province, majority of farmers in Gaza and Inhambane did not practice recommended practices for disease control, such as crop rotation. Mixed cropping of other rootknot nematode host crops (of different growth cycle lengths) was commonly observed in Gaza and Inhambane provinces. It was observed that the majority of farmers in Nampula province included fallows and rotation within their agricultural practices. Apart from rootknot galling damage, signs of fungal and viral diseases were evident on cowpea plants. Viral symptoms such as chlorosis, yellowing and mottling of plant leaves appeared were widespread in Gaza province as opposed to the other provinces.

3.4 Discussion

Results of this study have revealed that *M. incognita* and *M. javanica* are dominant rootknot nematode species affecting cowpea plants in Mozambique. In close agreement with the current study, *M. incognita* and *M. javanica* have been reported in a number of studies as some the most encountered rootknot nematode species affecting different crops in several tropical countries all over the globe (Adam *et al.,* 2007; Ma *et al*., 2007; Ploetz, 2009; Onkendi *et al.,* 2014). Besides *M. incognita* and *M. javanica*, the current study also identified another species *M. enterolobii* on one of the cowpea plant samples from Homoine district. The current study therefore further partially agrees with findings by Onkendi *et al.* (2014), Onkendi & Moleleki (2013b) that reported presence of *M. enterolobii* on potatoes and guava from Mozambique, Burkina Faso, the Democratic Republic of Congo, Malawi, Senegal, South Africa and Togo. However, this constitutes the first report of the presence of *M. enterolobii* specifically in cowpea in Mozambique. The occurrence of these species on cowpea further indicates a likelihood of wide spread cultivation of susceptible varieties of the crop in the country and possibly, the presence of aggressive populations of *Meloidogyne* spp. that may have evaded any potential resistance.

From the current study, it is discernable that cowpea fields from southern Mozambique, more so from Inhambane province, were infested with multiple species of rootknot nematodes, namely, *M. javanica, M. incognita* and *M. enterolobii*. Populations of *M. javanica* and *M. incognita* aggressive and virulent, respectively against resistant cowpea cultivars have been reported by Roberts *et al*. (1996). In addition, *M. enterolobii* has been reported to break resistance in some crops (Onkendi *et al*., 2014). Although this situation has not been well studied in cowpea, its possibility should not be ruled out. Therefore, the observed simultaneous presence of these species in southern Mozambique poses a need for effective control measures to be undertaken to reduce soil populations of these species. This can be achieved by identifying cowpea cultivars with multiple resistances to rootknot nematode species, whose prior dissemination in the worst affected areas, will help in reducing multiple infestation levels of the prevailing *Meloidogyne* spp.

The presence of these species could be attributed to the fact that *Meloidogyne* spp. are ubiquitous members of the soil fauna (Viketoft, 2007), have a wide host range which includes

crops and weeds (Mukhtar *et al.,* 2014) of possibly varying growth cycle lengths, which facilitate on and off-season nematode survival, and the subsequent re-infection of crop hosts such as cowpea. Besides reports by INIA (1996), this study also observed that cowpea was generally cultivated on sandy soils. Light textured soils (sandy soils) have been reported to be conducive for nematode survival (Olabiyi & Oladeji, 2014) and hence, may partly have contributed to the observed rootknot nematode damage levels.

High rootknot nematode damage levels observed in the current study, especially from cowpea fields in Inhambane province, may be attributed to poor farming practices, which may favor multiplication of nematodes. It was established in this study that in majority of the farmer fields, other rootknot nematode host plants or crops such as maize, cassava, peanuts in addition to cow pea were mixed cropped for more than one growing season. Apart from Nampula province, this practice was common in Gaza and Inhambane provinces. Ideally, these different crop types have different rootknot nematode resistance profiles. Poor cropping practices such as the ones observed herein may render use crop resistance inefficient. It has been observed that repeated cultivation of resistant cultivars for 3-8 years may to evoke outbreak of resistant rootknot nematode strains arising from selection pressure exerted against native perhaps initially less virulent rootknot nematode populations (Eddaoudi,1997). Integrated nematode management approaches such as crop rotation amongst others have been reported to have an appreciable control effect against rootknot nematodes by breaking their cycles and delaying nematode reproduction sequences (Seid *et al*., 2015; Sardanelli, 2010). Neglect of such practices is one of the contributing factors to high rootknot nematode disease pressure among host crops such as cowpea.

In general, low rootknot damage intensity was observed in Gaza and Nampula provinces, this may in part be due to possible limitations in wide "self-transmission" of rootknot nematodes. By virtue of their nature, nematodes as soil borne plant pathogens have limitations as far as their self-dispersal is concerned. They move slowly and are present in small areas. Besides, their level of genetic flow is also limited. Their dispersal is usually aided by external agents such as windy rains and erosions amongst other agents (Agrios, 2005). It is thus clear that temporal and spatial occurrence of the right combination of a set of dispersal conditions may be difficult and may not synchronize with the right developmental stages of the nematode. This leaves transmission of

these pathogens in the hands of unscrupulous agricultural practices that may not be common across agro-ecologies.

3.6 Conclusions

- Rootknot nematodes were found widely spread over all sampled localities with an overall prevalence of 56.9%.
- Three rootknot nematode species, namely: *M. incognita*, *M. javanica*, and *M. enterolobii* were identified to be associated with cowpea rootknot in the surveyed cowpea fields.
- High rootknot nematode diversity was observed from Inhambane province
- Over all, Inhambane province registered the highest rootknot nematode prevalence, incidence and gall indices, and hence rootknot nematode damage intensity.

3.7 Recommendations

- Screening of cowpea and other host crops grown with, before and after it for existing resistance against rootknot nematode will help in identification of resistance and possible abatement of rootknot nematode damage.
- Conducting of rootknot nematode resistance research must be under taken by cowpea breeders before variety release, especially in areas severely infested with rootknot nematodes. This should be conducted under field and greenhouse conditions for several successive crop seasons in order to test the durability of observed resistance. Identifying cowpea genotypes with multiple resistances to rootknot nematode species should be underscored in the search for resistance.
- When conducting resistance trials, isolates of rootknot nematode populations should collected from the various cowpea growing areas of southern Mozambique, as these have been observed to be infested with multiple species of rootknot nematodes.
- In addition to that, infested areas should be prioritized for receiving currently available rootknot nematode resistant cowpea cultivars.
- As part of agronomic research, conducting awareness and training of farmers on proper rootknot nematode management by agronomists will help in reducing rootknot nematode damage.

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CHAPTER FOUR:

CHARACTERIZATION OF COWPEA GENOTYPES FOR RESISTANCE TO *Meloidogyne javanica* IN MOZAMBIQUE

Abstract

Cowpea (*Vigna unguiculata* (L.) Walp.) is a broadly adapted and an important crop in Mozambique. The crop is cultivated almost exclusively by smallholder farmers in warm marginal environments of the country. One of the key field hindrances to the success of this crop are rootknot nematodes (*Meloidogyne* Spp.), that reduce its desired yield. Twenty five cowpea genotypes from the faculty of agriculture and forestry engineering at Eduardo Mondlane University, Maputo Mozambique were assessed for resistance against *M. javanica*. A pot experiment was conducted in a greenhouse at the Mozambique agricultural research institute (IIAM). A randomized complete block design was used and each genotype was replicated three times. Three weeks after planting, each plant was inoculated with 3000 second stage juveniles of *M. javanica*. Six weeks after inoculation, plants were uprooted and assessed for resistance using Canto-saenz's scheme based on root gall index and host efficiency. Out of the twenty five cowpea genotypes assessed, four were found to be resistant, sixteen were tolerant and five were susceptible. Line IT4S-2049 used as a negative check succumbed to damage from *M. javanica*.

Keywords: cowpea genotypes, *Meloidogyne javanica*, resistance assessment.

4.1 Introduction

Cowpea (*Vigna unguiculata* (L) Walp) is a broadly adapted and an important crop in Mozambique. The crop is cultivated almost exclusively by smallholder farmers in warm marginal environments of the country. Its production is mainly done in the provinces of Nampula, Inhambane, Zambezia, Gaza and Maputo (INE 2008, Chiulele *et al.,* 2011, Bulletin of tropical legumes, 2013). Its production comes fourth in ranking after maize, cassava and groundnut production (INE, 2008). Its preference by smallholder farmers and or resource poor households is mainly for its grain, young leaves and fresh pods that are used as food or vegetables and income (Chiulele *et al.*, 2011, Bulletin of tropical legumes, 2013). Providing about 25% protein from its grain (dry weight basis) the crop serves as a cheap source of protein (meat for the poor), carbohydrates, vitamins and minerals (Bressani, 1985; Davis *et al*., 1991). The crop is an important component of cereal and starchy tuber cropping systems, where it improves soil fertility, reduces soil erosion, suppresses weeds. The crop thus contributes to the good performance of crops grown with or after it (Tarawali *et al*., 2002; infonet biovision.com, 2015).

Despite the crop's usefulness, average cowpea yield in Mozambique has for long remained below 300 kg ha^{-1}. To a large extent, these low yields are as a result of biotic constraints such as insect pests, diseases, and several species of nematodes (INIA, 2000, 2003). Amongst nematodes, rootknot nematodes (*Meloidogyne* spp.) have been reported as one of the major constraints, affecting and causing yield loss in cowpea (Fery *et al*., 1994). Amongst other rootknot nematodes species, *M. incognita* and *M. javanica*, have been the most implicated species in the loss of cowpea yield (Swanson & Gundy, 1984). Chiulele (2010) reported susceptibility of cowpea to rootknot nematodes as one of the constraints contributing to low cowpea yields in Mozambique. Elsewhere, rootknot nematodes have been reported to result into 72% cowpea yield reduction in susceptible varieties (Crozzoli *et al.,* 1999). Babatola and Omotade (1991) reported 69% of cowpea grain yield loss resulting from rootknot nematode infestation. Such elevated yield losses threaten food, income and nutritional security and hence warrant urgent control intervention, through research.

Amongst other rootknot nematode control measures, effective control of rootknot nematodes has been remarkably achieved through application of synthetic nematicides, however,

majority of these nematicides have been banned from markets due to associated negative environmental and health effects (Onkendi, *et al.*, 2014). This poses a need to explore alternative control nematode control approaches. Amongst the various rootknot nematode control alternatives available, the use host resistance appears most reliable, since it does not only circumvent health and environmental downsides of synthetic nematicides, but it also saves expenses associated with their purchase.

Resistance to rootknot nematodes amongst cowpea varieties has been documented. A rootknot nematode resistance study conducted by Ononuju and Nzenwa (2011) reported that, three out of six cowpea varieties tested, were resistant to rootknot nematodes. In Mozambique, information regarding rootknot nematode resistance of cultivated local cowpea varieties is still scarce, yet very useful in the management rootknot nematodes. The challenge is that, resistance is rare and its efficacy is threatened by the capacity of rootknot nematodes to adapt (Barbary *et al.* 2016). Against this background, this study was aimed at characterization of 25 cowpea genotypes under a breeding program in Mozambique, for resistance to rootknot caused by *Meloidogyne javanica*. *M. javanica* was chosen for this study because it recorded the highest frequency among cowpea fields as shown in the previous chapter. Additionally, populations of this species that are aggressive against rootknot nematode resistant cowpea genotypes have been reported (Roberts *et al.*, 1996). Characterization of cowpea for resistance to *M. javanica* was seen as a way to facilitate cultivar recommendation and eventually contribute to effective management and or control of this species in the country.

The specific objective of this study was to identify cowpea genotypes that are resistant to rootknot caused by *Meloidogyne javanica*.

The study was conducted to test the hypothesis that, cowpea genotypes are resistant to rootknot caused by *Meloidogyne javanica*, and that more than one genotype is resistant.

4.2 Materials and methods

4.2.1 Description of the experimental site

The experiment was conducted under controlled conditions in a greenhouse at the Mozambique Agricultural Research Institute (IIAM: Instituto de Investigação Agrária de

Moçambique) in Maputo. Maputo, is at 25°55'S, 32°34'E, 39 m (128 ft) with a tropical wet and dry/savanna and pronounced dry season in the low sun months. The average temperature is 23°C (73.5°F). Average monthly temperatures vary by 6.5°C (11.7°F). Total annual Precipitation averages 769mm (http://www.maputo.climatemps.com).

4.2.2 Experimental procedure

A total of 25 cowpea genotypes were evaluated were evaluated for resistance to rootknot caused by *Meloidogyne* species. These genotypes were provided by a cowpea breeding program at the department of crop production Faculty of Agriculture and Forestry engineering (FAEF), Eduardo Mondlane University, main campus, Maputo Mozambique. Genotypes including IT84S-2049 and INIA-76 were included as internal controls, in which the former was used as negative control (resistant to *Meloidogyne* species) and the latter as a positive control (susceptible to *Meloidogyne* species). The choice of line IT84S-2049 was due to its complete resistance to diverse populations of rootknot nematodes (Roberts *et al.* 1996), while INIA-76 was established to be highly susceptible (Ndeve, personal communication). Table 6 provides a summary of the evaluated genotypes and their attributes.

4.2.3 Isolation and multiplication of rootknot nematode inoculum

Seedlings of a rootknot nematode susceptible tomato variety (Mayford) were used for multiplication of rootknot nematode inoculum. Seedlings were raised on sterile organic material (coco peat) before transplanting them into plastic pots containing 1:1 sterile mixture of sand and coco peat in a screen house. In the pots, seedlings stayed for three weeks before inoculation.

A root sample of a rootknot nematode infected cowpea plant was obtained from the experimental field of the faculty of agronomy and forestry engineering (Eduardo Mondlane University main campus). In the pathology laboratory at the same faculty in the Department of Crop Protection, the root sample was washed under a gentle stream of tap water to free it of soil debris. The sample was then bloated dry using blotting paper. It was suspended in a one liter solution containing 15mg of phloxine B stain for 5 minutes (Coyne *et al*., 2007).

Table 6: Origin and attributes of evaluated cowpea genotypes

Genotype	Origin	Type of material	Yield	Maturity	Seed size	Use type*
VAR-11	Mozambique	Landrace	High	Late	Large	Dual purpose
524B	USA	Improved variety	High	Early	Large	Grain
B21x2246-3-9	Mozambique	Breeding line	High	Early	Medium	Grain
B21x499-13-2	Mozambique	Breeding line	High	Early	Medium	Grain
INIA152xBambey-21-1	Mozambique	Breeding line	High	Medium	Large	Dual purpose
VAR-9	Mozambique	Landrace	High	late	Large	Dual purpose
VAR-4	Mozambique	Landrace	High	late	Large	Dual purpose
2246x503-9-1	Mozambique	Breeding line	High	Early	Medium	Grain
503xP24-17-3	Mozambique	Breeding line	High	Early	Medium	Grain
B21x2246-3-4	Mozambique	Breeding line	High	Early	Medium	Grain
034-50	USA	Breeding line	High	Early	Large	Grain
VAR-13	Mozambique	Landrace	High	Late	Large	Dual purpose
2246x503-9-5	Mozambique	Breeding line	High	Early	Large	Grain
VAR-3	Mozambique	Landrace	High	Late	Large	Dual purpose
I-41x18-9-1	Mozambique	Breeding line	High	Medium	Large	Dual purpose
499x18-1-13	Mozambique	Breeding line	High	Medium	Large	Grain
CB-46	USA	Improved variety	High	Early	Large	Grain
IT84S-2049	IITA	Improved variety	High	Early	Medium	Grain
B21x2246-4	Mozambique	Breeding line	High	Early	Medium	Grain
Nhantchengue	Mozambique	Landrace	High	Late	Large	Dual purpose
VAR-8	Mozambique	Landrace	High	Late	Large	Dual purpose
I-41x499-28-3	Mozambique	Breeding line	High	Medium	Large	Grain
IT-16	Mozambique	Improved variety	High	Early	Small	Grain
IT-1105-5	IITA	Improved variety	High	Early	Small	Grain
INIA-76	Mozambique	Improved variety	High	Early	Medium	Grain

*Use type indicates plant part harvested for consumption

The sample was then observed under a dissecting microscope (Leica EZ4, CME Microscope, Buffalo, N.Y., 14240 U.S.A., Leica Microsystems (Shweiz AG)) for isolation of single egg masses. A single female was carefully dissected from the root tissues with its egg mass. In a tapering bottom plastic bottle, eggs were incubated for 24 hours. Emerged second stage juveniles (J_2) were then used to inoculate a susceptible commercial tomato variety (Mayford) at three weeks after transplanting. Because of the mitotic parthenogenetic mode of reproduction of *Meloidogyne* species (Triantaphyllou, 1985), all the J_2 that hatched from the egg mass were considered as a clonal line. Multiplication went through several generations until

enough inoculum for characterizing 25 cowpea genotypes for resistance to rootknot was obtained.

4.2.4 Sowing,

Three seeds of each genotype were sown in one liter plastic pots containing sterile growth media of 1:1 ratio of sand and organic matter (coco peat). Plants in pots were adequately watered every after 24hours with clean tap water. After germination, seedlings in each pot were thinned to one plant.

4.2.5 Preparation of inoculum and inoculation of cowpea

A clonal isolate *Meloidogyne javanica* inoculum that had been multiplied on susceptible commercial tomato variety (Mayford) as described above was used to inoculate cowpea plants in pots. Nematode inoculum was collected from tomato plants following a procedure described by Coyne and Ross (2014). Tomato plants containing inoculum were carefully uprooted from their pots by gently tapping on the sides of the pot. Their roots were then washed under a gentle stream of clean tap water to free them of soil debris. Roots were then suspended in a one liter solution containing 0.5% sodium hypochlorite for 5 minutes (Coyne & Ross, 2014).

Above a 2liter bucket, individual tomato plants were then placed in a 53µm nested over a 25µm sieve. In order to collect all the nematode eggs from the plant, the sodium hypochlorite solution in which the plant had been suspended was also poured through the same 53µm nested over a 25µm sieve. During this process, each plant was rinsed 3 times using a gentle flash of tap water. Eggs retained in the 25µm sieve were then collected in a labeled container, and then incubated at room temperature using an extraction tray set up. The set up was checked after 24 hours for hatched second stage juveniles (J2). The suspension containing hatched J2 was then collected in a labeled beaker in preparation for standardization. The J2 in the suspension were then counted with dilutions where necessary, using a tally counter and a counting slide, under a microscope (4x objective and 10x eye piece). Three 1ml aliquots of the J2 suspension were pipetted using a 1ml pipette. An average count of the 3 aliquots was then calculated and adjusted to the total J2 suspension volume in a labeled beaker. The suspension was agitated prior to every count to even out the distribution of juveniles in the suspension. Agitation was accomplished by blowing air into the suspension using a pipette.

In the greenhouse, each cowpea seedling (aged 3 weeks) in a pot was inoculated with 3000 juveniles. During inoculation, three holes were made around the base of the plant using a pencil. Inoculum in a beaker was then agitated before pipetting off 1ml containing 1000 juveniles into each of the three holes. Holes were coved with soil and watering of the pots was halted for 36 hours. The set up was left to stand for 8 weeks. Plants were adequately watered with tap water every after 24hours during the entire experimental period.

4.2.6 Experimental design

The experiment was conducted following a randomized complete block design with 3 replicates and 25 plots per replicate. Each plot contained 3 plants (each plant in its own pot) of an individual genotype inoculated with rootknot nematodes as described above. Each pot represented a nematodes genotype treatment combination. The layout of the experiment is shown in annex 14. Figures in the lay out represent genotype codes used for the purpose of this study.

4.2.7 Data collection

At the end of 8weeks from inoculation, cowpea plants were assessed for rootknot nematode gall damage and number of rootknot nematode eggs. Cowpea plants of respective genotypes were carefully uprooted from their pots by gently tapping on the side of the pots. Plants were washed and dipped in a solution containing 15mg/liter of phloxine B for 10 minutes. Plants were then scored for nematode gall index using a rating scale ranging from 0-5; where: 0 = no galls on roots; 1 = 1 – 2 galls; 2= 3 – 10 galls; 3 =11 – 30 galls; 4 = 31 – 100 galls and 5 = 101 – above (Sasser *et al.*,1984).

Rootknot nematode eggs were extracted using the sodium hypochlorite method as described by Coyne and Ross (2014), by dipping individual roots of individual cowpea plants in a solution of 2.5% sodium hypochlorite for 10 minutes, to free the eggs from the gelatinous matrix suspending them.

Above a 2liter bucket, roots of individual cowpea plants were placed in a 53µm nested over a 25µm sieve. In order to collect all the nematode eggs from the plant, the sodium hypochlorite solution in which the plant had been suspended was also poured through the same 53µm nested over a 25µm sieve. During this process, each plant was rinsed 3 times using a

gentle flash of tap water. Eggs retained in the 25µm sieve were then collected in a labeled container. The suspension containing eggs and a few was then collected in a labeled beaker in preparation for counting. The suspension was then counted with dilutions where necessary, using a tally counter and a counting slide, under a microscope (4x objective and 10x eye piece). Three 1ml aliquots of the suspension were pipetted using a 1ml pipette. An average count of the 3 aliquots was then calculated and adjusted to the total egg suspension volume in a labeled beaker. The suspension was agitated prior to every count to even out the distribution of eggs. Agitation was accomplished by blowing air into the suspension using a pipette. An average count the 3 aliquots was then calculated and recorded.

The *Meloidogyne javanica* reproduction factor on each cowpea genotype was determined using a formula: $R = \frac{Pf}{Pi}$, where R = the average final *Meloidogyne javanica* egg count from the roots of each cowpea genotype, Pi = Original inoculum volume of *Meloidogyne javanica* and Pf = Final egg volume of *Meloidogyne javanica* for the individual cowpea genotypes (Oostenbrink,1966). The host status of each genotype was determined basing on mean cowpea genotype root gall index and mean rootknot nematode reproduction factor (R) using a rating scheme (Table 7.) modified from Canto-saenz (1983).

Table 7: Resistance rating scale for rootknot nematode

Root gall index	R-factor for host efficiency	Host status
≤2	≤1	Resistant
≤2	>1	Tolerant
>2	>1	Susceptible
>2	≤1	Highly susceptible

Modified from Canto-saenz (1983).

4.2.8 Data analysis

Data collected on root galling and nematode reproduction was entered in Microsoft excel and analyzed using STATA statistical package (Stata Version 12, StataCorp, College Station, Texas). Data was presented as a table of means and standard error.

4.3 Results

4.3.1 Reaction of cowpea genotypes to *M. javanica*

Out of the twenty five cowpea genotypes assessed for resistance to *M. javanica*, four were found to be resistant with low root gall indices (≤2) and low reproductive factors (≤1). Sixteen genotypes were found to be tolerant, with low root gall indices (≤2) and higher host efficiency ratios (>1). Five genotypes were found to be susceptible, with high root gall indices (>2) and reproductive factors (>1). Line IT4S-2049 used as a negative control was found to be tolerant, with a mean gall index of 1.22 ± 0.15 (≤2) and reproductive factor of 3.58 ± 1.00 (>1). Genotype INIA-76, used as a positive control, was found to be susceptible, with a mean gall index of 2.33 ± 0.17 (>2) and reproductive factor of 15.26 ± 6.08 (>1). The highest mean gall index (2.89 ± 0.20) and reproductive factor (49.1 ± 9.44) were recorded by genotype B21x499-13-2. Results of genotype assessment are presented in Table 8, annex 15 and 16.

Table 8: Suitability of cowpea genotypes to *M. javanica*

	Damage	Host efficiency	
Cowpea genotype	Nematode gall index*	(Mean reproductive Factor)**	Resistance rating
VAR-9	1.00 ± 0.00	0.79 ± 0.40	Resistant
VAR-4	0.67 ± 0.17	0.46 ± 0.30	Resistant
VAR-13	1.00 ± 0.00	0.22 ± 0.05	Resistant
VAR-8	1.00 ± 0.00	0.4 ± 0.13	Resistant
VAR-11	1.00 ± 0.00	3.19 ± 1.40	Tolerant
INIA152xBOMBEY-21-1	2.00 ± 0.17	8.98 ± 2.69	Tolerant
2246x503-9-1	1.89 ± 0.11	7.17 ± 2.12	Tolerant
B21x2246-3-4	1.89 ± 0.20	13.83 ± 1.95	Tolerant
034-50	1.67 ± 0.17	7.78 ± 1.79	Tolerant
2246x503-9-5	1.89 ± 0.11	23.2 ± 1.79	Tolerant
VAR-3	1.00 ± 0.00	1.1 ± 0.63	Tolerant
I-41x18-9-1	2.00 ± 0.17	38.29 ± 10.66	Tolerant
499x18-1-13	1.78 ± 0.15	14.14 ± 2.44	Tolerant
CB-46	1.78 ± 0.15	16.07 ± 4.51	Tolerant
LINE IT84S-2049	1.22 ± 0.15	3.58 ± 1.00	Tolerant
B21x2246-4	1.11 ± 0.11	2.72 ± 1.01	Tolerant
NHANTCHENGUE	1.22 ± 0.15	2.34 ± 0.52	Tolerant
I-41x499-28-3	2.00 ± 0.17	26.32 ± 15.13	Tolerant
IT-16	1.22 ± 0.15	2.32 ± 0.57	Tolerant
IT-1105-5	1.67 ± 0.24	5.19 ± 2.06	Tolerant
524B	2.22 ± 0.22	5 ± 0.51	Susceptible
B21x2246-3-9	2.89 ± 0.11	14.89 ± 6.03	Susceptible
B21x499-13-2	2.89 ± 0.20	49.1 ± 9.44	Susceptible
503xP24-17-3	2.44 ± 0.24	14.47 ± 4.22	Susceptible
INIA-76	2.33 ± 0.17	15.26 ± 6.08	Susceptible

*Gall index scale: 0 = 0 galls; 1 = 1-2 galls; 2 = 3-10 galls; 3 = 11-30 galls; 4 = 31-100, 5 = 100 + galls.

** The reproductive factor was the average final egg count divided by 3000 (number of eggs with which each pot was inoculated) eggs.

4.4 Discussion

From the current study, genotypes; VAR-9, VAR-4, VAR-13 and VAR-8 were found to be resistant to *M. javanica*, which indicates a likelihood of these genotypes carrying genes coding for resistance against rootknot nematodes. This makes these genotypes good candidates for exploitation of resistance as a nematode control approach. The crop rotation value of resistant cultivars has been established and documented. Including resistant crop cultivars in a rotation has been reported to suppress nematode reproduction on resistant genotypes, the result of which is relatively low final soil population densities of nematodes at the end of the crop season (Roberts *et al*., 2007)). According to Roberts (2002), these low densities have a lower damage potential to susceptible crops than the typically high populations remaining after a season of a susceptible crop. This therefore indicates that presence of resistant genotypes makes susceptible ones also equally important. Incorporation of resistant genotypes in rotations will therefore reduce yield losses in nematode susceptible, but high yielding cultivars planted in subsequent seasons, besides reducing financial costs incurred during control of nematodes.

Findings of the current study have identified line IT4S-2049 to succumb to nematode damage. Roberts *et al*. (1996) reported that nematode resistance in this line was completely effective against nematode isolates that are virulent to the resistance gene *Rk* present in cultivars such as CB5 and CB-46. Systematic genetic studies indicated that the resistance in IT84S-2049 was conferred by a single dominant gene, designated as *Rk2*. However, the allelic form in which a nematode resistance gene is expressed has been reported to cause a gene dosage effect, where heterozygous individuals register higher reproductive rates than homozygous individuals (Jacquet *et al*., 2005). Therefore, failure of line IT4S-2049 to effectively resist against *M. javanica* populations used in the current study may have resulted from the expression of the resistance gene in an allelic form that does not confer effective resistance.

From the current study, sixteen cowpea genotypes have been found to be tolerant to *M. javanica*. In addition, it is also clear that nematode resistant genotypes are very limited in number. From this point of view, the cultivation of these nematode tolerant genotypes should be accorded importance especially in crop rotation programs where they can serve as trap crops. However, parcels in a rotation, where these genotypes existed in the previous season should be planted with resistant genotypes in subsequent seasons in a bid to control nematode buildup.

Furthermore, it can be discerned from results of the current study that mean root gall indices recorded by tolerant genotypes was generally low while reproduction was overwhelmingly high. Scenarios in agreement with this observation in other crops have been documented. While working with *Arachis hypogea*, Garcia *et al.* (1996) found out that in some genotypes, root galling was not commensurate with nematode reproduction in nematode-host plant interactions. These authors proceeded by identifying and designating respective genes for suppression of root galling and nematode reproduction. Low root gall indices and high reproduction observed among tolerant genotypes in the current study is an indication of a general absence of mechanisms for suppression of nematode reproduction and presence of galling suppression mechanisms in these genotypes. It therefore theoretically appears that a combination of these two mechanisms would make these genotypes completely resistant against rootknot nematodes, a possibility of which deserves to be identified and exploited in these genotypes.

4.5 Conclusions

Four, sixteen and five genotypes are resistant, tolerant and susceptible to *M. javanica*, respectively. No genotype was found to be highly susceptible.

4.6 Recommendations

- Cowpea genotypes identified to be resistant under greenhouse conditions should also be tested for resistance to rootknot nematodes under field conditions.
- Identified resistance should be employed in improving farmers' preferred but nematode susceptible cowpea cultivars
- Genes coding for resistance (against galling and reproduction) in resistant genotypes should be identified, mapped and bred into susceptible and tolerant genotypes in order to reduce yield losses that may result from cultivation of those susceptible genotypes in rootknot nematode infested fields.
- Resistant cowpea genotypes identified from this study should be incorporated in crop rotation schemes as part of an integrated rootknot nematode management program. This will reduce the buildup of rootknot nematode populations in the soil and hence contribute to cowpea yield improvement

- The experiment should be repeated using the current rootknot nematode species (*M. javanica*) and other rootknot nematode species to test durability and consistence of observed resistance.

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CHAPTER FIVE: SUMMARY OF MAJOR FINDINGS AND THE WAY FORWARD

5.1 Introduction

Rootknot (root galling) caused by rootknot nematodes (*Meloidogyne* spp.) is one of the main biotic constraints threatening cowpea production. Nonetheless, published information on their identity, distribution among cowpea fields and resistance of local cowpea cultivars against these pathogens is very limited. Therefore, these circumstances pose a need to establish the identity, distribution and resistance status of local cultivars in order to improve cowpea production. This study was seen as a step towards effective rootknot nematode management through facilitating the making of informed decisions on control actions and guiding future research such as breeding for rootknot nematode resistance in Mozambique. This study was aimed at contributing to knowledge and information of the commonest local rootknot nematodes species and their distribution. The study commenced with the collection of cowpea root samples across the various cowpea growing areas in the country, for assessment of the extent of rootknot nematode damage, and identification of *Meloidogyne* species responsible for this damage. Thereafter, the study proceeded with a pot experiment to characterize some of the local cowpea genotypes for resistance to *Meloidogyne javanica*, one of the rootknot nematode species that was found to be locally wide spread among cowpea fields. This was aimed at identifying rootknot nematode resistant cowpea genotypes for potential use during management and control of these pathogens. This chapter provides an overview of the overall study, highlighting the study objectives, major findings and implication of the results to future rootknot nematode control research. The study comprised of three interrelated objectives as shown below:

1. To establish the distribution of rootknot nematodes and evaluate the incidence and intensity of the rootknot problem in cowpea growing areas in Mozambique;
2. To identify the rootknot nematode species (*Meloidogyne* spp.*)* affecting cowpea;
3. To identify cowpea genotypes resistant to rootknot caused by *Meloidogyne javanica*.

5.2 Summary of major findings

Each of the objectives was achieved with an independent study, and the findings of each objective are presented separately.

5.2.1 Intensity and distribution of *Meloidogyne* spp. within and across selected cowpea growing areas in Mozambique

A survey for rootknot nematode distribution, rootknot damage intensity (measured by incidence and severity) and species identity was conducted in the main cowpea growing areas in Mozambique, including eight districts in three provinces, namely Gaza, Inhambane and Nampula. Results obtained from this study include the following:

- Out of the 72 cowpea fields sampled, 56.9% were infested with rootknot nematodes.
- Among provinces, Inhambane provinces registered the highest (39.8%) mean rootknot nematode incidence.
- Among districts, Homoine district recorded the highest mean rootknot nematode incidence at 55.8%.
- Generally, rootknot nematode galling score across provinces was low, with highest mean galling score of 1.9 recorded in Inhambane province.
- Homoine district recorded the highest mean rootknot galling severity (2.1) compared to the rest of the districts in this study.

5.2.2 Identification of rootknot nematode species (*Meloidogyne* spp.) affecting cowpea in Mozambique

In order to identify to which *Meloidogyne* species the various rootknot nematode samples belonged, DNA was extracted from female nematodes and the samples analyzed using PCR primers specific to bar code region Nad5, in the mitochondria coding genome of tropical rootknot nematodes. The following results were obtained:

- Considering all the areas surveyed, the commonest rootknot nematode species encountered were *M. javanica* and *M. incognita*. However, the current study identified *M. enterolobii* in one of the cowpea fields sampled.

- *M. javanica* was found to be present in 1, 4, and 6 cowpea fields of Nampula, Inhambane and Gaza provinces, respectively.
- *M. incognita* was found to be present in 7 and 3 cowpea fields of Inhambane and Gaza provinces, respectively while as *M. enterolobii* was only in one cowpea field belonging to Inhambane province. From the 72 cowpea fields sampled,
- *M. javanica* registered the highest overall frequency index (15%) followed by *M. incognita* (14%) and *M. enterolobii* (1%).
- Gaza province registered the highest (22.2%) number of cowpea fields infested with *M. javanica* followed by Inhambane (14.8%) and Nampula (5.5%) province.
- *M. incognita* was observed from 25.9% and 11.1% of cowpea fields from Inhambane and Gaza provinces, respectively.

5.2.3 Characterization of cowpea genotypes for resistance to rootknot nematodes (*Meloidogyne javanica*) in Mozambique

To characterize cowpea for resistance to rootknot caused by *M. javanica*, 25 cowpea genotypes from the Faculty of Agriculture and Forestry Engineering, at Eduardo Mondlane University, Maputo Mozambique, were used. A pot experiment was conducted in a greenhouse at the Mozambique Agricultural Research Institute (IIAM) and the following results were obtained:

- Out of the twenty five cowpea genotypes assessed for resistance to *M. javanica*, 4 were found to be resistant with low root gall indices (≤2) and reproductive factors (≤1). 16 genotypes were found to be tolerant, with low root gall indices (≤2) and higher host efficiency ratios (>1). 5 genotypes were found to be susceptible, with high root gall indices (>2) and reproductive factors (>1).
- Line IT4S-2049 used as a negative control was found to be tolerant, with a mean gall index of 1.22 ± 0.15 (≤2) and reproductive factor of 3.58 ± 1.00 (>1).
- Genotype INIA-76, used as a positive control, was found to be susceptible, with a mean gall index of 2.33 ± 0.17 (>2) and reproductive factor of 15.26 ± 6.08 (>1).
- The highest mean gall index (2.89 ± 0.20) and reproductive factor (49.1 ± 9.44) were recorded by genotype B21x499-13-2

5.3 Implications of the findings to rootknot nematode management and control

Rootknot nematodes were found to be widespread across sampled areas with the most frequent species being: *M. javanica* and *M. incognita*. These findings implied that cowpea varieties resistant to these species ought to be developed and dissemination priority be given to areas with high rootknot nematode infestation. The resisted exhibited against *M. javanica* by some cowpea genotypes, implied that these genotypes can be used in the management and control of this species, including their incorporation into crop rotation programs.

5.4 The Way Forward

In general, this study found out that two (*M. javanica* and *M. incognita*.) of the most crop damaging tropical rootknot nematode species, were responsible for rootknot on cowpea across all rootknot nematode infested fields sampled. Therefore, control research should be initiated with priority on bringing the population of these species below crop damaging levels. Another survey should be conducted, in which the sample size and coverage should be increased, especially around the area where *M. enterolobii* was identified so as to establish the extent to which this species has infested cowpea fields, and thus aid in its control. Cowpea genotypes identified to be resistant to *M. javanica* should be screened under field conditions to test their ability to with stand nematode populations associated with cowpea fields.

ANNEXES

Annex1: Survey coordinates

Location	Latitude	Longitude
Macia	-25.0603	33.01597
Messano	-25.0069	32.98325
Chichango	-25.0814	33.10003
Chichango	-25.0929	33.08581
Mangume	-24.6995	33.61197
Mangume	-24.6983	33.61731
Godide	-24.6685	33.66544
Godide	-24.6772	33.65656
Machulane	-24.6114	34.03028
Macuacua	-24.4443	33.95469
Macuacua	-24.4655	33.92036
Nacoma	-15.1013	39.73539
Nacoma	-15.0686	39.74364
Meconta sede	-14.9796	39.87606
Corrane sede	-15.533	39.65969
Corrane sede	-15.5345	39.66283
Corrane sede	-15.5326	39.66511
Colocoto	-16.6455	39.2555
Colocoto	-16.6494	39.25956
Colocoto	-16.6502	39.25742
Namuato	-16.6628	39.28628
Namuato	-16.663	39.27869
Nange	-16.6244	39.25939
Nange	-16.6134	39.25997
Makungela	-24.3018	35.22708
Dongane	-24.3168	35.20517
Dongane	-24.3237	35.20681
Bambela	-24.2872	35.15631
Likaka	-24.2499	35.32728
Likaka	-24.2382	35.32044
Mangueira	-23.8874	35.19436
Macia	-25.0681	33.00569
Macia	-25.0538	33.01242
Messano	-25.033	32.99075
Messano	-25.031	32.99728
Chichango	-25.0834	33.09325
Canhavane	-24.6961	33.57442

Canhavane	-24.6951	33.57106
Canhavane	-24.6968	33.58011
Mangume	-24.6997	33.60281
Godide	-24.6724	33.66192
Makungela	-24.2917	35.23108
Makungela	-24.285	35.22161
Dongane	-24.3122	35.21114
Nhanombe	-24.5013	35.18517
Nhanombe	-24.4224	35.09731
Nhanombe	-24.4298	35.10239
Bambela	-24.2666	35.10525
Bambela	-24.2738	35.16958
Jangamo sede	-24.0898	35.31764
Jangamo sede	-24.0959	35.31653
Jangamo sede	-24.1125	35.31619
Likaka	-24.236	35.31369
Madeula	-23.8744	35.24508
Madeula	-23.8868	35.22497
Madeula	-23.8797	35.22275
Mangueira	-23.8839	35.18939
Mangueira	-23.9123	35.20244
Malengue	-23.8935	35.1965
Malengue	-23.9083	35.19711
Malengue	-23.9058	35.19286
Chicuango	-24.9468	33.99647
Chicuango	-24.9296	33.97719
Chicuango	-24.9423	33.99461
Machulane	-24.6096	34.03394
Machulane	-24.6104	34.04556
Macuacua	-24.4873	33.91789
Nacoma	-15.0736	39.74633
Meconta sede	-14.9668	39.88903
Meconta sede	-14.9877	39.87556
Colocoto	-16.6277	39.25811
Namuato	-16.6651	39.2835

Annex 2: Computation of provincial mean rootknot nematode incidence

```
. mean incidence, over(province)

Mean estimation                     Number of obs    =       72

         Gaza: province = Gaza
    Inhambane: province = Inhambane
      Nampula: province = Nampula
```

Over	Mean	Std. Err.	[95% Conf.	Interval]
incidence				
Gaza	21.14815	4.603264	11.9695	30.3268
Inhambane	39.88889	6.943265	26.04441	53.73337
Nampula	11.83333	5.988688	-.1077706	23.77444

Annex 3: kruskal-wallis equality of rank tests for rootknot nematode incidence across provinces

```
. kwallis incidence, by(province)

Kruskal-Wallis equality-of-populations rank test
```

province	Obs	Rank Sum
Gaza	27	950.50
Inhambane	27	1218.00
Nampula	18	459.50

```
chi-squared =     9.622 with 2 d.f.
probability =     0.0081

chi-squared with ties =    10.465 with 2 d.f.
probability =     0.0053
```

Annex 4: Dunn's pairwise comparison of provincial rootknot nematode incidence means

```
. dunntest incidence, by(province) ma(none)

Kruskal-Wallis equality-of-populations rank test
```

province	Obs	Rank Sum
Gaza	27	950.50
Inhambane	27	1218.00
Nampula	18	459.50

```
chi-squared =     9.622 with 2 d.f.
probability =     0.0081

chi-squared with ties =    10.465 with 2 d.f.
probability =     0.0053
```

Dunn's Pairwise Comparison of incidence by province
(No adjustment)

Col Mean- Row Mean	Gaza	Inhamban
Inhamban	-1.813954 0.0348	
Nampula	1.584542 0.0565	3.206991 0.0007

Annex 5: Computation of district mean rootknot nematode incidence

```
. mean incidence, over(district)

Mean estimation                   Number of obs    =        72

   Mandlakazi: district = Mandlakazi
       Bilene: district = Bilene
      Chibuto: district = Chibuto
    Inharrime: district = Inharrime
      Jangamo: district = Jangamo
      Homoine: district = Homoine
      Meconta: district = Meconta
         Moma: district = Moma
```

Over	Mean	Std. Err.	[95% Conf.	Interval]
incidence				
Mandlakazi	21.77778	7.626303	6.571362	36.98419
Bilene	24.33333	9.937303	4.518913	44.14775
Chibuto	17.33333	6.823163	3.728332	30.93833
Inharrime	24.66667	11.2645	2.205898	47.12744
Jangamo	39.11111	12.18884	14.80725	63.41497
Homoine	55.88889	11.55596	32.84697	78.93081
Meconta	18.88889	11.33265	-3.707778	41.48556
Moma	4.777778	3.398438	-1.998516	11.55407

Annex 6: kruskal-wallis equality of rank tests for rootknot nematode incidence across districts

Kruskal-Wallis equality-of-populations rank test

district	Obs	Rank Sum
Mandlakazi	9	331.50
Bilene	9	323.00
Chibuto	9	296.00
Inharrime	9	329.00
Jangamo	9	396.50
Homoine	9	492.50
Meconta	9	266.00
Moma	9	193.50

chi-squared = 13.888 with 7 d.f.
probability = 0.0532

chi-squared with ties = 15.105 with 7 d.f.
probability = 0.0347

Annex 7: Dunn's pairwise comparison of district rootknot nematode incidence means

Dunn's Pairwise Comparison of incidence by district
(No adjustment)

Col Mean- Row Mean	Mandlaka	Bilene	Chibuto	Inharrim	Jangamo	Homoine
Bilene	0.099835 0.4602					
Chibuto	0.416957 0.3384	0.317122 0.3756				
Inharrim	0.029363 0.4883	-0.070472 0.4719	-0.387594 0.3492			
Jangamo	-0.763443 0.2226	-0.863277 0.1940	-1.180400 0.1189	-0.792806 0.2139		
Homoine	-1.890989 0.0293	-1.990824 0.0233	-2.307946 0.0105	-1.920352 0.0274	-1.127546 0.1298	
Meconta	0.769315 0.2209	0.669480 0.2516	0.352358 0.3623	0.739952 0.2297	1.532758 0.0627	2.660304 0.0039
Moma	1.620847 0.0525	1.521013 0.0641	1.203890 0.1143	1.591484 0.0558	2.384290 0.0086	3.511836 0.0002

Col Mean- Row Mean	Meconta
Moma	0.851532 0.1972

Annex 8: Computation of provincial mean gall index

```
. mean gindex, over(province)

Mean estimation                     Number of obs    =       511

          Gaza: province = Gaza
     Inhambane: province = Inhambane
       Nampula: province = Nampula
```

Over	Mean	Std. Err.	[95% Conf.	Interval]
gindex				
Gaza	1.201087	.0406206	1.121283	1.280891
Inhambane	1.90099	.0818515	1.740183	2.061798
Nampula	1.384	.0982321	1.191011	1.576989

Annex9: Kruskal-wallis equality of rank tests for rootknot nematode gall indices on cowpea roots across provinces

```
. kwallis gindex, by(province)

Kruskal-Wallis equality-of-populations rank test
```

province	Obs	Rank Sum
Gaza	184	41094.00
Inhambane	202	61168.50
Nampula	125	28553.50

```
chi-squared =    33.667 with 2 d.f.
probability =     0.0001

chi-squared with ties =    56.729 with 2 d.f.
probability =     0.0001
```

Annex 10: Dunn's pairwise comparison of provincial gall index means

```
. dunntest gindex, by(province) ma(none)

Kruskal-Wallis equality-of-populations rank test
```

province	Obs	Rank Sum
Gaza	184	41094.00
Inhambane	202	61168.50
Nampula	125	28553.50

```
chi-squared =    33.667 with 2 d.f.
probability =     0.0001

chi-squared with ties =    56.729 with 2 d.f.
probability =     0.0001

                Dunn's Pairwise Comparison of gindex by province
                                (No adjustment)
Col Mean-
Row Mean |       Gaza    Inhamban
---------+--------------------
Inhamban |   -6.856143
         |      0.0000
         |
 Nampula |   -0.386133    5.746394
         |      0.3497      0.0000
```

Annex11: Computation of district mean gall index

```
. mean gindex, over(district)

Mean estimation                     Number of obs    =       511

   Mandlakazi: district = Mandlakazi
       Bilene: district = Bilene
      Chibuto: district = Chibuto
    Inharrime: district = Inharrime
      Jangamo: district = Jangamo
      Homoine: district = Homoine
      Meconta: district = Meconta
         Moma: district = Moma
```

Over	Mean	Std. Err.	[95% Conf.	Interval]
gindex				
Mandlakazi	1.288136	.0803661	1.130246	1.446025
Bilene	1.121212	.0551123	1.012937	1.229487
Chibuto	1.20339	.0756026	1.054859	1.351921
Inharrime	1.603175	.134898	1.33815	1.868199
Jangamo	1.955224	.1433476	1.673599	2.236848
Homoine	2.111111	.1406384	1.834809	2.387413
Meconta	1.69697	.1766955	1.349829	2.04411
Moma	1.033898	.0237622	.9872145	1.080582

Annex 12: Kruskal-wallis equality of rank tests for rootknot nematode gall indices on cowpea roots across districts

Kruskal-Wallis equality-of-populations rank test

district	Obs	Rank Sum
Mandlakazi	59	14145.50
Bilene	66	13875.50
Chibuto	59	13073.00
Inharrime	63	16486.50
Jangamo	67	20610.00
Homoine	72	24072.00
Meconta	66	16944.00
Moma	59	11609.50

chi-squared = 48.299 with 7 d.f.
probability = 0.0001

chi-squared with ties = 81.384 with 7 d.f.
probability = 0.0001

Annex 13: Dunn's pairwise comparison of district gall index means

Dunn's Pairwise Comparison of gindex by district
(No adjustment)

Col Mean- Row Mean	Mandlaka	Bilene	Chibuto	Inharrim	Jangamo	Homoine
Bilene	1.448424 0.0737					
Chibuto	0.867964 0.1927	-0.556488 0.2889				
Inharrim	-1.064446 0.1436	-2.568180 0.0051	-1.946524 0.0258			
Jangamo	-3.341355 0.0004	-4.936119 0.0000	-4.236449 0.0000	-2.300366 0.0107		
Homoine	-4.734750 0.0000	-6.401923 0.0000	-5.644762 0.0000	-3.701756 0.0001	-1.383887 0.0832	
Meconta	-0.832813 0.2025	-2.347926 0.0094	-1.724749 0.0423	0.247716 0.4022	2.579383 0.0049	4.003498 0.0000
Moma	2.052360 0.0201	0.660620 0.2544	1.184396 0.1181	3.150180 0.0008	5.457866 0.0000	6.886536 0.0000

Col Mean- Row Mean	Meconta
Moma	2.941857 0.0016

Annex 14: Experimental lay out

REPLICATE ONE						REPLICATE TWO						REPLICATE THREE					
18	18	18	25	25	25	28	28	28	15	15	15	6	6	6	23	23	23
17	17	17	13	13	13	7	7	7	19	19	19	16	16	16	11	11	11
4	4	4	26	26	26	2	2	2	10	10	10	2	2	2	15	15	15
28	28	28	15	15	15	23	23	23	3	3	3	17	17	17	9	9	9
19	19	19	8	8	8	24	24	24	1	1	1	3	3	3	27	27	27
16	16	16	10	10	10	8	8	8	13	13	13	7	7	7	24	24	24
9	9	9	1	1	1	22	22	22	18	18	18	1	1	1	4	4	4
22	22	22	5	5	5	12	12	12	9	9	9	21	21	21	19	19	19
6	6	6	12	12	12	25	25	25	11	11	11	12	12	12	8	8	8
23	23	23	27	27	27	6	6	6	17	17	17	13	13	13	20	20	20
2	2	2	3	3	3	5	5	5	26	26	26	26	26	26	14	14	14
11	11	11	7	7	7	27	27	27	20	20	20	5	5	5	22	22	22
21	21	21	20	20	20	4	4	4	21	21	21	10	10	10	18	18	18
24	24	24	14	14	14	16	16	16	14	14	14	25	25	25	28	28	28

Annex 15: Outputs for mean genotype gall index

Over	Mean	Std. Err.	[95% Conf.	Interval]
gallindex				
1	1	0	.	.
4	2.222222	.2222222	1.784309	2.660136
5	2.888889	.1111111	2.669932	3.107846
6	2.888889	.2003084	2.494159	3.283619
8	2	.1666667	1.671565	2.328435
9	1	0	.	.
10	.6666667	.1666667	.3382315	.9951018
11	1.888889	.1111111	1.669932	2.107846
12	2.444444	.2421611	1.967239	2.92165
13	1.888889	.2003084	1.494159	2.283619
14	1.666667	.1666667	1.338232	1.995102
15	1	0	.	.
16	1.888889	.1111111	1.669932	2.107846
17	1	0	.	.
18	2	.1666667	1.671565	2.328435
19	1.777778	.1469862	1.488125	2.06743
20	1.777778	.1469862	1.488125	2.06743
21	1.222222	.1469862	.9325696	1.511875
22	1.111111	.1111111	.8921543	1.330068
23	1.222222	.1469862	.9325696	1.511875
24	1	0	.	.
25	2	.1666667	1.671565	2.328435
26	1.222222	.1469862	.9325696	1.511875
27	1.666667	.2357023	1.202189	2.131144
28	2.333333	.1666667	2.004898	2.661768

Note:

Genotype1 = VAR-11; 2 = INIA-41; 3 = VAR-18; 4 = 524B; 5 = B21x2246-3-9; 6 = B21x499-13-2; 7 = VAR-22; 8 = INIA152xBAMBEY-21-1; 9 = VAR-9; 10 = VAR-4; 11 = 2246x503-9-1; 12 = 503xP24-17-3; 13 = B21x2246-3-4; 14 = 034-50; 15 = VAR-13; 16 = 2246x503-9-5; 17 = VAR-3; 18 = I-41x18-9-1; 19 = 499x18-1-13; 20 = CB46; 21 = IT4S-2049; 22 = B21x2246-4; 23 = NHANCHTENGUE; 24 = VAR-8; 25 = I-41x499-28-3; 26 = IT-16; 27 = IT-1105-5 and 28 = INIA-76

Annex 16: Outputs for mean nematode reproductive factor for individual genotypes

Over	Mean	Std. Err.	[95% Conf.	Interval]
reproductivefactor				
1	3.192222	1.401229	.4309442	5.9535
4	4.998889	2.060501	.9384427	9.059335
5	14.89	4.437979	6.144471	23.63553
6	49.1	15.34037	18.8701	79.3299
8	8.984444	1.814955	5.407874	12.56101
9	.7866667	.2822332	.2304949	1.342838
10	.4611111	.2160468	.0353669	.8868554
11	7.173333	1.441039	4.333605	10.01306
12	14.46778	3.054612	8.448326	20.48723
13	13.83	4.425598	5.108868	22.55113
14	7.777778	1.343891	5.129491	10.42606
15	.2188889	.0763298	.0684726	.3693052
16	23.20333	14.64188	-5.65012	52.05679
17	1.101111	.45006	.2142179	1.988004
18	38.28778	11.78942	15.05542	61.52013
19	14.13667	4.071104	6.114105	22.15923
20	16.07444	3.962678	8.265547	23.88334
21	3.582222	1.414752	.7942956	6.370149
22	2.723333	.8381743	1.071618	4.375049
23	2.343333	.3960675	1.562838	3.123828
24	.3966667	.1040299	.1916642	.6016692
25	26.31889	9.973687	6.664632	45.97315
26	2.32	.5044359	1.325953	3.314047
27	5.188889	1.448469	2.33452	8.043258
28	15.26222	4.37597	6.638888	23.88556

Annex 17: Identified *Meloidogyne* species and where they occur

Province	District	Locality	Latitude (°S)	Longitude (°E)	Species Present
Gaza	Chibuto	Canhavane	24°41'46.1"	33°34'27.9"	*Meloidogyne javanica*
Gaza	Chibuto	Canhavane	24°41'48.5"	33°34'48.4"	*Meloidogyne javanica*
Gaza	Chibuto	Godide	24°40'20.8"	33°39'42.9"	*Meloidogyne javanica*
Gaza	Mandlakazi	Chichuango	24°55'46.6"	33°58'37.9"	*Meloidogyne javanica*
Gaza	Mandlakazi	Machulane	24°36'34.7"	34°02'02.2"	*Meloidogyne javanica*
Gaza	Mandlakazi	Macuacua	24°29'14.1"	33°55'04.4"	*Meloidogyne javanica*
Inhambane	Inharrime	Nhanombe	24°30'04.8"	35°11'06.6"	*Meloidogyne javanica*
Inhambane	Inharrime	Nhanombe	24°25'47.2"	35°06'08.6"	*Meloidogyne javanica*
Inhambane	Jangamo	Bambela	24°16'25.5"	35°10'10.5"	*Meloidogyne javanica*
Inhambane	Jangamo	Likaka	24°14'09.7"	35°18'49.3"	*Meloidogyne javanica*
Nampula	Meconta	Meconta sede	14°58'00.3"	39°53'20.5"	*Meloidogyne javanica*
Gaza	Bilene	Messano	25°01'51.6"	32°59'50.2"	*Meloidogyne incognita*
Gaza	Chibuto	Mangume	24°41'59.0"	33°36'10.1"	*Meloidogyne incognita*
Inhambane	Inharrime	Makungera	24°17'30"	35°13'51.9"	*Meloidogyne incognita*
Inhambane	Inharrime	Nhanombe	24°25'20.5"	35°05'50.3"	*Meloidogyne incognita*
Inhambane	Jangamo	Bambela	24°25'31.8"	35°06'18.9"	*Meloidogyne incognita*
Inhambane	Jangamo	Jangamo sede	24°05'45.1"	35°18'59.5"	*Meloidogyne incognita*
Inhambane	Jangamo	Jangamo sede	24°06'45.1"	35°18'58.3"	*Meloidogyne incognita*
Inhambane	Homoine	Inhamussua	23°52'27.7"	35°14'42.3"	*Meloidogyne incognita*
Inhambane	Homoine	Malengue	23°54'20.9"	35°11'34.3"	*Meloidogyne incognita*
Gaza	Mandlakazi	chicuango	24°56'48.5"	33°59'47.3"	*Meloidogyne incognita*
Inhambane	Homoine	Malengue	23°54'29.7"	35°11'49.6"	*Meloidogyne enterolobii*