KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY (KNUST) KUMASI-GHANA.

PHENOTYPIC AND MOLECULAR SCREENING OF THIRTY TOMATO (*Solanum lycopersicum* L.) GERMPLASM FOR ROOTKNOT NEMATODES (*Meloidogyne incognita*) Chitwood, RESISTANCE.

A DISSERTATION SUBMITTED TO THE DEPARTMENT OF CROP AND SOIL SCIENCES OF THE COLLEGE OF AGRICULTURE AND NATURAL RESOURCES IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF M.Sc PLANT BREEDING.

BY

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DECLARATION

I hereby declare that, except for references cited in relation to other people's work, this work is the result of my original research and that this thesis has neither in whole nor in part been presented for any degree elsewhere.

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Dedicated to our baby, Reginald Okyere Danso

whose birth coincided with the timely completion

of this work.

ABSTRACT

This study was conducted to screen 30 tomato (*Solanum lycopersicum*) germplasm for root-knot nematodes (*Meloidogyne incognita*) resistance. Three experiments were conducted. Field experiment was done on a field naturally heavily infested with root– knot nematodes at Afari in the Atwima Nwabiagya district of Ashanti region of Ghana. A pot experiment was conducted in a plant house at Crops Research Institute. Molecular screening was also done at the Biotechnology Laboratory of the Crops Research Institute to detect markers for the gene that confers resistance to tomato (*Mi*) with specific primers (Mi23/F//Mi23/R). Six resistant cultivars - FLA 496-11-6-0, 2641A, "Adwoa Deede", Tima, Terminator F1 and 2644A were identified in the molecular screening.

The susceptible check (UC82) recorded the highest (2,508) J2/200 ml soil recovered from the rhizosphere of tomatoes which was significantly different (P=0.01) from the resistant check (VFNT) which recorded 208 J2/200ml soil in the field study.

The susceptible check (UC82) also recorded a significantly high number (108) J2/g root compared to the resistant check (VFNT) which recorded none.

The susceptible check, UC82, and the resistant check, VFNT, recorded mean gall indices of 4.0 and 0.0, respectively, on a scale of 0-5 in the field work.

VFNT and three other resistant cultivars, - FLA 496-11-6-0, 2641A and Adwoa Deede did not gall in the pot experiment. FLA 496-11-6-0, Adwoa Deede, Tima and the resistant cultivar recorded significantly high fruit yield (3.3, 5.8, 1.3 and 2.4 t/ha) respectively. The susceptible check, UC82, however, recorded significantly low fruit yield (0.4 t/ha).

Most of the susceptible cultivars identified recorded significant decreases in fresh and dry shoot weights with increasing inoculum densities (Pi = 0, 250, 500).

Six resistant cultivars identified in the molecular screening correlated well with the resistant phenotypes in both the field and pot experiments.

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is cultivated as an annual crop in most regions of the world and is a valuable source of several minerals and vitamins; particularly vitamins A and C (Vossen van der, 2004). It also contains lycopene which is responsible for the red colouration of the fruit when ripe, and reduces risk of cancer and other heart related diseases in humans (Pardee, 2007). All the savanna and transitional zone soils in Ghana favour tomato production (MoFA, 1987) and the crop has become one of the most important vegetable crops grown for both processing and fresh markets worldwide. It is an important "cash" crop in the forest, transitional and savannah zones of Ghana. Total production in Ghana increased from 28,400 ha in 1996 to 37,000 ha in 2000 (MoFA, 1987).

However, root-knot nematodes, *Meloidogyne* species, severely attack the vegetable crop, resulting in severe yield losses, and its infestation is the most common and serious problem associated with tomato cultivation in Ghana (Addoh, 1971; Hemeng, 1980). Root-knot nematodes are economically important plant parasitic pests and distributed worldwide. They are obligate parasites and parasitize thousands of different plant species including monocotyledons, dicotyledons, herbaceous and woody plants (Sikora and Fernandez, 2005). They are also pests of major food crops, vegetables, fruit and ornamental plants grown in tropical, subtropical and temperate climates of the world, infesting a wide variety of subsistence and cash crops and causing more economic damage than any other single group of plant parasitic nematodes (Mai, 1985). According to

Caveness (1978), there are very few annual or perennial crops that are not attacked by one *Meloidogyne* sp. or the other. Mahmood (1988) reported that, the root–knot nematode (*M. incognita*) alone infests hundreds of weedy plants in addition to crops of economic importance. Sasser (1989) aptly described them as the "hidden enemy" because, the economic loss they cause is enormous and the fact that, these pests cannot be readily seen with the naked eyes but protected in plant roots.

Root-knot nematodes are ubiquitous in Ghana and they attack many crops of economic importance. The magnitude of the problem could be appreciated by the fact that root-knot nematodes caused about 33% loss in tomatoes in a single season in Ghana (Addoh, 1971). Estimates of tomato crop losses in the tropics due to the pest ranged from 24 to 38% (Sasser,

1979). In India, tomato yield losses of 28-91% due to *Meloidogyne* species have been recorded (Bhatti and Jain, 1977). *Meloidogyne* species infestation is a limiting factor to the production of fresh tomatoes in Ghana and the need to search for a sustainable control measure has engaged the attention of many growers and researchers of the crop.

Occurrence of root–knot nematodes in the country was first reported in a tomato field near Accra. Three main species distinguished to be economically important in tomato production are; *M. javanica*, *M. incognita,* and *M. arenaria.* Besides *Meloidogyne* species, several other plant parasitic nematodes are prevalent in both cultivated and uncultivated fields in Ghana (Addoh, 1970).

According to Chitwood (2002), phytoparasitic nematodes are among the most difficult crop pests to control. Root-knot nematodes may be controlled by means of nematicides and crop rotation (Addoh and Amanquah, 1968). Reports by Peacock (1957) have shown that Ethylene dibromide and Tetrachlorobutadiene controlled nematodes in the coastal savanna zones of Ghana. Chemical

application for controlling root–knot nematodes has been extensively investigated (Colon *et al*., 1972; Kyron, 1973). Soil treatment with Thionazin and Aldicarb effectively controlled root–knot nematodes on tomatoes (Reddy and Sheshadri., 1971).

Most, if not all, of the existing control measures developed against root-knot nematodes have their weaknesses. Chemical application could be an effective nematode control measure in Ghana, but the high cost of nematicides discourages resource-challenged farmers from using them. There are also very few nematicides available for nematode control (Schroeder *et al*., 1993). There is an increasing public concern regarding the widespread use of agro chemicals that are hazardous to human health and the environment. Such concerns are driving the search for more environmentally friendly methods to plant pests and diseases control that will contribute to the goal of sustainability in tomato production. For these reasons, farm chemicals such as Methyl bromide are being withdrawn from the market (Anon, 2002; Thomas, 1996). The above demerits render the use of nematicides unattractive.

According to (Khan and Khan, 1985), host weeds of root-knot nematodes contribute greatly to the maintenance of inocula during intermittent fallowing periods, rendering most control measures ineffective. Physical control methods such as the use of heat, either as steam or as hot water treatment, have special defects. Steam sterilization of soil is expensive and cannot be used on a large scale. Hot water treatment, of planting materials could control plant parasitic nematodes in the propagules but faces possible re–infestation on nematode-infested lands. Other cultural methods, such as ploughing in the dry season, fallowing, and application of organic amendments, are not economically feasible. Crop rotation has not been very effective. This is because *Meloidogyne* species have extensive plant host ranges and this make selection of rotation crops very difficult.

Environmental problems associated with the use of nematicides (Sikora *et al.*, 2005) and unreliable results from crop rotation systems have resulted in a sense of urgency regarding the search for alternative plant parasitic nematodes management strategies (Kerry, 1990).

Therefore, emphasis is now being placed on cultural practices options such as the use of resistant cultivars of crops. Resistant cultivars of crops offer a promising strategy for nematode control (Roberts, 1992). Clerk (1974) emphasized that an ideal and cheapest way of controlling crop plant pests is by the use of resistant host varieties. According to Castagnone-Sereno (2002) plant resistance is the most effective and environmentally safe method to control nematodes. Current research in tomato focuses on improving yield, flavour, pest and disease resistance (Pardee, 2007).

Host resistance has often been derived from wild relatives of tomato and could be incorporated into adapted cultivars. Use of resistant cultivars could be an effective, economic and environmentally friendly strategy for managing root-knot nematodes. Resistances that prevent plant parasitic nematode infestation represent a realistic alternative to chemical nematicides.

Molecular marker-assisted-selection (MAS) has also provided potential for efficient development of disease and pest resistance in crop plants. Polymerase Chain Reaction (PCR) amplification, using sequence characterized regions, is considered to be a convenient system of using markers for screening in commercial breeding programmes (Heer *et al*., 1998.; Huang *et al*., 2000.; Mathews *et al*., 1998.; and Paran and Michelmore, 1993). Plant genetic improvement can benefit from the use of PCR markers. This is because it is efficient, can be carried out using young plant material (raised on petri dishes instead of pots), and is non-destructive, faster and cheaper to run than standard bioassays (Hussey and Janssen, 2002).

However, a successful plant breeding programme for plant parasitic nematode resistance depends on the identification of effective, resistance sources and the inheritance of the resistance (Niu *et al.*, 2007).

A major problem with vegetable production in Ghana is the risk of pesticide use to farmers and their families through improper formulations and wrong methods of application (Osei *et al.*, 2006). Against this backdrop, the use of plant resistance may be an effective and safe control measure. Nematode-resistance crop varieties could be viewed as the foundation of a successful integrated plant parasitic nematode management programme on all high value vegetable crops. The main objective of this work, therefore, is to screen 30 tomato germplasm for root-knot nematodes (*Meloidogyne* species) resistance.

Chapter Two

LITERATURE REVIEW

Nematodes are small, slender, colourless, roundworms of the phylum, *Nematoda*. Members of this phylum are distributed worldwide in all climatic zones, occurring often in great numbers wherever

suitable nutrients and environmental conditions are favourable (Caveness and Ogunfowora, 1985). Phytoparasitic nematodes are among the most difficult pests to control (Chitwood, 2002). They damage agricultural crops throughout the world, especially in the tropics where environmental factors favour survival and dispersal (Noe and Sikora, 1990). The root-knot nematodes are the most economically important (Williams-Woodward and Davis, 2001).

The genus *Meloidogyne,* belongs to the order, *Tylenchida* of which there are several species and races. Globally, over 90 species of the genus, *Meloidogyne,* have been described (Sikora and Fernandez, 2005). However, *Meloidogyne arenaria, M. javanica* and *M. incognita* are of the greatest agronomic importance, being responsible for at least 90% of all the damage caused by nematodes (Castagnone-Sereno, 2002).

They are polyphagous and attack most cultivated crops (Dickson and De Waele, 2005) and many common weeds of agricultural lands (Anwar *et al,*. 1992; Quènèhervè *et al.*, 1995 and Luc *et al.*, 2005). They are one of the most pervasive and damaging plant-parasitic nematodes and infect hundreds of plant species (Sakai and Carter, 1987), notably vegetables (Caveness, 1978). In a collaborative research work involving eleven centers in Europe, Africa, South America and the Caribbean to assess the occurrence and importance of *Meloidogyne* species, the pests were implicated as widespread and infecting food crops in all countries under the study (Trudgill *et al.*, 2000).

Meloidogyne species enter roots of susceptible plants as juveniles, select a feeding site of 3-5 cells and swell up in their chosen spots as they progress towards adulthood. They introduce a hormone-like substance into the plant cell, causing the plant to swell, producing galls, or rootknots. The males retain their slender profiles and leave the roots at adulthood, the fattened adult female remain

inside. They exude eggs into the soil, after the juveniles hatch, and the cycle begins again. Roots infected with *Meloidogyne* spp. usually have visible galls and excessive branching. Parasitized plants may be stunted. Root systems may be deformed, and the underground organs of plants such as potato, tubers and carrot may also be damaged and unmarketable (http://ocid.nacse.org/nematodes/index).

Since infestation with *Meloidogyne* causes the plants to have a shallow and defective root system with an impairment of secondary root growth, the plants become more susceptible to stress. Plant growth and fruit yields are reduced and mineral deficiencies are common. The pests cause normal galling of the roots which ultimately leads to wilting, stunted growth, chlorosis, early senescence and reduced yields (Luc *et al.*, 2005) of the crops attacked. Other symptoms include fewer feeder roots, lack of vigour and less resistance to drought conditions (Sikora and Fernandez, 2005).

A major problem facing sustainable tomato production in Ghana is how to control *Meloidogyne* species which cause characteristic galling to deform feeder roots. Some cultivated vegetables, including tomatoes, develop extreme root manifestations, signifying serious attacks by the pests (Addoh, 1971).

2.0 MANAGEMENT OF ROOT - KNOT NEMATODES.

The role that *Meloidogyne* species play in limiting food crops production, especially tomato, depends to a large extent on the farming system practiced. In general, *Meloidogyne* species will be less important under more extensive farming and varied growing systems typical of shifting cultivation and multiple cropping as in subsistence agriculture or in a widely spaced rotations of commercial farming system than in more intensive production where monocropping and narrow crop rotations are practiced (Netscher, 1978).

According to IFAS (1989), many of the techniques used for controlling *Meloidogyne* species on vegetables simultaneously control other phytoparasitic nematodes. Once populations of *Meloidogyne* species have developed in a field, it is virtually impossible to suppress and maintain populations at sufficiently low levels without repeated treatment, regardless of the control method practiced (Netscher, 1981). In view of this, a commercial farmer will not hesitate to utilize resistant cultivars or expensive nematicides to protect a crop (Radewald *et al.*, 1987). Control strategies should, therefore, be protective rather than curative from the onset at preventing the build-up of high population densities.

2.1 Cultural Practices.

Bridge (1996) emphasized that, the management of *Meloidogyne* and other pests can be achieved in sustainable and subsistence agricultural systems by the integration of different farming practices that fall into 4 broad strategies;

(1) Preventing the introduction and spread of root-knot nematodes by the use of nematode-free planting materials.

(2) Using direct, non-chemical, cultural and physical methods, particularly crop rotations and soil cultivations.

(3) Encouragement of naturally occurring agents, by understanding cultivation methods and appropriate use of soil amendments and

(4) Maintaining or enhancing the biodiversity inherent in traditional farming systems that use multiple cropping and multiple cultivars to increase the available resistance or tolerance.

2.1.1 Root–knot-free nurseries.

Only seedlings with roots free of galls should be selected for transplanting (Bridge, 1987). Nurseries must be free of root-knot nematodes in order to reduce dissemination into root–knotfree

production areas with contaminated transplants. Seedbeds should be selected on sites which previously were not planted to host plants. To reduce contamination, seedbeds should be prepared for dry season crops on land normally flooded during the wet season, e.g. in previous paddy fields (Sikora, 1988). Soils can be heated in drums or on old sheets of metal before being added to trays or plastic bags for seedling production. Solarization of small quantities of soil may also prove feasible. The burning of straw, paddy husk or saw dust has been suggested (Choudhury, 1981).

2.1.2 Land Rotation and Bush Fallow.

Page (1979) and Sikora *et al*. (1988) suggested designed land rotation and bush fallow practices to reduce the impact of root-knot nematodes in tropical vegetable cropping systems in Bangladesh and Niger, respectively. Tomato, eggplant, lettuce, and melon are very susceptible to root-knot nematodes (Netscher and Luc, 1974). In areas where the climate is characterized by a prolonged and severe hot dry season, fallow during the dry season followed by non-hosts during the wet season for a period of 2-3 years may result in the reduction of *Meloidogyne* populations (Duc, 1980). The effect of crop rotations may be seriously compromised, however, if susceptible weeds are present. Proper weed control can be a vital factor in nematode control.

The systems of crop rotation that have been developed to maintain soil fertility in different ecosystems and for nematode management, are to reduce initial populations of *Meloidogyne* to levels that allow the following crop to become established and complete early growth before being heavily attacked (Nausbaum and Ferris, 1973). Nematode pests were virtually absent in areas where land was plentiful and long periods of bush fallow was practiced; and where sweet potato was rotated with maize, groundnut and cassava (Bridge and Page, 1982). If a field was kept free of host crops during a fallow period, *Meloidogyne* would be sustained in the presence of alternative

weed hosts such as *Synedrella nodiflora*, *Echinochloa colona* and *Setaria barbata* (Sakai and Carter, 1987). For effective control, the fallow should be "clean or complete" with no flora on the field or the weeds growing should be non-host such as *Mucuna puriens* (Atu and Ogbuji, 1986). Soil population of root-knot nematodes can be reduced and yield increased by grass fallows. The local pasture or introduced grasses that are useful in managing *Meloidogyne* species include weeping love grass; *Eragrotis curvula*, Rhodes grass; *Chloris gayana*, Guinea grass; *Panicum maximum*, Pongola grass; *Digitaria decumbens*, Bahia grass; *Paspalum notatum* and Bermuda grass; *Cynodon dactylon* (Luc *et al*., 2005).

2.1.3 Root destruction.

Galled roots remaining in the field after harvest should be eliminated by uprooting and destruction by fire after drying. The spread of *Meloidogyne* will be retarded and the initial population reduced because they cannot survive and reproduce on the roots in the soil after harvest. It has been estimated that when soil temperatures are high, each month that the root system survives causes a 10-fold increase in *Meloidogyne* densities (IFAS, 1989).

2.1.4 Organic amendments.

The incorporation of organic material into the soil reduces *Meloidogyne* densities (Muller and Gooch, 1982). Oil cakes, sawdust, urea and bagasse have been used with some degree of success (Singh and Sitaramaiah, 1967). The decomposition of organic matter resulted in the accumulation of specific compounds that were nematicidal (Sitaramaiah, 1990). Neem cake significantly decreased the number of infected tubers of yams while saw dust reduced nematode population by 50% over the control (Sharma and Raj, 1987). Hemeng (1995) reported that, increasing the rate of poultry manure decreased plant-parasitic nematode population considerably with a corresponding

increase in yields of rice and resulted in 40% reduction in mean root galls in lettuce. Oil seed cakes of castor, mustard, neem and groundnut significantly reduced *Meloidogyne incognita* populations which improved plant growth (Alam, 1991). Chitin in combination with waste products from the paper industry has been used to reduce *Meloidogyne* populations (Culbreath *et al*., 1985).

2.1.5 Maintaining biodiversity and multiple cropping.

The intensification process of modern agriculture tends to be a destabilizing factor in sustainable agricultural systems. Altieri (1991), as well as Page and Bridge (1993), agreed that the reduction in crop diversity and trend towards genetic uniformity invariably increase problems associated with plant-parasitic nematodes. Egunjobi *et al*. (1986) stated that mixed cropping of maize and cowpea proved an effective means of nematode pests' management, with specific advantages for the maize in particular. *Meloidogyne* caused greater damage to crops grown under intensive modern systems than those grown under traditional agriculture (Ogbuji, 1979).

2.2 Physical Control Methods.

2.2.1 Flooding.

This strategy is effective when land is submerged under water for longer periods of time in order to kill *Meloidogyne* species by suffocation. Artificial inundation is, in most circumstances, a costly and uneconomic means of controlling plant-parasitic nematodes even for commercial farmers (Stover, 1979). Naturally-flooded areas are utilized every dry season by subsistence farmers to grow nematode–susceptible crops such as vegetables that are severely damaged by root-knot nematodes (Bridge, 1987). *Meloidogyne* densities dropped significantly when soils were flooded for prolonged periods of time and flooding of rice fields for three months gave acceptable control

for two succeeding vegetable crops (Thames and Stover, 1953). Degree of root-knot damage to vegetable was less severe in cropping systems based on paddy ricevegetable rotations than in rotations, without paddy rice when flooding was maintained for at least 4 months (Sikora, 1989). Crops grown in fields not flooded were frequently severely damaged by nematodes (IFAS, 1989).

2.2.2 Solarization.

Soil solarization could help resource-poor farmers in controlling *Meloidogyne* (Katan *et al*., 1976) when soil temperature is raised to 35^{o}C and above levels that would kill nematodes (Stover, 1979). Solarization was very effective in controlling root-knot nematodes (Stapleton and De Vay, 1986; Gaur and Parry, 1991). To be effective, exposure periods must be long (Egunjobi, 1992). The clear polyethylene film used in the method is expensive for commercial use. Its detrimental effect on potential biological control agents in the soil is thought to be minimal (Gaur and Perry, 1991). Black plastic with the simultaneous use of solar-heated water applied by drip irrigation increases hot water penetration into deeper soil horizons, and may be promising for high value crops (Saleh *et al*., 1988; Abu-Gharbieh *et al*., 1987).

2.2.3 Ploughing during the dry season.

Peacock (1957) adopted this method to control *Meloidogyne* successfully. Prasad and Chawla (1991) reported that summer ploughing of fallow land where temperatures reached 40-42^{o}C could reduce populations of *Meloidogyne* species by 40%.

2.2.4 Grafting.

This technique is well known and could, under certain circumstances, be used for *Meloidogyne* control in the absence of other alternatives. Movra *et al.* (1992) have reported on examples of

grafting vegetable crops such as egg plant unto resistant tomato root stocks to control root-knot nematodes when there were no resistant cultivars of the crop itself.

2.3 Resistance and tolerance as a management strategy.

2.3.1 Non-hosts crops.

Root-knot nematodes are extremely polyphagous; therefore, relatively few non-host plants are available for control through crop rotation. Unfortunately, there are many reports of *Meloidogyne* populations parasitizing plants which have been reported as non-hosts, an important factor in developing rotation-based control systems (Netscher and Taylor, 1979). Fodder and green crops which are considered non-hosts to species of *Meloidogyne* can be used in developing rotations.

They include *Arachis hypogaea*, *Crotalaria fulva*, *Crotalaria grahamiana, Crotalaria retusa, Eragrostis curvula, Glycine javanica, Panicum maximum,* and *Stylosanthes gracilis*. Good host plants of *Meloidogyne* species in one part of the world are not necessarily hosts to all populations of that species (Southards and Priest, 1973). Therefore, all crops being considered for rotation must be tested for host status to local populations before rotation schemes are recommended. Local shade trees as well as plants being selected for wind-breaks, e.g. Adansonia *digitata*

(Taylor *et al.*, 1978) or *Prosopis juliflora* (Netscher and Luc, 1974) are good hosts. Conversely, *Azadirachta indica, Anacardium occidentale* and *Eucalyptus camaldulensis* are resistant to rootknot nematodes. *Carica papaya* however is a good host. Roots of some non-host crops could react to root-knot nematode penetration with local necrosis.

2.3.2 Resistance.

The use of resistant cultivars is an elegant, economical and environmentally safe method for controlling root-knot nematodes (Netscher and Mauboussin, 1973). There are few sources of resistance amongst crops susceptible to *Meloidogyne* species. Cultivars resistant to different species of *Meloidogyne* have been bred or selected especially in tomato and pepper (Netscher and Sikora, 1990). Amosu (1976) screened 35 cultivars of tomato for resistance to root-knot nematodes and the cultivars; Atkinson, Nematex, Rossol, VEN 8, and Ife I were resistant. Odihirin (1976) listed some crops with natural immunity to root-knot nematodes.

Two types of resistance have been reported by Howard (1970): A) Plants might be resistant to invasion or B) There might be resistance after penetration, resulting in juveniles not being able to complete their development and reproduction. Tolerance – when plants sustain little injury even if heavily infested, such plants can be of considerable value under natural conditions (Cook and Evans, 1987). In most cases, the genetic basis for resistance is determined by one major gene (Gilbert and McGuire, 1956; Hare, I957). Genetic barriers make it extremely difficult to introduce genes of the "wild" species into cultivated ones. Modern techniques like protoplast culture and somatic hybridization may make it possible to create viable hybrids and attempts have been made to develop inter-specific hybrids. In some cases, "wild" species can be used as resistant rootstock of susceptible grafts (Dunay and Dalmasso, 1985). They concluded that a list of resistant cultivars should be used with caution. It is often based on a limited number of field observations and does not guarantee that a cultivar is resistant to all populations of *Meloidogyne*. It has been emphasized that resistant cultivars of crops susceptible to *Meloidogyne* do not necessarily protect the crop against all species of the genus. Races may exist which are able to break resistance. In some cases, the *Mi* gene does not confer immunity to *Meloidogyne incognita*, *M. javanica* and *M. arenaria*

(Sauer and Giles, 1959). Resistant cultivars should be used judiciously and with caution or should be tested by using small micro-plots with the cultivars in question (Roberts *et al*., 1986).

Dropkin (1969) showed that at 28°C, the resistant cultivar, Nematex, was highly resistant to *Meloidogyne incognita*, whereas at 32°C, it was susceptible. A breakdown in resistance due to high soil temperatures has been observed (Sikora *et al.*, 1973; Berthou *et al*., 1989). In areas with extreme temperatures, cultural practices, such as appropriate watering and mulching, may reduce soil temperature to counteract and prevent loss of resistance.

2.4 Chemical Control.

Chemical control consists of the application of botanical or organic synthetic compounds that have a killing, inhibiting or repulsive effect on injurious organisms threatening mankind (Oudejans, 1991). Chemicals used for controlling nematodes are called nematicides. Maqbool *et al*. (1985) reported that two systemic chemicals, aldicarb and carbofuran, were effective in control of root-knot nematodes in cauliflower. Before that time, aldicarb had been used to control root-knot nematodes effectively on potatoes (Gill, 1976). Phenamiphos 1-3-D and Carbofuran, each at 5kg ai/ha, were recommended for the control of root-knot nematodes in the northern savanna zone of Ghana whilst the rate of 10kg ai/ha for each of them gave remarkable results in the transitional zone (Hemeng, 1980). Phorate, a systemic chemical, was effective in reducing root-knot nematode populations and number of galls but was not as effective as aldicarb and carbofuran (Jagdale *et al*., 1985). Stephen *et al*. (1989) showed that, Phenamiphos 40% EC and Mical 10%G applied at the recommended rates were effective in controlling *Meloidogyne javanica* on egg plants. Yields increased 59% and 55%, respectively, compared with the untreated control.

Nematicides can be applied effectively by surface and drip irrigation (Overman, 1974; Johnson, 1985; IFAS, 1989). Alternative approaches such as dip treatment or treatment of transplants in nurseries (Mateille and Netscher, 1985) and seed coating (Schiffers *et al*., 1985) have been suggested.

2.5 Biological control.

Progress has been made regarding the incorporation of nematode parasites or antagonists into the soil for controlling root-knot nematodes on vegetables (Kerry, 1987). Dube and Smart (1987) have reported that *Paecilomyces lilacimus* could suppress the root-galling and stunting of tobacco by *Meloidogyne incognita*. A strain of *Arthrobotrys irregularis* grown on rye grain reduced root-knot galling and increased tomato yields when it was introduced in the soil at 140g/m^2 (Caryol and Frankowski, 1979; Caryol, 1983). *Pasteuria penetrans* is an obligate parasite of *Meloidogyne* species (Birchfield and Antonpoulus, 1976). Addition of the bacterium, *Pasteuria penetrans*, to the soil, reduced galling of roots caused by root-knot nematodes (Stirling, 1984). Brown and Smart (1985) showed that *Pasteuria penetrans* decreased the pathogenecity of field population of *Meloidogyne incognita*. The spore form can resist both drought and exposure to non-fumigant nematicides (Mankau and Prasad, 1972).

The protozoal parasite, *Duboscquia penetrans*, attacks many plant-parasitic nematodes, including *Meloidogyne incognita* and *M. javanica*, by penetrating the cuticle, destroying the reproduction system and preventing egg production (Luc *et al.*, 2005). Stirling (1991) proposed that a natural biological control in farm soils could be encouraged by reduced tillage, generally more effective in conserving beneficial antagonists than under intensive cultivation. According to Sikora (1978), penetration and development of *Meloidogyne incognita* in tomato was significantly reduced by

Glomus mosseae in glass house studies. A promising group of microorganisms that may be effective in reducing nematode damage is the plant health-promoting *rhizobacteria* (Sikora, 1988; Oostendorp and Sikora, 1989) which could be applied as seed dressings or as drench treatment for transplants. Application through drip-irrigation systems may be an effective method of post-planting application (Zavaleta-Meija and Van Gundy, 1982). Altieri (1984) established that, traditional farming systems could build-up diversity in the soil as well as in crops, and they usually enjoy a high degree of natural pest control. Many workers (Stirling, 1991; Morgan-Jones and Kabana, 1987; Kerry, 1987) have enumerated naturally occurring organisms that are antagonistic to root-knot nematodes. They include; *Vesicular arbuscular mycorrhiza* (VAM), *Pasteuria penetrans* and predaceous organisms such as *Enchytraeids, Collembolla* and other nematodes. They stated that these microbes provide effective control of root-knot nematodes, particularly in low-input, traditional agriculture. Farming systems with a more stable ecosystem enhances natural control of root-knot nematodes. Results in a pot experiment showed that, the African marigold, *Tagetes erecta*, was most effective from eight weeks after planting (Osei, 2000) and there was a significant reduction of *Meloidogyne incognita* juvenile population. Root gall index was also at minimum.

Several writers (Taylor and Sasser, 1978; Johnson and Fassuliotis, 1984; IFAS, 1989) have chronicled the principles and main components of effective nematode control programmes in vegetables as well as other food crops.

2.6. Evaluation of Resistance and Terminology for Plant Response.

For a complete evaluation of plant response to nematodes, two parameters should be measured. These are nematode reproduction and then the damage caused by the nematodes (Jones, 1956). Host efficiency is usually not correlated with damage (Hijink and Oostenbrink, 1968), and a low

level of parasitic damage may be due to either a level of resistance in the host or to a low level of parasitic ability in the parasite. The host and the parasite are single system, and the terminology about the system should be based on the system rather than on one or the other of its components (Robinson, 1980).

The terms "non-host", "non-efficient host" (pf/pi < 1) and "efficient host" (pf/pi > 1) were proposed to describe the plant when only nematode reproduction is measured (Jones, 1956). When both parameters are measured, the terms used to describe the plants' response are "immune" (non-host, no damage), "resistant" (non-efficient host that suffers no damage). The term, "intolerant", was proposed for a "non-efficient host" that suffers damage and could be distinguished from an efficient host that suffers damage (Cook, 1974). The distinction between "susceptible" and "intolerant" is important, but intolerant, a negative term, cannot be used to differentiate one possibility of four, where each possibility has two components (De Morgan's law of logics). In other words, resistant or susceptible plants are also intolerant, and tolerant or susceptible plants are also non-resistant. However, the resistant and susceptible are used indiscriminately to describe plant response in relation to nematode reproduction or to describe damage caused by the nematode. The use of the term to define two distinct phenomena is confusing and should be avoided (Cook, 1974). Some definitions of plant response in which the terms are used indiscriminately are; "non-host" (plants with pre-infectional resistance); "immune" (Plants' ability to prevent infection with no disease or pest expression); "resistant" (Plants cannot prevent entrance by the parasite but are able to prevent, restrict, or retards its development, or penetration by nematode juveniles does not occur) and "tolerant" (Plant survives or gives a satisfactory yield at a level of infection that causes economic loss on other varieties of the same species (Fassuliotis, 1979).

Host efficiency is measured by gall index, number of egg masses/g of root; females/g of root or index of reproduction (number of eggs developing in a resistant cultivar as a percentage of those developing on a susceptible cultivar (Fassuliotis, 1967 and 1979; Veech, 1978).

At high nematode densities, there is sometimes gall formation and the egg masses may appear later. If the evaluation is based only on gall or egg mass index before 50 days, the results may be misinterpreted. Gall index is a useful criterion for general screening; but negative results should be retested (Fassuliotis *et al.*, 1970; Golden and Shafer, 1958; Santo *et al.*, 1980). Damage caused by nematodes is determined by relating pre-plant nematode densities (initial population) to growth and yield of annual crops. The minimal density that causes a measurable reduction in plant growth or yield is regarded as the damage threshold density. Threshold density varies with nematode species, race, plant variety and the environment. Infection of non-efficient and efficient hosts by low densities of *Meloidogyne* species may enhance growth and yield (Madamba *et al.*, 1965; Olthof and Potter, 1972) or cause severe damage. Some damage threshold densities of *Meloidogyne incognita,* depending on temperature, are as follows; 300081000 eggs/plant for soybean, 0.005-0.02 eggs/g soil for tomato, 0.005-0.03 egg/g soil for sweet potato and 0.04-2 eggs/g soil for tomato (Ferris, 1978).

2.6.1 Biochemical Mechanisms of Incompatibility.

Reports of biochemical mechanisms of incompatibility are numerous. Root diffusates (e.g Cucurbitacin) can repel or (e.g mangosa) can be toxic to the nematode (Veech, 1981). The death of the nematode after appearance of necrosis indicates the plants' response to the presence of feeding of the nematodes inside the roots by production and accumulation of toxic substances such as nicotine; proline; glyceollin; phytoalexins such as hemigossypol; methoxyhemigossypol;

gossypol and dimethoxygossypol. These substances may affect nematodes behaviour or cause death and disintegration of the nematode. The incompatible response is faster in some incompatible plants than in others. This suggests quantitative instead of qualitative differences since the final response is the same (Canto-Sánez and Brodie, 1984). Another interesting hypothesis is that, hydroxyproline concentration increases and cells develop cyanide-insensitiverespiration in incompatible plants when they are invaded by nematodes (Zacheo *et al.*, 1977).

2.6.2 Correlation between Parts of the Plant.

It is important to determine if there is a correlation between the responses of the different parts of the plant. Sweet potato tubers were not infested when artificially inoculated (Khana and Nirula, 1964). There are several reports with other crops that incompatibility is inherent to a particular part of the plant (either top or roots) and it is not translocated (McClure *et al.*, 1974; Peacock, 1957). Temperature requirements for symptom manifestation may also be different for different parts of the plant (Canto-Sánez and Brodie, 1984).

2.6.3 Factors affecting plant response to *Meloidogyne* species infection.

2.6.3.1 Temperature.

Temperature affects nematode survival (Dropkin *et al.*, 1969; Fassuliotis and Bhatt, 1982; Peacock, 1957) and distribution (Nirula and Raj, 1971), embryogenesis and hatching (Barker *et al*, 1969; Tyler, 1933; Vrain and Barker, 1978), migration and penetration (Duo, 1970; Jatala and Russel, 1972; Prot and Van Grundy, 1979), development (David and Triantaphyllou, 1967; Nardacci and Barker, 1979; Peacock, 1957; Tyler, 1933; Vrain *et al.*, 1978), and symptom expression in the plant (Joubert and Rappard, 1971). Temperature varies among populations of *Meloidogyne incognita* - thermotypes (Ritter, 1973; Thomason and Lear, 1961) and with each host-parasite

combination (Peacock, 1957). However, some plants and cultivars of the same plant species do not respond similarly (Dropkin *et al*., 1969). The equilibrium density for nematode reproduction may also be different among plants of the same crop (Canto-Sánez and Brodie, 1984).

Under environmental stress (except nutritional), nematode reproduction was high (Fawole and Mai, 1979; Wallace, 1969). The highest temperature for hatching was reported to be 27ºC (Dropkin *et al.*, 1969; Wallace, 1969). Nematode life cycle was completed faster at high temperatures; therefore more generations were produced (David and Triantaphyllou, 1967; Peacock, 1957; Tyler, 1933). Moreover, at high temperatures, fewer males developed (David and Triantaphyllou, 1967).

The differential plant responses to nematodes at high temperatures are probably due to quantitative differences in the enzymatic reactions occurring in the plant-nematode relationship.
The progressive increase in host efficiency with temperature increase supports this hypothesis.

2.6.3.2 Plant age at inoculation time.

Nematodes' effect on the growth of susceptible plants is influenced by plant age at inoculation. Older plants have more roots already differentiated which the nematode usually does not penetrate (Christie, 1949). Thus, more roots remain undamaged (Jaffe and Mai, 1979). High nematode density in older plants is probably a function of the greater availability of roots and less individual competition (Fawole and Mai, 1979). The scarce root systems of younger plants at transplanting may cause a concentration of juveniles around the root tips. When this situation occurs, root tip growth can stop, resulting in a dramatic reduction in the size of the root system (Canto-Sánez and Brodie, 1984). The number of eggs/g root is usually higher in young plants and response of young plants to root-knot nematodes, if not qualitatively, is sometimes quantitatively different from that of older plants. These differences should be taken into consideration in experiments with small

seedlings, where it is assumed that seedlings' response is the same as that of older plants (Golden and Shafer, 1958; Loos, 1953).

2.6.3.3 Origin of the plant.

Nematode reproduction in some susceptible plants is greatly affected by the origin of the plant. In potato, for example, the nematode *pf* (final population density) is higher in plants originating from cuttings and tubers, while number of eggs/g of root is higher in seedlings (Canto-Sánez and Brodie, 1984).

2.7 Rating scheme for resistance to nematode infection.

Nematode resistance can be defined as the character or characters of a plant that inhibits nematode reproduction. The various methods for rating plants for resistance to root-knot nematodes have been reviewed (David and Triantaphyllou, 1967). Plants vary greatly in their response to root-knot nematodes infection. Upon infection, most genotypes show a galling effect within 24 hr and reproduction is evident within 20 days. However, within some plant species, response to infection may vary greatly. Some plants may gall following penetration by juveniles, but nematode reproduction is significantly reduced (Fassuliotis and Dukes, 1972; Fox and Miller, 1973; Golden and Shafer, 1958). Most workers use galling and reproductive response in evaluating plants for resistance. An index scale of 0–5 (Barker, 1978) is most commonly used to indicate the severity of galling and degree of reproduction of the nematode. 0 = no galling or reproduction (1% or less of the susceptible check plant), 2 = light galling or reproduction (2-10% of the susceptible check plant), 3 = moderate galling or reproduction (11–25% of the susceptible check plant) and 5 = heavy galling or reproduction (51 – 100% of the susceptible check plant). Plants falling into the categories of 0, 1, 2, 3, are considered immune, highly resistant, very resistant and moderately resistant,

respectively. Population of the same species can vary in aggressiveness if they are from different sources (Sasser and Carter, 1985).

2.8 Identification of sources of resistance.

The most limiting factor in the expansion of food crops with root-knot nematode resistance is the lack of genetic material among some plant species. It is obvious that, a breeding programme for root-knot nematode-resistance requires resistant germplasm from which traits can be transferred to plants. Transfer of resistance is greatly simplified if resistant germplasm can be found in adapted cultivars or in advanced breeding lines containing otherwise good horticultural qualities. Over the years, many tomato cultivars and breeding lines that have been developed from rootknot nematode-resistant genotypes can be selected for use in tomato breeding programmes (Khana and Nirula, 1964).

2.9 Factors influencing resistance.

Several factors may alter the expression of resistance. Genetic resistance to *Meloidogyne* species is sensitive to soil temperatures above 28ºC. Tomato, beans and sweet potato lose resistance in elevated temperatures (Dropkin, 1969; Fassuliotis *et al.*, 1970; Holtzman, 1965; Jatala and Russel, 1972). High soil temperatures appeared to be the reason why resistance of root-knot nematodes was not effective in Florida, U.S.A (Walter, 1967). Reproduction of *Meloidogyne incognita* at elevated soil temperatures may be race dependent (Arujo *et al.*, 1983) and that, race 4 reproduces better on resistant tomato genotypes at 32.5ºC than race 1. Application of exogenous kinetin to tomato seedlings altered their expression for resistance (Dropkin *et al.*, 1969). Tissue culture

techniques which use plant hormones in the media for plant regeneration from tissues may reverse the resistance of plants to root-knot nematodes (Fassuliotis and Bhatt, 1982).

2.10 Categories of resistance.

Classification of resistance phenomena may express the relative success or failure of a plant pest to survive, develop, and reproduce on plant species, or the classification may describe the relative damage to the host plant in qualitative and quantitative terms. Resistance is usually measured by using susceptible cultivars of the plant species as controls. Immunity, which represents complete inadequacy for plant pests, is an absolute term, but it is rarely encountered in plants within a host species. The terms host plant and immune exclude each other. Plants of a non-host species would not ordinarily be classified for resistance and, therefore, be considered immune. A host plant can be more or less resistant but not immune. An immune plant is a nonhost. Any degree of host reaction less than immunity is resistance; more than immune is impossible. It must be remembered that, the term immunity does not permit qualifying adjectives such as “comparatively”, “more”, “most”, “rather”, “somewhat”, or “very”. Painter, (1951) used the following scale to classify degrees of decreasing resistance;

Immunity: An immune cultivar is one that specific plant parasites will never consume or injure under any known condition. Thus defined, there are few, if any, cultivars immune to the attack of specific plant pests known to attack cultivars of the same plant species.

High resistance: It is demonstrated by a cultivar that has qualities that result in small damage by specific plant parasite under a given set of conditions.

Low resistance: It indicates qualities that cause a cultivar to show less damage or infestation by specific plant parasite than the average for the crop considered.

Susceptibility: A susceptible cultivar shows average or more than average damage by specific plant parasite.

High susceptibility: The cultivar shows high susceptibility when more than average damage is caused by a specific plant parasite.

The terms indicate the classes used by most workers in plant resistance as it is observed on the field, without analysis of the mechanisms involved. Intermediate resistance is sometimes spoken of as moderate resistance, which, may result from one of the least three situations. A cultivar denoted as moderately resistant may consist of phenotypically similar plants, some of which have high and others low resistance, because of differences in physiological characteristics. In contrast, moderately resistant cultivars may be made of plants derived from a single clone, which is heterozygous for incompletely dominant genes that confer high resistance when homozygous. Moderately resistant plants may also be homozygous for genes which under given environmental conditions, produce plants that are moderately injured or infested (Painter, 1951).

2.11 Future research needs and outlook.

How would we manage or suppress the nematode problems in the future if environmentally safe and inexpensive nematicides are not available? Clearly, intensive efforts must be devoted to the development of genetic solutions to the problem. If resistant genotypes are included in cropping systems, target nematode population could be suppressed (Ferry and Dukes, 1984; Wyatt *et al.*, 1980). Also, resistant or tolerant cultivars make low dosage rates of less potent, non-fumigant nematicides a more practical and viable management option. These compounds applied in

combination with resistant or tolerant lines may make the difference between success and failure of a crop. Much research is needed to expand the list of crops and cultivars with resistance to root-knot nematodes. In crops in which resistant genes are readily available (sweet potato, pepper, cowpea, bean etc), conventional breeding methods can be used to produce new cultivars readily (Canto-Sánez and Brodie, 1984). The recognition of races within *Meloidogyne incognita* and *M. arenaria* opens new areas for research. Many, if not all, of the cultivars already known to have some resistance would have to be re-evaluated.

Nematologists and Plant Breeders are challenged to develop resistance to two or more of the important *Meloidogyne* species in those crops that carry resistance to none or only one species.

The International *Meloidogyne* Project (IMP) was instrumental in providing a worldview of rootknot nematodes and their distribution. From the data collected, a crop having resistance to *M. incognita* and *M. javanica* would be resistant to 82% of the major *Meloidogyne* populations around the world. Tomato cultivar carrying resistance to *Meloidogyne incognita*, *M. javanica* and *M. arenaria* would be resistant to 90% of the root-knot populations (Jones, 1956).

The effect of temperature stress on the expression and reversal of resistance will have to be addressed further. Germplasm with tolerance to soil temperatures above 28^{o}C is needed so that resistant plants developed in the temperate climates will be useful in more tropical climates. Advances in plant biotechnology, often referred to as “genetic engineering”, have emerged which Plant Breeders could use in the transfer of genetic factors into breeding lines (Barton and Bill, 1983). The degree to which this new technology would be used for plant breeding to transfer root-knot nematode resistant genes needs further investigation. Somatic hybridization may have scientific value in that; genes may be transferred between incompatible species. Whether protoplast fusion can be utilized to transfer root-knot nematode resistance to horticultural crops could be

exploited (Sink, 1984; Golden and Shafer, 1958). Recent advances in recombinant DNA (gene splicing) may prove useful in plant protection (Barton and Bill, 1983). Movement of single genes known to express resistance to root-knot nematodes could be possible by the recombination of this genetic information into plants at the molecular level.

Evidence abounds that genetic variability is often generated with cell culture techniques (Giamalba *et al.*, 1963). Although resistance to *Meloidogyne* species has been observed to be reversed after tissue culture; it is possible that some plants derived from tissue culture methods may yield variants with increased resistance and should be evaluated. Nematologists and Plant Breeders could utilize these new technologies to develop *Meloidogyne* species–resistant plant lines. Nematological involvement in a plant breeding programme for root-knot nematode resistance can be both challenging and rewarding and a great deal of gratification results from participation in the development of a cultivar that thrives in fields heavily infested with root-knot nematodes (Johnson, 1985). The task is daunting but not impossible.

2.12 Root-knot nematode resistance gene (*Mi*) in tomato.

The gene *Mi*, which confers resistance to several isolates of root-knot nematodes, *Meloidogyne* species, is present in modern and commercial tomato cultivars (Williamson, 1998; Seah *et al.*, unpublished data). According to them, it is the only source of resistance against the pest in modern tomato cultivars. This resistance gene was introgressed into cultivated tomato from *Solanum peruvianum* in the 1940's (Smith, 1944). A cloning of this gene has revealed that, it encodes a member of the plant resistance protein family characterized by the presence of putative nucleotide-binding-site and a leucine-rich repeat (Williamson, 1998). According to the same author, analysis

of transgenic plants revealed the unexpected results that the *Mi* gene also confers resistance to potato aphids.

Although highly effective under many conditions, the *Mi* gene fails to confer resistance under high soil temperatures and *Mi* virulent nematode isolates have been identified in many areas of the world (Williamson, 1998). Hadisoeganda and Sasser (1982) also concluded that the most important source of root-knot nematodes resistance in tomato is conferred by the *Mi* family of genes from the wild tomato *Lycopersicon peruvianum*, providing an effective resistance to *Meloidogyne incognita, M. javanica* and *M. arenaria;* and opportunistic organisms such as the soil-borne bacterial pathogen, *Ralstonea solanacearum* (Deberdt *et al.*, 2003). *Mi gene* also provides resistance to the aphid, *Macrosiphum euphorbiae* (Rossi *et al.*, 1998), biotypes Q (Nombela *et al.*, 2003) and B (Jiang *et al*., 2001) of *Bermisia tabaci*.

Most commercial tomato cultivars now available come from a cross by which the *Mi* gene has been introgressed from *Lycopersicum peruvianum* into *Solanum lycopersicum*, using embryo culture (Medina-Filho and Stevens, 1980).

The *Mi* locus is located at least 40 Mbp from the linked *Aps-1* gene (Zhong *et al.*, 1999), which codes for the enzyme acid phosphotase that had been used as a marker for root-knot resistance in the past (Rick and Fobes, 1974).

The *Mi* gene was discovered 66 years ago in an accession (P.1. 128657) of *Lycopersicum peruvianum* (Mill.), of a wild relative of the edible tomato (*Solanum lycopersicum*) that was grown in the western coastal region of South America (Cap *et al.*, 1991). The gene has been isolated, cloned and sequenced (ElekçioğÍu and Devran, 2004). The resistance was transferred and expressed in F1 plants derived from a cross between *L. peruvianum* (P.1. 128657) and *S.*

lycopersicum 'Michigan State Forcing' made by Smith (1944). The gene is located on the short arm of chromosome 6. This chromosome has been mapped in considerable detail, and multiple markers for other traits linked to it have been identified (Messequer *et al*., 1991; Williamson *et al*., 1994). *Mi* gene has been isolated by a positional cloning approach (Kaloshian *et al*., 1998) and DNA sequence analysis has been carried out to identify *Mi* candidates. Sequencing revealed two genes, *Mi-1.1* and *Mi-1.2* that were 95% identical to each other, and encoded proteins with a high similarity to previously cloned plant resistance genes (Milligan *et al*., 1998). In the same work, complementation analysis showed that, the introduction of *Mi-1.2*, but not *Mi-1.1*, to susceptible tomato plants was sufficient to confer a nematode-resistant plant phenotype with the same spectrum of resistance as that of *Mi*.

Resistance genes that differ from the *Mi* gene in properties could be identified to provide a resource for broadening the base of the root-knot nematode resistance in tomato.

2:13 Molecular marker techniques in today's plant breeding.

Genetic markers, differences in the DNA sequences of chromosomes derived from different progenitors, can be visualized in several ways. Morphological mutations, sometimes called visible markers, can be visualized by just looking at the individual. Isozymes or protein variants require separation by electrophoresis and are visualized by calorimetric activity assays for the relevant enzyme in crude extracts from living tissues. DNA markers are visualized either by the use of radioactivity (autoradiography), fluorescence, or by direct chemical staining of the DNA.

The molecular marker techniques commonly used include;

- Restriction Fragment Length Polymorphism (RFLP) (Tanha Maafi *et al.*, 2003).
- Random Amplified Polymorphic DNAs (RAPDs) (Da Cocceicao *et al.*, 2003).

- Amplified Fragment Length Polymorphism (AFLP) (Semblat *et al.*, 1998).
- Simple Sequence Repeats (SSR) or Microsatellites (He *et al*., 2003).
- Sequence Characterized Amplified Region (SCAR) (Fourie *et al.*, 2001).

It is important that plant breeders select the most appropriate techniques for plant molecular studies, given the constraints of time, money, technical expertise or other resources that they may face. The choice of a technique therefore rests with the researcher. In the current work, a codominant SCAR marker (Mi-23) that is tightly linked to the *Mi-1.2* gene (Seah *et al*., unpublished data) would be employed to amplify PCR fragments of thirty (30) tomato germplasm in a marker-assisted-selection.

2:14 Primers for amplifying root-knot nematode-resistance genes (*Mi*) in tomatoes.

The principal means of developing nematode-resistant tomato cultivars is by traditional breeding aided by marker-assisted selection to detect the *Mi* gene. Co-dominant CAPS markers such as REX-I (Williamson *et al.*, 1994) and Cor.Mi (Contact Cornell University Foundation, Ithaca, New York) are widely used to assay for the *Mi* gene in tomato. Although these markers are generally reliable, El Mehrach *et al.* (2005) found that, both gave false positives for nematode resistance with germplasm derived from Ih902 (F1, F2, Ve), which has begomovirus-resistance reportedly introgressed from *Solanum habrochaites* (listed as 902 in Vidavsky and Czosnek, 1998), but is susceptible to root-knot nematodes.

The region on the short arm of chromosome 6 where the *Mi-1* gene locus is located is well characterized genetically and physically (Seah *et al*., 2004, 2007). The *Mi-1* locus in both resistant and susceptible tomatoes consist of two clusters with three and four copies of *Mi* gene homologues, which in resistant tomatoes are separated by approximately 300 kb. Comparison of sequence

downstream of *Mi-1.2* with a conserved region from *S. lycopersicum* led to the development of primers (Mi23/F and Mi23/R) that flanked an indel within this conserved region (Seah and Williamson, unpublished data). The sequence of Mi23F is 5'-TGG AAA AAT GTT GAA TTT CTT TTG-3', and Mi23R is 5'-GCA TAC TAT ATG GCT TGT TTA CCC-3'.

This co-dominant SCAR (Sequence Characterized Amplified Region) marker has the advantage over previous PCR-based markers in that restriction enzyme digestion of the amplified product is not required, and it does not give false positive fragments with begomovirus-resistant breeding lines derived from *S. habrochaites* (Vidavsky and Czosnek, 1998) and *S. chilense* (Ty-1 locus) (Agrama and Scott, 2006). Additionally, M23 may be useful for tomato breeders introgressing other traits located in the resistance gene cluster on the short arm of chromosome 6.

Chapter Three

MATERIALS AND METHODS

EXPERIMENT 1

3.1 Field screening of tomato lines for root-knot nematode resistance.

Thirty lines of tomato were field-tested for their resistance to root-knot nematodes (*Meloidogyne* species), between October 2009 and January 2010 at Afari near Nkawie in the Atwima Nwabiagya District of the Ashanti Region of Ghana.

3:1:1 The experimental site.

Afari (Semi deciduous rainforest zone) is a vegetable growing area in the Ashanti region of Ghana. Due to the continuous cropping of vegetables, it has become a hot spot for nematodes, particularly *Meloidogyne* species. The area falls in the forest agro - ecological zone. The land had previously been cropped with Okra (*Abelmoschus esculentus)* in the year 2000 and had since been left to fallow. The vegetation cover was predominantly elephant grass (*Pennisetum purpureum)* interspersed with guinea grass, (*Panicum maximum)* and oil palm (*Elais guineensis)*.

The type of soil at the site is loamy clay.

3.1.2 Tomato bioassays.

Soil samples were taken randomly at the start of the field trial by walking in a zigzag fashion with a 5cm soil augur from 90 plots, each measuring (2.7x7 m) to a depth of about 20 cm. From each

plot three samples were taken from which a composite sample was taken. The soil samples were put into mini plastic pots with dimension (12x12x9 cm). Two-week-old tomato (cv. Petomech) seedlings which had been germinated on tissue paper in a 9 cm Petri dish were transplanted into the infested field soil in pots, one seedling per pot. The tomato cultivar was chosen because it is susceptible to root-knot nematodes. The pots were arranged in a randomized complete block design in a green-house. The tomato grew for eight weeks and root gall index (0– 5) determined.

3:1:3 Field preparation, Layout and experimental design.

The land was not ploughed but manually slashed with cutlass in order to maintain the stability of the nematode community. It was also not burnt for the same reason. Stumping was done with mattocks and hoes. The debris was also manually collected. Linning and pegging was done at a planting distance of 90x70 cm. The experimental design used was Randomized Complete Block (RCBD) with 3 replications partitioned by 2 alleys of 1 m each. Each plot had 3 rows. The middle row was the test row from which data was taken. The total land area was 1,863 m^2.

Tomato was planted on ridges prepared manually with hoes. Each ridge was 7 m long.

3:1:4 Soil sampling.

Soil sampling was done at planting time using the methodology explained at 3:1:2 with a soil augur up to a depth of about 20 cm to determine the initial population (Pi) of *Meloidogyne* species. The final sampling was done at harvest time. The difference in population levels between the initial and the final on the different plants together with population/g root would help determine the resistance status of the materials.

3:1:5 Tomato lines used for the screening.

Thirty tomato lines were assembled from both local and international sources for screening for root-knot nematode resistance. Below is a table showing the characteristics of the materials.

Table 1.0 Tomato cultivars evaluated.

Accession	Code	Source
FLA 496-11-6-1-0	015	AVRDC, TAIWAN
CLN 2641A	030	AVRDC, TAIWAN
FLA 653-3-1-0	C4	AVRDC, TAIWAN
MONEYMAKER	012	SOUTH AFRICA
ADWOA DEEDE	038	GHANA
ROMA (JAM) VF	010	UNITED STATES OF AMERICA
PARONA	034	GHANA
IGPH	C5	AVRDC, TAIWAN
AKOMA	036	GHANA
TERMINATOR (FI)	040	GREEN SEEDS, INDIA
H24	C2	AVRDC, TAIWAN
ROMA	032	GHANA
FLA 456-4	013	AVRDC, TAIWAN
RANDO	037	GHANA
FLA 505 (BL1172)	014	AVRDC, TAIWAN
FLA 478-6-3-0	016	AVRDC, TAIWAN
SLUMAC	003	HOLLAND
TIMA	006	FRANCE
RED CLOUD	001	HOLLAND
WOSOWOSO	035	GHANA
RIO GRANDE	002	HOLLAND
GH PETOMECH	007	GHANA/FRANCE
ROMA (VF)	004	BURKINA FASO
BIEMSO	039	GHANA
BK PETOMECH	020	GHANA/BURKINA FASO
POWER	033	GHANA
VENTURA (F)	005	BURKINA FASO/FRANCE
2644A	031	AVRDC, TAIWAN
VFNT	R1	TGRC/V. Williamson
UC82	S1	TGRC/ V. Williamson

VFNT and UC82 were used as resistant and susceptible checks, respectively.

3:1:6 Nursery preparation and management.

An open area close to the field with well-drained soil was chosen for the nursery. Two nursery beds with dimension (7 x 1 m) each were constructed by digging up to about 20 cm deep into the soil. Soil of the beds was well prepared by breaking up all lumps. Foreign materials were removed.

The beds were sterilized by burning dry maize straw on the surface. An insecticide – nematicide (Bastion-carbofuran 30g/kg) was sparingly applied by broadcast to protect the seedlings against soil arthropods and nematodes. Prior to sowing, the surface of the soil was lightly forked and treaded-on. Seeds were sown in drills that were about 15 cm apart and covered lightly with moist soil. Water was applied with a watering can with a fine nose. Shade was provided with palm fronds and gradually adjusted according to local weather conditions. Prior to two weeks to transplanting, the shade was completely removed for hardening. Visibly weak seedlings were thinned out. Watering was done manually when necessary. Stirring was also periodically done to improve aeration. Weeds were carefully removed manually from among the seedlings regularly to prevent competition between the weeds and tomato seedlings for soil nutrients.

3:1:7 Transplanting.

Transplanting was done late in the afternoon on 30th October 2009 when the seedlings were three weeks old. Only vigorous and healthy-looking seedlings which were almost of the same height and size were selected for transplanting. Planting holes were made big enough to contain the roots of the seedlings. The seedlings were set in the planting holes, covered with soil and firmed gently around the base. Transplanted seedlings were later watered sparingly with a watering can with a fine nose. Each ridge contained 10 stands (1 seedling/stand). The total plant population was 2,957.

3:1:8 Nematode extraction and counting.

Soil sub-samples from the 90 plots were stored briefly in the refrigerator at a temperature of 4^{O}C and processed for nematodes. The modified Baermann funnel method of extraction (Whitehead and Hemmings, 1965) was used. The procedure involved spreading thinly 100 g of soil on a 2 - ply tissue paper nested in a plastic basket. The plastic baskets with their contents were placed in

shallow trays and placed on a level bench. Tap water was added gently by the side of each tray until the soil was just moist. Each set-up was left for 48 hr after which period the baskets were lifted and tilted to allow water at the bottom of the baskets to drain into the tray. The water in the tray was gently shaken and poured into a beaker. The nematode suspensions were left for 24 hr for the nematodes to settle by gravitational force - concentration period, after which the supernatant was poured off. The nematodes in the samples were heat-killed in an oven and fixed with a few drops (2 or 3) of 4% formaldehyde (formalin) for a short-term preservation. Each concentration was poured into a counting tray (Doncaster, 1962) for microscopic examination and counting.

3:1:9 Fertilization.

Starter fertilizer (15-15-15) was applied 10 days after transplanting (DAT) on 13th November 2009 at a recommended rate of 250 kg/ha. Holes were made with a dibber at both sides of each stand at about 5 cm away for placement of the fertilizer. A nitrogenous fertilizer, (Sulphate of ammonia) was also applied at 28 (DAT) on 1st December 2009 at the same rate of application.

3:1:10 Weed management and earthening-up.

Weeds were effectively controlled manually with hoe and cutlass. The experimental plot and its immediate surroundings were never allowed to be weedy. Earthening-up was done with a hoe at 5 weeks after transplanting (WAT). Weeding was done twice.

3:1:11 Data collection.

Three stands were randomly selected and tagged on the test row for all data collections. The following parameters were measured. The heights of the tomato plants were taken with a tape measure from the third to the seventh week after transplanting on weekly basis. The stem girth was

taken with vernier caliper (Tricle brand) from 3-7 weeks after transplanting (WAT) every week. Fruits of the tagged plants were collected, counted and weighed with a weighing scale. Root gall index was determined on a scale of 0-5. Fresh shoot biomass was taken at harvest with a weighing scale. Dry shoot biomass was taken after samples were oven-dried at 80^{o}C for 48 hr.

Second stage juveniles (J2)/g root was also determined.

Figure 1.0 Field testing of tomato cultivars for *Meloidogyne* species resistance.

3:1:12 Statistical analyses.

Nematode data were log count transformed, using √(x+0.5) to comply with the assumption of normal distribution. Continuous data such as height, girth, weight and yield of treatments were however not transformed. Statistical analyses were performed, using Genstat Release 8.1 (Lawes Agricultural Trust, VSN International).

EXPERIMENT 2

3.2. Pot experiment.

The pot experiment was conducted in a plant house at the CSIR-Crops Research Institute, Fumesua.

3:2:1 Nematode cultures.

Rook-knot nematodes (*M. incognita)* were identified through perineal pattern (CIH, 1978). The species were originally collected in March, 2007 from infected tomato roots in a field at Fumesua, near Kumasi, Ghana.

In August 2009, one egg mass of *M. incognita* was collected from the roots, and cultured on a susceptible tomato cultivar, Petomech, in the plant house at 25^{o}C minimum. Nematode eggs were extracted from tomato roots by shaking for 3 min in 0.05% Sodium hypochlorite (NaOCl) solution and rinsing for 2 min under running tap water (Stanton and O' Donnell, 1994). Extracted eggs were incubated at 22^{o}C on modified Baermann trays (Rodriguez – Kabana and Pope, 1981) for collection of infective second stage juveniles (J2) which were used in inoculating the tomato plants.

Figure 2.0 Cultures of *M. incognita* on tomato cv. Petomech.

3:2:2 Soil Sterilization.

Soil for the pot experiment was steam-sterilized, using a metal barrel at the Crop and Soil

Science Department of the College of Agriculture and Natural Resources at the Kwame Nkrumah University of Science and Technology in Kumasi. Black soil was taken from old refuse dump and mixed with river sand in a ratio of 3:1. The mixture was steam-sterilized at a temperature of 102ºC for 24 hr. The steam sterilizer had 2 chambers. The lower chamber contained water and the upper part, soil. The soil was covered with jute sack to prevent the steam from escaping. Heat was supplied from pieces of firewood from beneath. Heated soil was allowed to cool down for 24 hr before use.

3:2:3 Soil sterilization test.

To determine the effectiveness of the soil sterilization method, nematodes were extracted from 20 sub-samples. Each sample weighed 100 g. Extraction was done by the Whitehead and Hemmings (1965) method as described above (3.1.8). No nematodes were recovered from the soil samples which proved the effectiveness of the soil sterilization.

3:2:4 Raising tomato seedlings.

Thirty (30) different tomato lines were germinated on tissue paper in Petri dishes and nursed in sterilized soil in the plant house.

3:2:5 Extraction of *Meloidogyne* species eggs.

Eggs were extracted from plant house cultures of *Meloidogyne* species built on susceptible tomato, Petomech, for eight weeks, using the sodium hypochlorite (NaOCl) method (Taylor and Sasser, 1978; Hussey and Barker, 1973). The extracted eggs were incubated on modified Baermann funnel (RodrÍguez - Kábana and Pope, 1981) for second stage juveniles (J2) that were used for inoculation. The procedure involved carefully uprooting infested tomato plants from pots and

washing the roots under running tap water to remove the soil particles. The roots were cut into 1.0 cm long pieces with a sharp kitchen knife on a clean wooden board and macerated with an electrical blender (SANYO brand). Hundred grams (100 g) of macerated roots was placed in a jar and enough sodium hypochlorite (NaOCl) solution added to cover the macerated roots. The NaOCl solution was prepared by taking 1 part of NaOCl and 4 parts of tap water (1:4). The jar with its content was covered tightly with its lid and vigorously shaken for about 4 minutes. The NaOCl solution containing *Meloidogyne* species eggs and the root debris was quickly poured through 200 µm sieve nested over 500 µm sieve. The 200 µm sieve was gently tapped at the side so that the eggs were washed from it into the 500 µm sieve. The residual NaOCl in the 2 sieves were rinsed several times by placing them under slow running tap water, and the eggs collected from the 500 µm sieve into a 200 ml beaker.

In order to have sufficient inocula of eggs, the process was repeated several times with the rest of the chopped and macerated infested roots. The supernatant was poured off carefully, leaving a concentrated egg suspension, which was topped with distilled water to the 200 ml beaker for easy determination of the number of eggs in the suspension.

3:2:6 Counting of second stage juveniles (J2).

The number of J2 in aqueous suspension was determined by the use of counting tray. One millilitre of the aqueous suspension was collected with a pipette, after bubbling air into the suspension, and poured into a counting dish for counting. The counting was repeated 3x and the number of J2 estimated by finding the mean of the three counts.

3:2:7 Determination of concentration of second stage Juveniles (J2) in water suspension.

To determine the number of (J2)/unit of the suspension, the suspension was stirred continuously and a pipette was used to draw 1 ml aliquot of the suspension. The 1 ml aliquot of the (J2) suspension was transferred into a Doncaster (1962) counting tray. To ensure uniform distribution of (J2) in the counting tray, enough distilled water was added and the end-point of the pipette was used to spread the aliquot evenly in the dish. The counting dish has 10 channels. Second stage juveniles (J2) in all the channels were counted. The counting process was repeated 3x, each time the suspension was well-stirred to ensure a uniform distribution of the (J2) before an aliquot was taken for counting. The counting was done under a dissecting microscope at magnification 100x, using a tally counter to ensure accuracy.

First counting (1ml) = 208

Second counting (1ml) = 210

Third counting (1ml) = 206

Total density = 208+210+206

Number of (J2) per ml = 624/3 = 208

Therefore, where the inoculum level is 250 (J2)/pot, these would be contained in 250/208 = 1.2 ml. The 500 J2 /pot inoculum level would be contained in 1.2 ml x 2 = 2.4 ml.

3:2:8 Methodology.

The 30 different tomato cultivars' were replicated 3x with 3 inoculum levels making a total of 270 treatments. The inoculum levels were 0, the control treatment, 250 and 500 J2/plant. The experiment was set-up in Completely Randomised Design (CRD). The tomato seedlings that were nursed in sterilized soil (3:2:4) were transplanted into the 270 plastic pots at two weeks after germination. Two weeks after transplanting, the seedlings were inoculated with *M. incognita* juveniles as per the inoculum levels specified above with the aid of a graduated pipette.

Figure 3.0 Set-up of pot experiment.

3:2:9 Data Collection.

Root gall index, reproduction factor (Rf = Pi/Pf), fresh and dry shoot biomass and J2/g root were determined as described above (3:1:11).

3:2:10 Harvesting of Plants.

The tomato plants were harvested 8 weeks after inoculation (WAI) by uprooting the roots from the soil. To ensure easy removal of the plants, judicious watering was done to loosen the soil to expose the knotted or otherwise roots.

The cultivars reaction to root galling was determined as follows:

Highly resistant	0 – 0.4
Resistant	0.5 – 1.0 Moderately
resistant	1.1 – 1.5
Tolerant	1.6 – 2.5 Moderately
tolerant	2.6 – 2.8
Susceptible	2.9 – 3.5
Highly susceptible	3.6 – 5.0

3:2:11 Statistical Analyses.

All the data were analyzed as described above (3:1:12).

EXPERIMENT 3

3:3 Molecular screening to detect *Mi* genes in tomato.

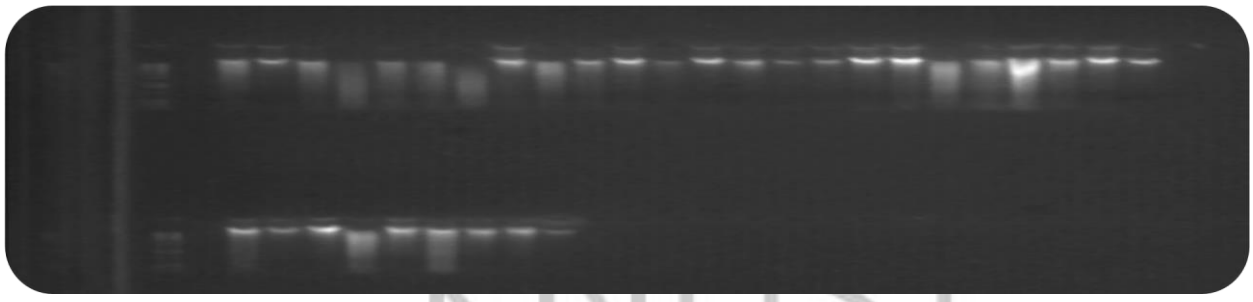
3.3.1 Tomato DNA isolation protocol.

Tomato DNA isolation, followed Egnin *et al*. (1998).

Young leaf samples were collected and placed on ice-packs prior to grinding. Both ends of the leaves were cut, with forceps, leaving about 3-5 cm long sample. Two hundred (200) milligram of the leaf sample was put into 1.5 ml eppendorf tubes and ground to fine texture soon after freeze-drying with liquid nitrogen. 800 µl Buffer A (lysis buffer) was added to each sample and incubated at 90ºC for 10 minutes, votexing every 5 minutes and cooled to room temperature for 2 minutes. Four hundred (400) µl 5M potassium acetate was added and mixed gently by inversion for 5-6 times. The samples were further incubated on ice for 30 minutes with shaking and centrifuged at 13, 000 rpm for 10 minutes. The supernatant phase was transferred into new eppendorf tubes. One (1) volume of ice-cold isopropanol, $1/10^{th}$ of 3M sodium acetate were added and mixed for 10x by inversion. The samples were further incubated at -20ºC for 1 hr. and centrifuged at 13,000 rpm for 10 minutes. Supernatant was poured off and the pellets washed with 800 µl of 80% ethanol.

The samples were further centrifuged at 14,000 rpm for 5 minutes and the DNA pellets dried at room temperature. Five hundred (500) µl 1X TE Buffer was added to dissolve the pellets. 4 µl RNase A was added and incubated at 37ºC for 30 minutes. Two hundred and fifty (250) µl of 7.5M ammonium acetate was added to each sample, incubated on ice for 30 minutes and centrifuged at 13,000 rpm for 5 minutes. The supernatant was transferred into new 1.5 ml eppendorf tubes. Seven hundred (700) µl isopropanol was added, mixed by inversion on ice and centrifuged at 13,000 rpm for 15 minutes. The supernatant was discarded and the pellets washed with 1 ml 80% ethanol. Samples were further centrifuged at 14,000 rpm for 5 minutes. The DNA pellets were dried at room temperature after the supernatant had been discarded. The pellets were dissolved in 200 µl 1X TE Buffer and the quality of the DNA checked on 0.8% agarose gel.

M-ve 1 2 3 4 5 ……24



M-ve 25……33

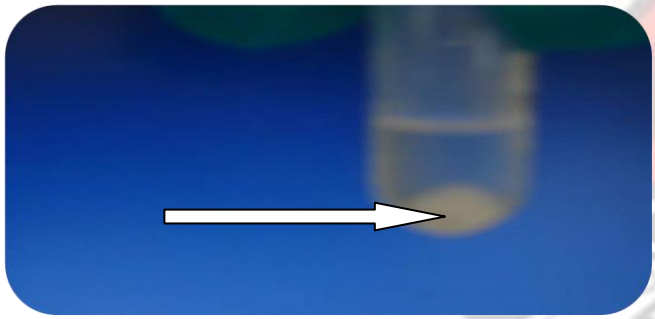


Figure 4.0 Total genomic DNA of 33 tomato cultivars.

3.3.2 PCR PROTOCOL.

DNA was extracted from fresh leaves of tomato plants by using the protocol of Egnin *et al*. (1998). PCR was carried out in 1x µl reaction containing 6.07 µl PCR water, 1.00 µl 10x PCR Buffer, 0.90 µl $MgCl_2$ (25mM), 0.40 µl dNTPs (10mM), 0.25 µl (10 µM) each for the forward and reverse primers and 0.125 µl Taq DNA polymerase. One (1) µl DNA was added to 9 µl of each reaction mix PCR tube. The tubes were then covered and placed in the thermocycler (Mycycler-BIO-RAD), using the standard-2 as indicated in the following cycles $94^{O}C$ for 3 min, $94^{O}C$ for 3 min, $57^{O}C$ for 1min, $72^{O}C$ for 1 min, $72^{O}C$ for 10 min, $94^{o}C$ for 30 sec, $57^{O}C$ for 1min, and $72^{O}C$ for 1min. These cycles were followed by a final extension of $72^{O}C$ for 10 min and held at $4^{O}C$. Amplified fragments were separated by electrophoresis through 1.5% agarose in 100

ml 1x TAE buffer, then stained with 4 µl ethidium bromide and visualized under UV light and a photograph taken.

Chapter Four

RESULTS

4.1 Results in field experiment.

4.1.1 Mean tomato plant heights (cm).

Results from Table 2.0 show the mean tomato plant height (cm) at 6 weeks old. Treatments fell within plant height range of 33 cm - 81 cm. Wosowoso and Adwoa Deede recorded the highest height (81cm) that was significantly different (P = 0.05) from the lowest height (33 cm) recorded by cultivar, 2644A. The resistant (VFNT) and susceptible (UC82) checks recorded 51 cm and 50 cm, respectively, which were not significantly different (P = 0.05) from each other but different from Wosowoso and Adwoa Deede.

4.1.2 Mean tomato plant stem girth (mm).

Table 2.0 also presents the results of the mean tomato plant stem girth (mm) at 6 weeks old. The cultivar Roma VF had the largest stem girth (1.1mm). However, this performance was not significantly different (P = 0.05) from that of the susceptible (UC82) check which recorded 1.0 mm. Strikingly, 2644A which recorded the least plant height also had the least stem girth (0.6 mm) which was significantly different (P = 0.05) from that of the susceptible check (UC82). The resistant (VFNT) check had 0.78 mm and was not significantly different from that of the susceptible (UC82) check.

Treatment	Plant height (cm)	Stem girth (mm)
FLA 496-11-6-0*	64	0.85
2641A*	64	0.85
FLA 653-3-1-0	46	0.68
Money maker	68	0.95
Adwoa Deede*	81	0.82
Roma (JAM) VF	66	0.76
Parona	64	0.93

Igph	47	0.97	Table
Akoma	66	0.90	
Terminator FI*	65	1.03	2.0
H24	47	0.92	
Roma	69	1.06	Mean
FLA 456-4	70	0.90	
Rando	73	0.97	
FLA 505 (BL1172)	74	0.81	
FLA 478-6-3-0	62	0.85	
Slumac	55	0.85	
Tima*	51	0.90	
Red cloud	53	0.90	
Wosowoso	81	0.90	
Rio grande	60	0.78	
GH Petomech	58	0.71	
Roma (VF)	44	1.13	
BK Petomech	52	0.86	
Biemso	60	0.91	
Power	66	0.87	
VFNT	51	0.78	
UC82	50	0.97	
Ventura (F)	54	0.92	
2644A*	33	0.60	
Lsd	**14.81**	**0.23**	
Cv%	**1.80**	**16.1**	

tomato plant heights (cm) and stem girth (mm) at 6 WAT.

Data are means of three replications. * Cultivars that amplified for resistance with molecular screening.

4.1.3 Mean tomato fruit number/ha, fruit yield t/ha, and fresh and dry shoot weights/plant.

Table 3.0 presents fruit number/ha, fruit yield (t/ha), and fresh and dry shoot weights (g). Significant differences (P=0.05) were observed amongst treatments in all the parameters under evaluation. Adwoa Deede recorded the highest fruit number/ha (426, 319). The treatment Igph did not yield any fruit. The resistant check (VFNT) recorded significantly higher (111, 170) fruit number/ha than the susceptible check (UC82) which recorded a paltry (26, 316) fruits/ha.

Similarly, Adwoa Deede and wosowoso, recorded significantly highest yield 5.8 t/ha and 5.4 t/ha respectively. There was significant yield difference between the resistant check (VFNT) 2.4 t/ha and the susceptible check (UC82) which recorded 0.4 t/ha.

Again, Table 3.0 indicates the mean fresh shoot weight/plant of the cultivars. Terminator F1 recorded the heaviest fresh shoot weight (521 g) which was significantly different from the check materials (VFNT and UC82) which recorded 139 g and 99 g, respectively. The mean fresh shoot weight of the resistant cultivar (VFNT) was not significantly different from that of the susceptible cultivar (UC82). BK Petomech and 2644A had the same fresh shoot weight (61 g). The least fresh shoot weight was recorded by FLA 653-3-1-0 (36 g).

Expectedly, significant differences were observed amongst treatments (P = 0.05) in dry shoot weights (Table 3.0). The mean dry shoot weight ranged between 6 and 40 g/plant. Wosowoso

recorded the highest dry shoot weight (46 g) which was significantly different from FLA 653-31-0 which recorded the least (6 g). There was no significant difference between the resistant check, VFNT (23 g) and the susceptible check, UC82 (18 g).

Treatment	Fruit No/ha	Fruit yield (t/ha)	Fresh shoot wt (g)	Dry shoot wt (g)
FLA 496-11-6-0*	21 053	3.3	104	19
2641A*	15 789	0.3	136	14
FLA 653-3-1-0	63 158	0.9	36	6
Money Maker	57 895	2.0	72	10
Adwoa Deede*	426 319	5.8	352	52
Roma (JAM) VF	89 474	0.4	150	26
Parona	63 158	1.3	228	33
Igph	0	0	94	13
Akoma	63 158	1.9	179	38
Terminator FI*	5 263	0.1	521	40
H24	21 053	0.4	81	12
Roma	210 528	4.8	335	43
FLA 456 – 4	10 526	0.3	77	14
Rando	94 737	2.1	144	41
FLA 505(BL1172)	10 526	0.2	126	20
FLA 478-6-3-0	42 105	0.7	85	16
Slumac	94 737	3.4	107	27
Tima*	57 895	1.3	124	24
Red Cloud	15 789	0.5	178	38
Wosowoso	32 631	5.4	293	46
Rio grande	15 263	0.4	126	16
GH Petomech	4 737	1.0	152	15
Roma (VF)	1 578	0.2	103	16
BK Petomech	42 105	0.7	61	19
Biemso	57 895	2 .0	186	27
Power	121 054	4.0	169	29
VFNT (R)	115 170	2.4	139	23
UC82 (S)	26 316	0.4	99	18
Ventura F	63 158	1.1	100	17
2644A*	21 053	0.5	61	10

Lsd	**21.0**	**0.4**	**110.0**	**17.0**	Table
Cv (%)	**17.5**	**8.6**	**15.4**	**14.0**	
					3.0
*Cultivars that amplified for resistance with molecular screening.					Mean fruit

number, yield, and fresh and dry shoot weight of treatments.

4.1.4. **Mean second stage juveniles (J2)/200 ml soil**.

Table 4.0 presents the mean population of second stage juveniles (J2) recovered from the tomato rhizosphere at harvest. The susceptible (UC82) recorded the highest mean population (2,508). This recovery was significantly different (P=0.05) from all the other treatments except Rando which recorded (1,765). The least mean population was recovered from the cultivar, H24 (67). The resistant VFNT recorded a significantly low mean population of 208. The six cultivars which showed amplification with primers in PCRs as resistant to the root-knot nematodes in the molecular screening (FLA 496-11-6-0, 2641A, Adwoa Deede, Terminator F1, Tima and 2644A) recorded significantly low densities; (292, 358, 341, 75, 423 and 550) respectively, and were not different from the resistant check (VFNT).

4.1.5. **Mean second stage juveniles (J2)/g root.**

Mean population of second stage juveniles (J2) recovered from gram root of indicate significant differences amongst treatments (Table 4.0). The susceptible check (UC82) had 108 which was significantly different from the highest population (1,025) recovered from FLA 456-4. This recovery was significantly different from the other treatments. VFNT (resistant check), Ventura F and 2644A were the same with no juveniles extracted from them.

4.1.6. **Mean gall index (0-5)**.

Table 4.0 also presents the results of mean gall index on a scale of 0-5. Mean gall indices scored by the cultivars ranged between 0 and 4. The maximum index of 4 was scored by the cultivars; Rio grande, Roma VF, Ghana Petomech, Burkina Petomech, Power, Slumac, FLA 456-4 and the

susceptible check (UC82). Besides the known resistant check VFNT, three cultivars which came out resistant in the molecular work, FLA 496-11-6-0, Terminator F1 and Tima did not gall at all.

Table 4.0 Mean J2/200ml soil, J2/g root and gall indices (0-5) in field trial.

Treatment	**J2/200 ml soil**	**J2/g root**	**Gall indices**
FLA 496-11-6-0*	292 (4)	16 (1)	0.0 (0.4)
2641A*	358 (4)	17 (1)	1.0 (0.5)
FLA 653-3-1-0	283 (4)	450 (5)	1.1 (0.9)
Money Maker	741 (5)	308 (4)	1.7 (0.5)
Adwoa Deede*	341 (4)	83 (1)	0.3 (0.2)
Roma (JAM) VF	675 (5)	17 (1)	0.3 (0.2)
Parona	108 (3)	150 (2)	2.3 (0.5)
Igph	460 (5)	42 (1)	2.3 (0.5)
Akoma	191 (4)	150 (2)	2.3 (0.5)
Terminator F1*	75 (3)	17 (1)	0.0 (0.4)
H24	67 (3)	92 (1)	2.3 (0.5)
Roma	225 (3)	158 (2)	3.0 (0.6)
FLA 456-4	392 (4)	1 025 (12)	4.0 (0.6)
Rando	1 765 (5)	0	0.3 (0.2)
FLA 505(BL1172)	152 (3)	17 (1)	3.0 (0.6)
FLA 478-6-3-0	635 (5)	183 (2)	2.3 (0.5)
Slumac	498 (4)	42 (1)	4.0 (0.6)
Tima*	423 (4)	0	0.0 (0.4)
Red cloud	431 (5)	442 (5)	2.0 (0.5)
Wosowoso	309 (4)	33 (1)	1.3 (0.5)
Rio grande	300 (4)	49 (1)	4.0 (0.6)
BK Petomech	117 (2)	133 (2)	4.0 (0.6)
Roma VF	316 (4)	333 (4)	4.0 (0.6)
GH Petomech	725 (5)	333 (4)	4.0 (0.6)
Biemso	309 (4)	25 (1)	1.7 (0.6)
Power	474 (5)	17 (1)	4.0 (0.6)
VFNT (R)	208 (4)	0	0.0 (0.4)
UC82 (S)	2 508(6)	108 (2)	4.0 (0.6)
Ventura F	417 (4)	0	1.3 (0.3)
2644A*	550 (4)	0	1.0 (0.1)
Lsd	**1562.5 (2.2)**	**401.8 (13.8)**	**3.0 (0.4)**
Cv%	**13.1 (4.5)**	**13.5 (13.3)**	**20.0 (42.2)**

In (x+1) and √(x+0.5)
transformed data used
in ANOVA
parenthesis.

4.2 Results of pot experiment.

4.2.1 Mean gall index of tomato.

Table 5.0 presents the results of mean gall index of initial populations of (250) and (500) second stage juveniles (J2) per plant. The analysis of (Pi = 0) has not been presented since no galls were recorded. The following cultivars, FLA 496- 11-6-0, 2641A, 'Adwoa deede', Parona, Rio grande, and VFNT (resistant check) did not gall (0) at Pi = 250. 'Power' had the highest index (4.3). The susceptible check (UC82) recorded a mean gall index of 2.0 at (Pi = 250). When tested at a higher inoculum level (Pi = 500), seventeen cultivars recorded the highest mean gall index of 5.0. The susceptible check UC82 recorded 4.7 which was not different from the other seventeen cultivars.

Table 5.0 Gall index of tomato (0-5) in pot experiment.

Treatment	**Pi = 250**	**Pi =500**
FLA 496-11-6-1-0*	0.0	0.0
2641A*	0.0	0.0
FLA 653-3-1-0	1.7	5.0
Money maker	1.3	5.0
Adwoa deede*	0.0	0.7
Roma (JAM) VF	2.0	5.0
Parona	0.0	0.7
Igph	1.3	4.0
Akoma	1.0	5.0
Terminator FI*	1.7	0.0
H24	1.0	5.0
Roma	2.7	5.0
FLA 546-4	2.3	5.0
Rando	1.7	5.0
FLA 505 (BL 1172)	1.0	5.0
FLA 478-6-3-0	2.0	5.0
Slumac	1.7	5.0
Tima*	1.0	0.3
Red cloud	2.3	5.0
Wosowoso	2.0	0.3
Rio grande	0.0	5.0
GH Petomech	2.3	5.0
Roma (VF)	2.3	5.0
BK Petomech	2.7	5.0
Biemso	3.0	0.0
Power	4.3	5.0
VFNT	0.0	0.0
UC82	2.0	4.7
Ventura F	2.3	4.7
2644A*	4.3	4.7
Lsd	**0.9**	**0.5**
Cv%	**5.3**	**1.0**

*Cultivars that amplified for resistance with molecular Screening

4.2.2 Mean fresh and dry shoot weights (g).

Table 6.0 indicates the mean fresh and dry shoot weights over the three initial population levels Pi (0, 250, and 500)/plant. At the highest inoculums level (Pi=500), 'Money maker' recorded the highest fresh and dry shoot weights (19.1 g) and (4.7 g) respectively. Roma recorded the lowest fresh shoot weight (3.8 g) while FLA 478-6-3-0 recorded the lowest dry shoot weight (0.3 g).

Table 6.0 Mean fresh and dry shoot weights in pot experiment

	Fresh weight			Dry weight		
Treatment	**Pi 0**	**Pi 250**	**Pi 500**	**Pi 0**	**Pi 250**	**Pi 500**
FLA 496-11-6-0*	9.1	9.1	9.1	3.5	3.9	3.7
2641A*	13.0	13.9	13.7	3.3	3.0	1.7
FLA 653-3-1-0	17.4	14.0	8.0	3.9	3.7	1.2
Money Maker	27.3	21.1	19.1	4.8	4.2	4.7
Adwoa Deede*	12.6	12.5	12.5	4.6	3.4	3.3
Roma (JAM) VF	17.5	15.9	12.6	3.4	3.3	1.1
Parona	10.4	6.6	6.6	3.9	3.3	0.9
Igph	11.6	11.6	6.5	2.4	1.7	1.5
Akoma	10.3	7.0	6.4	3.1	2.6	1.3
Terminator FI*	9.2	9.2	9.0	3.3	3.1	3.3
H24	17.6	11.9	9.2	1.6	1.2	0.9
Roma	12.2	8.2	3.8	3.1	2.6	2.2
FLA 456 – 4	7.5	6.9	5.8	4.4	3.3	0.7
Rando	18.8	16.3	12.4	1.5	1.1	0.7
FLA 505(BL1172)	12.2	8.2	7.9	2.3	1.7	0.9
FLA 478-6-3-0	8.9	7.5	6.8	3.3	2.7	0.3
Slumac	10.5	9.5	8.2	2.5	2.4	1.9
Tima*	16.3	16.2	16.2	2.7	2.6	2.4
Red Cloud	5.7	4.8	4.6	2.3	1.8	1.2
Wosowoso	9.3	7.2	4.8	2.6	2.3	2.0
Rio grande	13.1	6.7	5.1	3.6	3.6	2.2
GH Petomech	10.8	9.7	7.3	3.0	1.3	1.0
Roma (VF)	16.7	13.3	8.6	3.2	1.6	1.3
BK Petomech	11.5	10.2	9.3	3.1	2.4	1.6
Biemso	11.8	8.2	6.0	2.1	1.0	0.5

Power	15.7	9.8	7.1	3.8	2.5	1.2
VFNT	8.7	8.7	8.7	3.1	3.0	3.0
UC82	11.8	10.6	7.1	3.1	1.9	0.9
Ventura F	17.2	15.1	12.3	3.3	2.4	1.2
2644A*	12.7	12.6	12.6	3.1	3.1	3.1
Lsd	**2.4**	**4.5**	**7.4**	**1.3**	**0.5**	**3.2**	**1.5**	**0.4**
Cv%	**2.4**			**1.6**			**13.7**	**2.7**

*Cultivars that amplified for resistance with molecular screening.

4.2.3 Reproduction factor.

Table 7.0 presents the results of reproduction factor (Rf=Pf/Pi) of the root knot nematodes on the cultivars under evaluation in the pot experiment. At the highest initial population density level (Pi=500), the susceptible check (UC82) had the highest Rf (56.6) which was signficantly different (P=0.01) from all the other cultivars. The pest could not reproduce on the resistant cultivar (VFNT). The reproduction factor was therefore 0.

Table 7.0 Reproduction fator (Rf = Pf/Pi) in pot experiment.

Treatment	**Pi = 250**	**Pi = 500**
FLA 496-11-6-1-0*	0.7 (0.4)	0.9 (0.5)
(2641A)*	0.0	0.0
FLA 653-3-1-0	3.5 (1.9)	1.4 (1.1)
Money maker	0.7 (0.4)	1.8 (1.1)
Adwoa Deede*	0.3 (0.2)	0.3 (0.2)
Roma JAM (VF)	5.2 (2.3)	8.6 (1.7)
Parona	2.9 (1.7)	2.6 (1.2)
Igph	5.7 (1.7)	2.3 (1.2)
Akoma	2.7 (1.7)	1.7 (1.1)
Terminator F1*	0.3 (0.2)	0.5 (0.3)
H24	38.3 (4.2)	2.4 (1.2)
Roma	4.8 (2.3)	5.7 (1.7)
FLA 456-4	3.7 (1.9)	2.4 (1.2)
Rando	4.3 (2.0)	2.4 (1.2)
FLA 505 (BL 1172)	3.5 (1.9)	2.4 (1.2)
FLA 478-6-3-0	2.8 (1.7)	3.5 (1.7)
Slumac	8.6 (2.1)	5.6 (1.5)
Tima*	0.0	1.1 (0.4)
Red cloud	3.8 (1.9)	3.4 (1.3)
Wosowoso	2.2 (1.4)	3.0 (1.3)
Rio grande	2.9 (1.7)	3.3 (1.4)
GH Petomech	10.0 (2.9)	6.3 (1.6)
Roma (VF)	10.5 (3.0)	0.9 (0.5)
BK Petomech	10.5 (3.1)	6.3 (1.6)

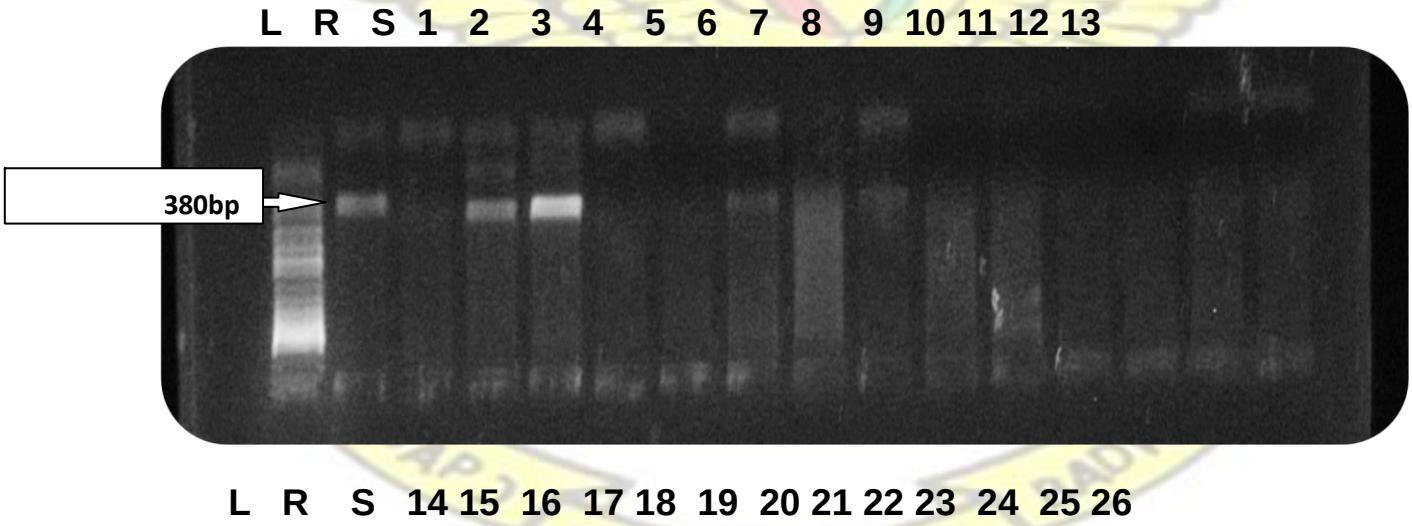
Biemso	2.6 (1.6)	1.9 (1.2)
Power	9.2 (2.9)	8.6 (1.7)
VFNT (Resist. check)	0.3 (0.3)	0.0
UC82 (Suscept. Check)	4.2 (2.0)	56.6 (2.4)
Ventura F	2.3 (1.5)	2.9 (1.3)
2644A*	0.3 (0.3)	0.3 (0.2)
Lsd	**20.3 (2.1)**	**17.0 (0.6)**
Cv%	**28.8 (9.1)**	**49.9 (7.3)**

√ (x+0.5) transformed data used in ANOVA in parenthesis.

*Cultivars that amplified for resistance with molecular screening.

4.3 Results of molecular work

4.3.1 Polymerase chain reaction products.



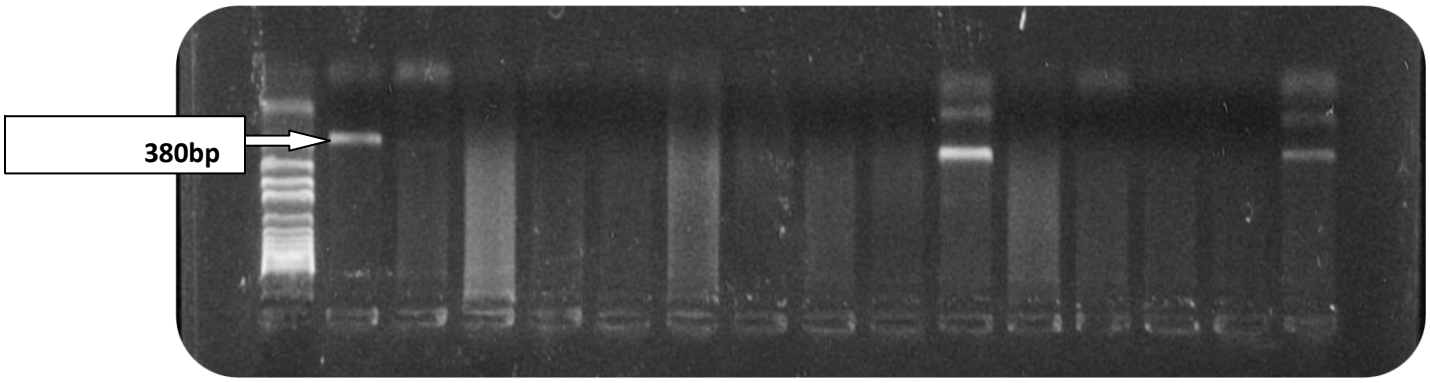


Fig. 5.0 PCR amplification to detect tomato *Mi* gene using Egnin *et al.*, 1998 DNA extraction method and primers Mi23F and Mi23R. From left: L (200 bp ladder), R (resistant cv. VFNT), S (susceptible cv. UC82), 1 (FLA 505-BL 1172), 2 (2641A), 3 (Wosowoso), 4 (FLA 496-11-6-0), 5 (Adwoa deede), 6 (TLB111), 7 (Terminator FI), 8 (3008A), 9 (Roma-JAM VF), 10 (BK Petomech), 11(Roma VF), 12 (Ventura F), 13 (Slumac), 14(Red Cloud), 15 (Rando), 16 (Akoma), 17 (GH Petomech), 18 (Floradade), 19 (FLA 478-6-3-0), 20 (Money maker), 21 (Tima), 22 (Rio grande), 23 (Parona), 24 (Biemso), 25 (Power), 26 (2644A).

Table 8.0 Binary table Absent = 0, Present = 1

CULTIVARS (26)	SCORE
2644A	1
FLA 505 (BL 1172)	1
WOSOWOSO	0
FLA 496-11-6-1-0	0
ADWOA DEEDE	1
TLB 111	0
TERMINATOR FI	1
3008A	0
ROMA (JAM) VF	0
BK PETOMECH	0
ROMA VF	0
VENTURA F	0
SLUMAC	0
RED CLOUD	0
RANDO	0
AKOMA	0
GH PETOMECH	0
FLORADADE	0
FLA 478-6-3-0	0
MONEY MAKER	0

TIMA	1
RIO GRANDE	0
PARONA	0
BIEMSO	0
POWER	0
2641A	1

9.0 Estimation of heterozygotes.

Genotypes	(*RR*), (*Rr*)	*Rr*	Total	Allele freq.
Genotype freq. (expected)	$p^2 + 2pq$	q^2	1	p=0.12
Number of individuals	6	20	26	q=0.88
Genotype freq. (observed)		$q^2 = 0.77$		1

Genotype frequency of susceptible cultivars (rr) $q^2 = 0.77$

Frequency of recessive allele $q = \sqrt{0.77} = 0.88$ p

+ q = 1, therefore p = 1 - 0.88 = 0.12

Expected number of heterozygous (*Rr*) can be estimated as follows:

2pqN, where N = sample size, 2(0.12) (0.88) (26) = 5.49 = 5

Chapter Five

DISCUSSION

Generally, root-knot nematode resistance or tolerance is tested by measuring plant performance and rating symptoms, such as root galls. Because growers are interested in yield and quality of products, this is an important criterion. However, the rate of nematode reproduction should also be determined.

Good plant performance in the presence of parasitic nematodes may result from plant resistance or tolerance. It could be reasoned that, a resistant variety is a poor host and does not support high nematode populations. Many levels of plant resistance occur, varying from immune plants on

which no nematodes develop to those supporting high populations as in susceptible varieties (Kehr, 1966). A tolerant variety is a good host that expresses low susceptibility.

The vigour of a plant influences resistance to nematodes (Kehr, 1966). In the current work, varieties 'Wosowoso' and 'Adwoa Deede' demonstrated resistance potential by recording the highest plant height (81cm) in the field, significantly low population per 200 ml soil, J2/g root and gall index.

The most common reaction of nematodes to resistant plants may be failure of all or a high percentage of females to develop to maturity even though infective stages of nematodes penetrate plant tissue (Kehr, 1966). In most cases, development of females does not proceed further than the third stage. Plant resistance may cause not only slower nematode development but also production of fewer eggs by females.

Substances given off by plant, which stimulate hatching of nematode eggs or attract infective juveniles to roots, may not necessarily be related to resistance of plants to nematodes (Jones, 1956). Stimulants or attractants from roots of immune or resistant plants are sometimes more potent than such substances from susceptible ones (Jones, 1956).

It was observed from this study that, the susceptible check (UC82) recorded the highest J2/200 ml soil which was significantly different from all the cultivars studied in the field. Again the susceptible check, recorded a significantly high J2/g root compared with the resistant check (VFNT), which recorded no J2/g root. A similar trend was observed in mean gall index. Nematodes penetrate roots of most resistant plants, but often in smaller numbers than they do in roots of susceptible plants (Robinson, 1980).

The resistant check (VFNT), and three other cultivars identified as resistant materials in the molecular screening, FLA 496-11-6-0, 2641A and "Adwoa Deede" did not gall in the pot experiment. Nematode populations in the root region of resistant plants sometimes decline at a more rapid rate than can be explained by starvation and it is presumed that toxins of plant origin are responsible (Vargas *et al*., 1996). The other suppressive mechanism could be the formation of wound periderm or corky layers, which wall off and retard the development of nematodes in plant tissue (Hijink and Oostenbrink, 1968).

FLA 496-11-6-0, Adwoa Deede and Tima, amongst the resistant cultivars identified, recorded significantly high fruit yield (3.3, 5.8, and 1.3 t/ha) respectively. The susceptible check (VFNT) recorded significantly low fruit yield (0.4 t/ha) principally due to the effect of nematode parasitism. The resistant check, VFNT, did not yield as high as three of the resistant cultivars identified in this study, suggesting that there were better cultivars in the collections.

Most of the cultivars decreased significantly in fresh and dry shoot weights (g) with increasing population density. However, the six cultivars identified as resistant; FLA 496-11-6-0, 2641A, Adwoa Deede, Tima, Terminator F1 and 2644A did not decrease significantly in fresh and dry shoot weights.

Using DNA extracted according to the protocol of Egnin *et al*. (1998), primers M23/F and M23/R amplified a 380-bp DNA fragments for the resistant allele. PCR products of the cultivars FLA 505-BL 1172, 2641A, Adwoa Deede, Terminator FI, Tima and 2644A in the lanes 1, 2, 5, 7, 21, and 26 lanes, respectively, amplified 380-bp expected of resistant tomato genotypes to the Mi-1 locus with primers M23/F and M23/R. They amplified the same locus with the resistant check (VFNT) as expected. Their resistance was confirmed through their performances in both the pot

and field experiments. In another study, the homozygous resistant line carrying the Mi gene, the homozygous susceptible line B and 13 F_2 plants from a crossing of line A and line B were used to test resistance to *M. javanica.* Following bioassay, 11 resistant and 2 susceptible strains were determined. The resistant parent (line A) and the resistant F_2 plants had a mean gall index of 1. The susceptible parent (line B) and the susceptible F_2 plants had a gall index that averaged 5 (Devran and Elekçioğlu, 2004). In the current work, the resistant cultivars identified and the resistant check (VFNT) had mean gall indices of 0.5 and 0.2 in the field and pot experiments, respectively. The susceptible cultivars identified and the susceptible check (UC82) also had average gall indices of 4.0 and 4.9 in the field and pot experiments, respectively.

In molecular screening of two parents (A and B) and 13 individuals with CI/2 and C2S4 primer combination, the DNA banding patterns of PCR amplification products correlated well with the known resistant or susceptible phenotype. The parents and their F_2 progenies were examined with the PCR-based primers REX-F2 and REX-R2. One major DNA band (approximately 750bp) was amplified for resistant and susceptible plants. In the same study, when Mi gene specific primers (1/2 and C2S4) were used; resistant and susceptible plants were distinguished from each other whereas resistant heterozygous individuals were not distinguished (Williamson *et al*., 1994).

In the current work, the resistant and susceptible checks were distinguished from each other whilst heterozygous (M/mi) and homozygous (Mi/Mi) resistant cultivars were not distinguished. When data analysis was done via Binary Table following the Hardy-Weinberg principle in population genetics, (1 = present and 0 = absent) with the formula 2pqN; where p = resistant allele, q = susceptible allele and N = sample size; 5 heterozygous individuals were determined out of the 6 resistant cultivars identified in the molecular screening.

According to Seah *et al*. (Unpublished data), the susceptible genotypes M82-1-8 and Gh13 (mi/mi) and the resistant genotypes Motelle and Gh2 (Mi/Mi) gave PCR fragments 430-bp and 380-bp, respectively, as expected of susceptible and resistant genotypes. In the current work, the resistant check VFNT also gave the same PCR fragment of 380-bp as expected of resistant cultivars. The susceptible check (UC82) however, did not amplify, presumably because of nonspecificity at the primer-binding sites or errors in PCR conditions (Palumbi *et al.*, 1991).

When six commercial hybrids (Celebrity, Charanta, Crista, Dominique, Tequila and Viva Italia) with reported resistance to root-knot nematodes were tested with the primers Mi23/F and Mi23/R, all of them (hybrids) had the three banded pattern associated with heterozygous plants for the *Mi-1* locus and Rodeo gave the expected single 380-bp, fragment for the homozygous resistant genotypes (Mi/Mi) (Seah *et al*., unpublished data).

When the same primers (Mi23/F and Mi23/R) were used in this study, two out of the six resistant cultivars identified in the molecular screening also had the three banded pattern associated with heterozygous plants for the *Mi-1* locus while the resistant check (VFNT) gave the expected single major band (380-bp).

Chapter Six

CONCLUSION

The six resistant tomato cultivars - FLA 505-BL 1172, 2641A, "Adwoa deede", Terminator FI, Tima and 2644A, identified in the molecular screening correlated well with their resistant phenotypes in both the field and pot experiments.

FUTURE RESEARCH

The high cost of direct control measures and persistence of nematodes in the soil point to the need for emphasis on breeding resistant varieties of plants. The development of many plant varieties with nematode resistance will result in untold benefits to growers.

There is the need to expand the tomato germplasm base through introductions from other regions and screening for resistance to root-knot nematodes by Marker-Assisted-Selection (M.A.S) must be advocated and intensified. There is also an urgent need for introgression of resistance genes into the backgrounds of promising but susceptible lines that are adaptable to our local conditions.

The biochemical basis for resistance in plants to nematodes has been little studied. Research in this area should aid in developing resistant varieties of plants.

Bibliography

Abu-Garbieh, W.I., Saleh, H.M. and Abu-Blan, H.A. (1987). Effect of solarization using different thickness of black plastic on soil-borne pathogens. Workshop, *Advances in Soil Plastic technology*. Amman, Jordan; 25.

Addoh, P.G. and Amanquah, S. Y. (1968). The effectiveness of Temik, a systematic pesticide for controlling root-knot nematodes and its influence on the growth and seed yield of kenaf (*Hibiscus cannabis* L). *Ghana Journal of Agricultural Science*. 1: 55-57.

Addoh, P.G. (1970). The root-knot nematode problem in Ghana: host and non-host plants of *Meloidogyne* species. *Ghana Journal of Agricultural Science.* 3: 3-12.

Addoh, P.G. (1971). The distribution and economic importance of plant-parasitic nematodes in Ghana. *Ghana Journal of Agricultural Science*. 4: 21-32.

Agrama, H.A. and Scott, J.W. (2006). Quantitative trait loci for Tomato yellow leaf curl virus and Tomato mottle virus resistance in tomato. *Journal of American Society of Horticultural Science*. 131: 267-272.

Ahuja, S. (1979). The effect of root-dip treatments on infestation of brinjal by *Meloidogyne incognita*. *Nematologica Mediterranea,* 6: 133 - 134.

Alam, M.M. (1991). Control of plant parasitic nematodes with oil seed cakes on some vegetables in field. *Pakistan Journal of Nematology*, 9: 21-30.

Altieri, M.A. (1984). Pest management technologies for peasants: farming system approach. *Crop Protection*. 3: 87-94.

Altieri, M.A. (1991). Increasing biodiversity to improve insect pest management in agro ecosystems. In: *The biodiversity of microorganisms and invertebrates: Its role in sustainable agriculture*. ed. Hawksworth, D.L. Wallingford, UK: CAB Int. 300. 14: 165-182.

Amosu, J. O. (1976). Report on *Meloidogyne* Research at University of Ife, Ile-Ife. *Proceedings of the research planning conference on root-knot nematodes*, Meloidogyne *species*. 38-40.

Anon (2002). Protection of Stratospheric Ozone: Incorporation of Clean Air for Reductions in Class 1 group controlled substances. Federal Register. 65: 795-804.

Anwar, S.A.; Rauf, C.A. and Gora, S.D. (1992). Weeds as alternate hosts of phytonematodes. *Afro-Asian Journal of Nematology*. 2: 41-47.

Arujo, M.T; Dickson, D.W.; Augustine, J.J. and Bassette, M.J. (1983). Reproduction of two races of *Meloidogyne incognita* in tomato plants grown at high temperature. *Journal of Nematology*. 15: 640-641.

Atu, G. and Ogbuji, R. O. (1986). Root-knot nematode problems with intercropped yam (*Dioscorea rotundata*). *Phytoprotection*, 67: 35-38.

Barker, K.R. (1978). Determining nematode population responses to control agents. In: Methods for evaluating plant fungicides, nematicides and bactericides. Zerh, E. (ed.). Pp. 114-125. American Phytopathological Society, St. Paul, Minnesota.

Barton, K.A. and Bill, W.J. (1983). Prospects in plant genetic engineering. *Science*. 219: 671-675.

Berthou, F.; Ba-Diallo, A. and Guiran, G. (1989). Caractèrisation chez les nèmatodes *Meloidogyne* Goeldi (Tylenchida) de types virulents vis á_vis du gène *Mi* dela tomate dans zones maraichères au Sènègal – *Agronomie*, 9: 877-885.

Bhatti, D. S. and Jain, R. K. (1977). Estimation of loss of okra, tomato and brinjal yields due to *Meloidogyne incognita*. *Indian Journal of Nematology*, **7**: 37-41.

Birchfield, W. and Antonpoulus, A. A. (1976). Scanning electron microscope observations, *Duboscquia penetrans* parasitizing root-knot larvae. *Journal of Nematology*, 8: 277-283.

Bridge, J. (1996). Nematode management in sustainable and subsistence Agriculture. *Annual Review of Phytopathology***,** 34: 201-205.

Bridge, J. and Page, S. L. J. (1982). Plant nematodes in Papua New Guinea: Their importance to crop plants. *Report of a plant nematode survey in Papua New Guinea*. Commonwealth Institute of Parasitology, St. Albans, UK: CAB, 99.

Bridge, J. (1987). Control strategies in subsistence Agriculture. In: *Principles and Practice of Nematode Control in Crops*, ed. R.H. Brown, B. K. Kerry, New York: *Academia*. 12: 389-420.

Brown, S.M. and Smart, G.C. (1985). Increased crop yields following application of *Bacillus penetrans* to field plots infested with *Meloidogyne incognita*. *Soil Biological Biochemistry*. 17: 483-486.

Cap, G.B.; Roberts, P.A.; Thomason, I.J. and Murashig, T. (1991). Embryo culture of *Lycopersicon esculentum* X *L. peruvianum* hybrid genotypes possessing heat-stable resistance to *Meloidogyne incognita*. *Journal of America Horticultural Science*. 116: 1082-1088.

Canto-Sánez, M. and Brodie, B.B. (1984). The nature of potato incompatible response to *Meloidogyne incognita* (Kofoid and White 1919) Chitwood 1949 and correlation between root and tuber resonse. *Journal of Nematology*. 16: In press.

Caryol, J.C. (1983). Lutte biologique contres les *Meloidogyne* au moyen *d'Aethrobotrys irregularis. Revue de Nématologique,* 6: 265-273.

Caryol, J. C. and Frankowski, J. P. (1979). Une mèthode de lute biologique contre les némathodes á galle des racines appurtenant au genre *Meloidogyne. P*èpinièristes *Horticulteur MaraÏchers. Revue Hortilcole,* 143: 15-23.

Castognone-Sereno, P. (2002). Genetic variability in parthenogenetic root-knot nematodes *Meloidogyne* spp. and their ability to overcome plant resistance genes. *Nematology*. 4(5): 605-608.

Caveness, F.E. and Ogunfowora, A.O. (1985). Nematological studies worldwide. In: *Cowpea: Research, Production and Utilization*. ed, S.R. Singh and K.O. Rachie. 273-285.

Caveness, F.E. (1978). Root-knot nematodes on selected food crops in Nigeria. In: *proceedings of the 2nd IPM Research Planning Conference on* Meloidogyne *species*, February 20-24 1978, Abidjan, Ivory Coast, 70-78.

Chitwood, D.J. (2002), Phytochemical based strategies for nematode control. *Annual Review of Phytopathology*. 40: 221-249.

Choudhury, B. C. (1981). Root–knot problem on various crop plants in Bangladesh. *Proceedings of the Third Research Planning Conference on* Meloidogyne *species*. *Region VI*, July 20-24, Jakarta: 142-145.

Christie, J.R. (1949). Host parasite relationship on the root-knot nematodes. *Meloidogyne* spp. 111. The nature of resistance in plants to root-knots. *Proceedings. Helminthological Society*. Washington. 16: 104-108.

CIH. (1978). Commonwealth Institute of Helminthology. Description of Plant Parasitic Nematodes. Sets 1–8. CAB International, Wallingford, UK.

Clerk, G.C. (1974). Crops and their diseases in Ghana, University of Ghana, Legon. Ghana Publishing Corporation.

Colon, F. M.; Ayda, A. and Cueba, D. (1972). Preliminary results of experiment with nematicides for the control of nematodes attacking tomato (*Lycopersicon esculentum* Mill) in sandy soils. *Helminthology*. 1145. Abstract.

Cook, R. and Evans, K. (1987). Resistance and Tolerance. In: *Principles and practices of nematodes control in crops*, ed. Brown R.H. and Kerry B.R. 12: 179-231.

Cook, R. (1974). Nature and inheritance of nematode resistance in cereals. *Journal of Nematology*. 6: 165-174.

Culbreath, A.K.; Rodriguez-Kabana, R. and Morgan-Jones, G. (1985). The use of hemicellulosic waste matter for reduction of the phototoxic effect of chitin and control of root-knot nematodes. *Nematropica.* 15: 49-75.

David, R.G. and Triantaphyllou, A.C. (1967). Influence of environment and sex differentiation of root-knot nematodes. (I). Effect of infective density, age of the host plant and soil temperature. *Nematologica*. 10: 102-110.

Deberdt, P.; Quénéhervé, P.; Darrase, A. and Prior, P. (2003). Increased susceptibility to bacterial wilt in tomatoes by nematodes galling and the role of the *Mi* gene in resistance to nematodes and bacterial wilt. *Plant Pathology*. 48: 408-4.

Devran, Z. and Elekçioğlu, H.I. 2004. The screening of F2 plants for the root knot resistance gene, Mi by PCR in tomato. *Turkish Journal of Agriculture*. 28: 253-257.

Dickson, D.W. and De Waele, D. (2005). Nematode parasites of peanuts. In: *Plant parasitic nematodes in subtropical and tropical agriculture*. (2nd edition). eds. Luc, M.; Sikora R.A. and Bridge, J. CABI Wallingford, UK. 393-436.

Doncaster, C.C. (1962). Counting dish for nematodes. *Nematologica*. 7: 334-336.

Dropkin, V.H.; Helgeson, J.B. and Upper, C.D. (1969). The hypersensitivity reaction of tomato resistance to *Meloidogyne incognita*: Reversal by cytokinins. *Journal of Nematology*. 1: 55-61.

Dube, B and Smart, Jr.G.C. (1987). Biological control of *Meloidogyne incognita* by *Paecilomyces lilacinus* and *Pasteuria penetrans*. *Journal of Nematology*, 9: 222-227.

Duc, T. M. (1980). *Système de cultures irrguèes á partir des eaux sous-terraines*. Collection C.I.E.H.-CEFIGRE. Bamako, February, 1980.

Dunay, M. C. and Dalmasso, A. (1985). Multiplication de *Meloidogyne javanica*, *M. incognita*, et *M. arenaria* sur divers *Solanum*. *Revue de Nématologie*, 8: 312-334.

Duo, F.D. (1970). Climatic influence on the distribution pattern of plant parasitic and soil inhabiting nematodes. *Meded. Landbouhogesch. Wageningen*. 70-72: 64-80.

Egnin, M.; Mora, A. and Prakash C.S. (1998). Factors enhancing *Agrobacterium tumefaciens* mediated gene transfer in peanut (*Arachis hypogaea* L.). *In vitro cellular and development of biology-plants.* 34: 310-318.

Egunjobi, O. A. (1992). The place of cultural strategies in the management of Africa nematode problems of food crops. *Paper presented at the 1st Regional Symposium on the Biology and Control of Nematode pests of food crops in Africa*, University of Ibadan, Nigeria, July 26-29, 1992.

Egunjobi, O.A.; Akonde, P.T. and Caveness, F.E. (1986). Interaction between *Pratylenchus safaensis*, *Meloidogyne javanica* and *Rotylenchulus reniformis* in sole and mixed crops of maize and cowpea. *Review of Nematologica*. 9: 61-70.

Elekçioğİu, Ĩ.H. and Devran, Z**.** (2004). The screening of F_2 plants for the root-knot nematode resistance gene *Mi* by PCR in tomato. *Turkish Journal of Agriculture*. 28: 253-257.

EI Mehrach, K.; Mej**i**a, L.; Gharsallah-Couchane, S.; Salus, M.S.; Martin, C.T.; Hatimi, A.;

Vidavsky, F.; Williamson, V. and Maxwell, D.P. (2005). PCR-methods for tagging the *Mi-1* locus for resistance to root-knot nematode in begomovirus-resistant tomato germplasm. *Acta Horticulturae* 695: 263-270.

Fassuliotis, G. (1967). Species of *Cucumis* resistant to the root-knot nematode, *Meloidogyne incognita acrita*. *Plant Disease Reporter*. 51: 720-723.

Fassuliotis, G. and Dukes, P.D. (1972). Disease reactions of *Solanum melongena* and *S. sisymbrifolium* to *Meloidgyne incognita* and *Verticillium alboatrum*. *Journal of Nematology*. 4: 222.

Fassuliotis, G. (1979). Plant breeding for root-knot nematode resistance. In: *Root-knot nematodes* (*Meloidogyne* species) *systematics, biology and control*. ed. Lambert, F. and Taylor, C.E. 425-453 pp.

Fassuliotis, G. and Bhatt, D.P. (1982). Potential of tissue culture for breeding root-knot nematodes resistance into vegetables. *Journal of Nematology*. 14: 10-14.

Fassuliotis, G.; Deakin, J.R. and Hoffman, J.C. (1970). Root-knot nematode resistance in snap beans: breeding and nature of resistance. *Journal of American Society of Horticultural Science.* 95: 640-645.

Fawole, B. and Mai, W.F. (1979). Influence of plant age, height intensity, nematode inoculum levels and their interactions on tomato growth and reproduction of *Meloidogyne hapla*.

Journal of Nematology. 11: 199-201.

Ferris, H. (1978). Development of nematode damage functions and economic thresholds using *Meloidogyne incognita* on tomatoes and sweet potatoes. *Journal of Nematology*. 10: 286-

287.

Ferry, R.L. and Dukes, P.D. (1984). Feasibility of breeding bell peppers (Capsicum annum) for resistance to the southern root-knot nematode, *M. incognita*. *Hortscience*. 19: 545

(Abstr).

Fourie, H.; Zijlstra, C. and McDonald, A.H. (2001). Identification of root-knot nematodes species occurring in South America using SCAR-PCR technique. *Nematology*. 3: 675-

680.

Fox, J.A. and Miller, L.I. (1973). Comparison of gall and egg-mass indices of two races of *Meloidogyne incognita* on differential host. *Phytopathology*. 63: 801. (Abstract).

Gaur, H. S. and Perry, R.S. (1991). The use of soil solarization for control of plant-parasitic nematodes. *Nematological Abstract*. 60(4): 153-167.

Giamalba, M.J.; Martin, and Hernandez, T.P. (1963). Sweet potato variety to species and races of root-knot nematodes. *Phytopathology*. 53: 1187-1189.

Gill, J.S. (1976). Studies on *Heterodera avenae*, the causal organism of "Molya" disease of wheat and barley. Ph. D Thesis, Punjab University, India.

Gilbert, J. C. and McGuire, D. C. (1956). Inheritance of resistance to severe root-knot from

Meloidogyne incognita in commercial type tomatoes. *American Society of Horticultural Science Proceedings*, 68: 437-442.

Golden, A.M. and Shafer, T. (1958). Unusual response of *Heperis matrnalis* L. to root-knot nematodes (*Meloidogyne species*). *Plant Disease Reporter*. 42: 1163-1166.

Hadisoeganda, W.W. and J.N. Sasser. (1982). Resistance of tomato, bean, southern pea and garden pea cultivars to root-knot nematodes based on host susceptibility. *Plant Disease*, 66: 145-150.

Hare, W.W. (1957). Inheritance of resistance to root-knot nematodes in pepper. *Phytopathology*, 47: 455-459.

He, G.; Meng, R.; Newman, M.; Guoqing, G.; Pittman, R.N. and Prakash, C.S. (2003). Microsatellites as DNA markers in cultivated peanut. *BMC Plant Biology*. 3: 381-390.

Heer, J.A.; Knap, H.T.; Mahalingam, R.; Shipe, E.R.; Rao-Arelli, A.P. and Mathews, B.F. 1998. Molecular markers for resistance to multiple races of soybean cyst nematode (SCN) as applied to advanced germplasm. *Molecular Breeding*. 4: 359-367.

Hemeng, B.M.S. (1995). Organic matter amendment. A potential for nematode control. *Proceedings of seminar on organic and sedentary agriculture*. MOFA, Ghana and GTZ, Accra. p 26-28.

Hemeng, O. B. (1980). Efficacy of selected nematicides for the control of root-knot nematodes, *Meloidogyne* species in Ghana. *Ghana Journal of Agricultural Science*. 13: 37-40.

Hijink, M.J. and Oostenbrink, M. (1968). Vruchtwisseting ter bestrijdjing Van plantenziekten en plagen. Versl. Meded. Plziektent Dienst. Wageningen, Sep. Ser. 368. 7.

Holtzman, O.V. (1965). Effect of soil temperature on resistance of tomato to root-knot nematode, *Meloidogyne incognita*. *Phytopathology* 55: 990-992.

Howard, H.W. (1970). Eelworm resistant varieties. In: *Plant Nematology*, ed. J.F. Soutley, S. Chand and Co. New Delhi, 262-268.

Huang, C.C.; Cui, Y.Y.; Weng, C.R.; Zabel, P. and Lindhout, P. (2000). Development of diagnostic PCR markers closely linked to the tomato powdery mildew resistance gene *OI-1* on chromosome 6 of tomato. *Theoretical and Applied Genetics*. 101: 918-924.

Hussey, R.S. and Janssen, G.J.W. (2002). Root-knot nematodes, *Meloidogyne* species. In. Plant Resistance to Parasitic Nematodes. ed. Starr, J.L.; Cook, R. and Bridge, J. CABI Publishing.

Hussey, R.S. and Barker, K.R. (1973). A comparison of methods for collecting inocula for *Meloidogyne* species, including a new technique. *Plant Disease Reporter*. 57: 1025-1028.

IFAS (1989) - Institute of Food and Agricultural Sciences, (1989). Entomology and Nematology Department, and Cooperative Extension Service, University of Florida, Gainesville. *Florida Nematode Control Guide.* 138.

Jagdale, G.B.; Pawar, A.B. and Darekar, K.S. (1985). Studies on the control of root-knot nematodes. *International Nematology Network Newsletter*. 2(1): 10-13.

Jaffe, B.A. and Mai, W.F. (1979). Growth reduction of apple seedlings by *Pratylenchus penetrans* as influenced by seedling age at inoculation. *Journal of Nematology*. 11: 161-165.

Jatala, P. and Rusell, C.C. (1972). Nature of sweet potato resistance to *Meloidogyne incognita* and the effect of temperature on parasitism. *Journal of Nematology*. 4: 1-7.

Jennings, P.R. (1980). In: Breeding plants for resistance to insects, ed. Fowden G. Maxwell- Texas A and M University, The Rockefeller Foundation, by John Wiley and Sons, Inc.

Jiang, Y.X.; Nombela, G. and Muniz, M. (2001). Analysis by DC-EPG of the resistance to *Bemisia tabaci* on a *Mi*-tomato line. *Entomologia Experimentalis et Applicata*. 99:

295-302.

Johnson, A.W. (1985). The role of nematicides in nematodes management. In: *An Advanced Treatise on Meloidogyne* species *Volume 1. Biology and Control*, ed. Sasser J.N. and Carter, C.C. Raleigh, North Carolina State University Graphics: 249-267.

Johnson, A.W. and Fassuliotis, G. (1984). Nematode parasites of vegetable crops. In: *Plant and Insect Nematodes*, ed, Nickle, W.R. Marcel Dekker Inc., New York and Basel: 323-372.

Jones, F.G. (1956). Soil population of beet eelworm (*Heterodera schachtii, Schum*.) in relation to cropping. II. Micro and field plot results. *Annual Biological Review*. 44:25-56.

Joubert, T.G. and Rappard, C.E. (1971). Roodepleat Albesto: eelworm-worm tomato cultivar. *Farming in South Africa*. 46(10): 3, 11, and 14.

Kaloshian, I.; Yaghoobi, J.; Liharska, T.; Hontelez, J.; Hanson, D.; Hogan, P.; Jesse, T.; Wijbrandi J.; Simons, G.; Vos, P.; Zabel, P. and Williamso, V.M. (1998). Genetic and physical localization of the root-knot nematode resistance locus *Mi* in tomato. *Molecular Genetics*. 257: 376-385.

Katan, J.; Greenberger, A.; Alon, H. and Grinstein, A. (1976). Solar heating by polyethylene mulching for the control of diseases caused by the soil-borne pathogens. *Phytopathology*, 66: 113-139.

Kerh, A.H. 1966. Current status and opportunities for the control of nematodes by plant breeding. Pp. 126-138. *In* Pest control by chemical, biological, genetic and physical means. (A symposium). Agricultural Research Services. USDA, ARS 33-110.

Kerry, B.R. (1987). Biological control. In: *Principles and practice of nematode control in crops*. ed. Brown, R.H. and Kerry, B.R. London, Academic Press Inc. 233-263.

Kerry, B.R. (1990). An assessment of progress towards microbial control of plant parasitic nematodes. *Supplement to the Journal of Nematology*. 22: 621-631.

Khana, M.L. and Nirula K.K. (1964). Breeding potatoes for resistance to root-knot nematodes. *Current Science*. 33: 314.

Khan, A.A. and Khan, M.W. (1985). Root-knot nematodes infecting some common weeds in vegetable fields of Western Uttah Pradesh, India. *International Nematological Network Newsletter*. 2(4): 15-16.

Kyron, N.C. (1973). Effects of soil treatments on root-knot nematodes and tomato yields under field conditions. *Plant Disease Reporter*. 57: 10-13.

Loos, C.A. (1953). *Meloidogyne brevicanda*, a cause of root-knot of mature tea in caylon, Proc. Helminthological Society. Washington. Pp 83-9.

Mai, F.W. (1985). Plant parasitic nematodes: their threat to Agriculture. In: *Advanced Treatise on Meloidogyne,* 1*: Biology and Control*, pp 11-19. ed. Sasser, J.N. and Carter C.C. Raleigh, North Carolina: Department of Plant Pathology and the U.S. Agency for International Development.

Madamba, C.P.; Sasser, J.N. and Nelson, L.A. (1965). Some characteristics of the effects of *Meloidogyne species* on unsuitable host crops. *North Carolina Agricultural Experimental Station Technical Bulletin*. 169: 1-34.

Mahmood, I. (1988). Two possible new hosts of *Meloidogyne incognita*. *International Nematology Network Newsletter*, 5(1): 20.

Mankau, R. and Prasad, N. (1972). Possibilities and problems in the use of sporozoan endoparasite for biological control of plant-parasitic nematodes. *Nematropica*. 2: 7-8.

Maqbool, M.A.; Hasmid, S. and Ghaffer, A. (1985). Effect of Albicard and Carbofuran on the control of root-knot nematodes in cauliflower (*Brassica oleracea*). *International Nematology Network Newsletter*. 2(3): 24-25.

Mateille, T. and Netscher, C. (1985). Temporary protection of egg-plant from *Meloidogyne incognita* by minute quantities of isazophos and aldicarb applied at seedling stage. *Revue de Nématologie,* 8*:* 41-44.

Mathews, B.F.; Macdonald, M.H.; Gebhardt, J.S. and Devine, T.E. (1998). PCR markers residing close to the *Rhg4* locus conferring resistance to soybean cyst nematode race on linkage group A of soybean. *Theoretical and Applied Genetics*. 97: 1047-1052.

McClure, M.A.; Ellis, K.C. and Nigh, E.L. (1974). Resistance of cotton to root-knot nematode, *M. incognita*. *Journal of Nematology*. 6: 17-20.

Medina-Filho, H.P. and Stevens, M.A. 1980. Tomato breeding for resistance: survey of resistant varieties for horticultural characteristics and genotypes of acid phosphotases. *Acta Horticulturae* . 100: 383-391.

Messequer, R.; Ganal, M.C.; De Vicente, M.C.; Young, N.D.; Bolkan, H. and Tanksley, S.D. (1991). High resolution RFLP map around the root-knot nematode resistance gene (*Mi*) in tomato. *Theoretical and Applied Genetics*. 82: 529-536.

Milligan, S.B.; Bodeau, J.; Yaghoobi, J. Kaloshian, I.; Zabel, P. and Williamson, V.M. (1998). The root-knot resistance gene *Mi* from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. *Plant Cell*. 10: 1307-1319.

MoFA (1987). Ministry of Food and Agriculture–Policy Planning Monitoring and Evaluation Department (PPMED) handbook, MoFA, Accra.

Movra, L.; Menella, G. and D'amore, R. (1992). Innesta della melanzana (*Solanum melongena*) quale mezzo per la difesta da patogeni tellurieie l'aumento Dell rese. (II). Contributo.

Colt. Protte 21: 85-93. In. Nematode management in sustainable Agriculture. ed. Bridge, J., 1996.

Muller, R. and Gooch, P. S. (1982). Organic amendments in nematode control. An examination of the literature. *Nematropica*. 12: 319-326.

Morgan-Jones, G. and Kabana, R. (1987). Fungal biological control for the management of nematodes In: *Vistas on nematology*. ed. Veech, J.A. and Dickson. Hyattsville MD: Society of Nematology, 509. 14: 94-99.

Nardacci, J.F. and Barker, K.R. (1979). The influence of temperature on *Meloidogyne incognita* on soybean. *Journal of Nematology*. 11: 62-70.

Nausbaum, C. J. and Ferris, H. (1973). The role of cropping systems in nematode management. *Annual Review of Phytopathology*. 11: 423-440.

Netscher, C. and Mauboussin, J. C. (1973). Résultats d'un essai concernant lefficacité compare dune variéte resistance et de certains nématicides contre *Meloidogyne javanica. Cahiers O. R. S. T. O. M. Serie Biologie*. 21: 79-102.

Netscher, C. and Taylor, D. P. (1979). Physiologic variation within the genus *Meloidogyene* and its implications on integrated control. In: *Root-knot nematodes* (Meloidogyne *species)*;

Systematics, Biology and Control, ed. Lamberti, F. and Taylor, C. E. London, Academic Press: 269-294.

Netscher, C and Sikora, R.A. (1990). Nematode parasites of vegetables. In: *Plant parasitic nematodes in subtropical and tropical agriculture*, ed. Luc. M.; Sikora R.A. and Bridge J. Wallingford, UK: CAB International 62. 3: 237-283.

Netscher, C. and Luc. M. (1974). Nematodes associès aux cultures maraÏchères en Mauritanie. *Agronomic Tropicale Nogent*. 29: 697-701.

Netscher, C. (1978). Morphological and physiological variability of species of *Meloidogyne* in West Africa and implications of their control. *Mededelingen Landbouwhoge School*, Wageningen, 46.

Netscher, C. (1981). Preliminary studies on the control of *Meloidogyne* species. *Proceedings of the 3rd Research Planning Conference on root-knot nematodes*, Meloidogyne *species. Regions IV and V.* Ibadan, Nigeria: 247-253.

Nirula, K.K. and Raj, B.T. (1971). Vertical distribution of *Meloidogyne incognita* in potato fields. *Indian Phytopathology*. 24: 375-378.

Niu, C.; Hinchcliffe, D.J.; Cantell, R. G.; Wang, L.; Roberts, P.A.; and Zang, J.F. (2007). Identification of molecular markers associated with root-knot nematodes resistance in upland rice. *Crop Scienc*e. 47(3). 951-960.

Noe, J.P. and Sikora, R.A. (1990). Effect of tropical climates on the distribution and host parasite relationship of plant parasitic nematodes. In: *Plant parasitic nematodes in subtropical and tropical agriculture*. eds. Luc. M.; Sikora, R.A. and Bridge, J. CABI, Wallingford, UK. 583-597.

Nombela, G.V.M.; Williamson, V.M.; and Muniz, M. (2003). The root-knot nematode resistance gene *Mi-1.2* of tomato is responsible for resistance against White Fly, *Bemisia tabaci*. *Molecular Plant Microbe Interactions*. 16: 645-649.

Odihirin, R.A. (1976). Report on *Meloidogyne* Research at the University of Ibadan. *Proceedings of the research planning conference on root-knot nematodes*, Meloidogyne *species*. 11-13.

Ogbuji, R.O. (1979). Shifting cultivation discourages nematodes. *World Crops,* 31: 113-114.

Olthof, T.H.A. and Potter, J.W. (1972). Relationships between population densities of *Meloidogyne hapla* and crop losses in summer maturing vegetables in Ontario. *Phytopathology*. 62: 981-986.

Oostendorp, M. and Sikora, R. A. (1989). Seed treatment with antagonistic rhizobacteria for the suppression of *Heterodera schachtii* early root infection of sugar beet. *Revue de N*èm*atologie*, 12: 77-83.

Osei, C.K.; Offei - Bonsu, K.; Gyasi – Boakye, S.; Asiedu, E.; Berchie, J. N. (2006). Promotion of healthy vegetable production in Ghana: Experiences and Challenges, *Ghana Journal of Horticulture.* 5: 129 – 135.

Osei, K. (2000). Effect of Africa Marigold (*Tagetes erecta* L.) on root-knot nematodes (*Meloidogyne incognita*), MSc. Thesis, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. pp 43-55.

Oudejans, J.H. (1991). Components of integrated pest management. In: *Agro-pesticides: properties and functions in integrated crop protection.* United Nations, Bangkok. 15-25.

Overman, A. J. (1974). Nematicides in linear drip irrigation for full-bed mulch of tomato. *Proceedings of the Soil and Crop Science Society of Florida, U.S.A* 34: 197-200.

Page, S.L.J. (1979). An assessment of the importance and control of plant-parasitic nematodes of vegetable crops in Bangladesh, O. D. M. Report on visit to Bangladesh. Ascot, Berks. , UK. Ministry of Overseas Development, 36.

Page, S.L.J. and Bridge, J. (1993). Plant nematodes and sustainability in tropical agriculture. *Experimental Agriculture*. 29: 139-154.

Painter, R.H. (1951). Insect resistance in crop plants. Pub., the Macmillan Co., New York, 520.

Palumbi, S.; Martin, A.; Romano, S.; McMillan W.O.; Stice, L. and Grabowski, G. (1991). The simple fool's guide to PCR. Department of Zoology and Marine Laboratory, University of Hawaii.

Paran, I. and Michelmore, R.W. (1993). Development of PCR-based markers linked to downy mildew resistance gene in lettuce. *Theoretical and Applied Genetics*. 85: 985-993.

Pardee, W.D. (2007). 'Tomato' *Microsoft student 2008* (DVD), Redmond, W/A. Microsoft Corporation.

Peacock, F.C. (1957). Studies on root-knot nematodes of the genus *Meloidogyne* in the Gold Goast. Part 1. Cooperative development on susceptible and resistant host species. *Nematologica*. 2: 27-84.

Prasad, D. and Chawla, M.L. (1991). Importance of environmental factors in the management of phytonematodes. In: *The fragile environment*. New world environmental service, 5, New Delhi, Ashih. ed. Mohan, I. 115-125.

Prot, J.C. and Van Gundy, S.D. (1979). Influence of soil type and temperature on the migration of *Meloidogyne incognita* juveniles towards roots. In: *XI ONTA Meeting Abstracts*. Charleston, South Carolina. 26.

Quénéhervé, P., Drob, F. and Topart, P. (1995). Host status of some weeds to *Meloidogyne* species, *Pratylenchulu*s species, *Helicotylenchus* species and *Rotylenchulus reniformis* associated with vegetables cultivated in potytunnels in Martinique. *Nematropica.* 25:

149-157.

Radewal, J.D.; Mckenry, M.V; Roberts P.A. and Westerdall, B.B. (1987). The importance of soil fumigation for nematode control. *California Agriculture*: 41: 16-17.

Reddy, N.C. and Sheshadri, R. (1971). Studies on some systematic nematicides. Evaluation for systemic nematicides and contact action against the root-knot nematode, *M. incognita*. *Indian Journal of Nematology* 1: 199-208.

Rick, C.M. and Fobes, J.F. 1974. Association of an allozyme with nematode resistance. *Tomato Genetics Cooperative*. 24: 25.

Ritter, M. (1973). Life cycles and development of *Meloidogyne* species. *European Mediterranean, Plant Protection Organ (OEPP/EPPO) Bulletin*. 9: 53-59.

Roberts, P. A.; May, D. and Mathew, W. C (1986). Root-knot resistance in processing tomato, *California Agriculture*. 40: 24-26.

Roberts, P.A. (1992). Current status of the availability, development and use of host plant resistance to nematodes. *Journal of Nematology*. 24: 213-227.

Robinson, R.A. (1980). New concepts in breeding for disease resistance. *Annual Review of Phytopathology*. 18: 189-210.

Rodriguez-Kabana, R. and Pope, M.H. (1981). A simple incubation method for the extraction of nematodes from soil. *Nematropica*. 11: 175-185.

Rossi, M.; Goggin, F.L.; Milligan, S.B.; Kaloshian, I.; Wallman, D.E. and Williamson, V.M. (1998). The nematode resistance gene *Mi* of tomato confers resistance against the potato aphid. *Proceedings of the National Academy of Science,* USA. 95: 9750-9754.

Sakai, V.W. and Carter, C.C. (1987). Hosts and non-hosts of the root-knot nematode, *Meloidogyne incognita*. *Crop Nematode Research and Project*, 6-53.

Saleh, H.; Abu-Garbieh W.I.; and Al-Banna, L. (1988). Effect of solarization combined with solar-heated water on *Meloidogyne javanica*. *Nematologica*. 34: 290-291.

Santo, G.C.; O' Bannon, J.H.; Finley, A.M. and Golden, A.M. (1980). Occurrence and host range of a new root-knot nematode in the Pacific Northwest. *Plant Disease Reporter*. 64: 591-592.

Sasser, J.N. (1954). Identification and host parasite relationship of certain root-knot nematodes, *Meloidogyne species*. *University Md. Agricultural Experimental Station Bulletin*. A-77.31.

Sasser, J.N. (1979). Economic importance of *Meloidogyne* in tropical countries. In: *Root-knot nematodes* (Meloidogyne *Species*). *Systematics, Biology and Control*. ed. Lamberti, F. and Taylor, C.E. 359-374.

Sasser, J.N. (1989). Plant Parasitic nematodes, The Farmer's Enemy. North Carolina State University, Raleigh, North Carolina. 115.

Sasser, J.N. and Carter, C.C. (1985). An advanced treatise on *Meloidogyne* species, Volume I, Publication of plant pathology and the U.S. Agency for International Development. Printed by North Carolina State University Graphics.

Sauer, M.R. and Giles, J.E. (1959). A field trial with root-knot resistant tomato varieties. *C. S. I. R. O. Australian Irrigation Station Technical Paper*, 3: 1-10.

Schiffers, B.C.; Fraselle, J. and Jaumin, L. (1985). Comparsison d'efficité contre le *Ditylenchus dipsaci* (Kuhn) Fil. de 4 traitements nmaticides incorporés aux enrobages de semences de fèverolle (*Vicia faba* L.). *Medelingen Landbouwhge school*, Wageningen. 50: 797-807.

Schroeder J.; Stephen H.; Thomas and Leigh Murray (1993). Yellow and purple nut sedge and chile peppers host southern root-knot nematodes. *Weed Science*. 41(1), 150-156.

Seah, S.; Yaghoobi, J.; Rossi, M.; Gleason, C.A. and Williamson, V.M. (2004). The nematode resistance gene, *Mi-1*, is associated with an inverted chromosomal segment in susceptible compared to resistant tomato. *Theoretical and Applied Genetics*. 108: 1635-1642.

Seah, S.; Telleen, A.C. and Williamson, V.M. (2007). Introgressed and endogenous *Mi-1* gene clusters in tomato differ by complex rearrangement in flanking sequences and show sequence exchange and diversifying selection among homologues. *Theoretical and Applied Genetics*. 114: 1289-1302.

Seah, S.; Williamson, V.M.; Garcia, E.B.; MejÍa, L.; Salus, S.M.; Martin, C.T. and Maxwell, D.P. (Unpublished data). Evaluation of a co-dominant SCAR marker for detection of the *Mi-1* locus for resistance to root-knot nematodes in tomato germplasm.

Semblat, J.P.; Wajinberg, E.; Dalmasso, A.; Abad, P. and Castagnone-Sereno, P. (1998). High resolution fingerprinting of parthenogenetic root-knot nematodes using AFLP analysis. *Molecular Ecology*. **7**: 119-125.

Sharma, R.K. and Raj, D. (1987). Effect of nematicides and organic amendments on root-knot nematodes infecting potato. *International Nematology Network Newsletter.* 4(1): 8-10.

Sikora, R.A.; Sitaramaiah, K. and Singh, R.S. (1973). Reaction of root-knot nematode resistant tomato cultivars to *Meloidogyne javanica* in India. *Plant Disease Reporter*. 57: 141-143.

Sikora, R.A. (1978). Einfluss der endotrophen Mykorrhiza (*Glomos mossea*) auf das Wirt Parasit-Verhaltnis von *Meloidogyne incognita* in Tomaten. *Zeitschrift fur Pflanzenkkrakheiten und Pflanzenschutz,* 85: 197-202.

Sikora, R.A.; Reckhaus, P. and Adamou, I. (1988). Presence, distribution and importance of plant-parasitic nematodes in irrigated agricultural crops in Niger. *Mededelingen van de Faculteit Landbouwwetenschappen, Rijsuniversiteit,* Gent, Belgium, 53: 821-834.

Sikora, R.A. (1988). Interrelationships between plant health promoting rhizobacteria , plant-parasitic nematodes and soil microorganisms. *Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit.* Gent, Belgium, 53: 867-878.

Sikora, R. A. (1989). Importance of plant parasitic and entomopathogenic nematodes to vegetable production in the Phillipines. *GTZ Report, Eschborn*. 37.

Sikora, R.A. and Fernandez, E. (2005). Nematode parasites of vegetables. In: *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture* (2nd edition). ed. Luc. M.; Sikora, R.A. and Bridge, CABI Wallingford, UK. 319-392.

Sikora, R.A.; Bridge, J and Starr, J. L. (2002). Management practices: an overview of integrated nematode management technologies. In: *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*, (2nd edition). ed. Luc, M.; Sikora, R.A. and Bridge, J. CABI Wallingford, UK. 793-825.

Singh, R. S. and Sitaramaiah, K (1966). Incidence of root-knots of okra and tomatoes in oil cake amended soil. *Plant Disease reporter*. 9: 668-672.

Singh, R. S. and Sitaramaiah, K. (1967). Effect of decomposing green leaves, sawdust and urea on the incidence of root - knot to okra and tomato. *Indian Phytopathology*. 20: 349-355.

Sink, K.C. (1984). Protoplast fusion for plant improvement. *Proceedings of the American Society of Horticultural Science*. 19: 33-37.

Sitaramaiah, K. (1990). Mechanisms of reduction of plant parasitic nematodes in soil amended with organic materials. In. *Nematode management in sustainable and subsistence agriculture*, ed. Bridge, J. 217.

Southards, C.J. and Priest, M.F. (1973). Variation in pathogenicity of seventeen isolates of *Meloidogyne incognita. Journal of Nematology*, 5: 63-67.

Stanton, J.M. and O'Donnell, W.E. (1994). Hatching, motility and infectivity of root-knot nematodes, *Meloidogyne javanica* following exposure to sodium hypochlorite. *Australian Journal of Experimental Agriculture*. 34: 105-108.

Stapleton, J.J. and De Vay, J. E. (1986). Soil Solarization: a non-chemical approach for management of plant pests. *Crop Protection*. 5: 190-198.

Stephen, Z.A.; Michbass, A.H. and Shashir, C.W. (1989). Control of root-knot nematodes on egg plant by nematicides. *International Nematology Network Newsletter*. 6(3): 25-26.

Stirling, G.R. (1991). Biological control of plant parasitic nematodes. *Progress, problems and prospects*. Wallingford, UK: CAB Int. 282.

Stirling, G.R. (1984). Biological control of *Meloidogyne javanica* with *Baccilus penetrans*. *Phytopathology*. 74: 55-60.

Stover, R.H. (1979). Flooding of soil for disease control. In: *soil disinfection (developments in agricultural and managed forest ecology)*, ed. D. Mulder, Amsterdam, The Netherlands: Elsever. 19-28.

Tanha Maafi, Z.; Subbotin, S.A. and Moens, M. (2003). Molecular identification of cyst-forming nematodes (Heteroderdae) from Iran and a phylogeny based on ITS-rDNA sequences. *Nematology*. 5: 99-111.

Taylor, D.P.; Netscher, C. and Germani, G. (1978). *Adansonia digitata* (Baobab), a newly discovered host for *Meloidogyne* species and *Rotylenchulus reniformis*. Agricultural Implications. *Plant Disease Reporter*, 37: 187-192.

Taylor, A.L. and Sasser, J.N. (1978). *Biology, Identification and control of root-knot nematodes (Meloidogyne* species*)*. Cooperative Publication Department of Plant Pathology, North Carolina State University and U.S. Agency for International Development, Washington D. C., *North Carolina State University Graphics*, 111.

Thames, W.H. and Stover, W.N. (1953). A preliminary trial of lowland culture rice in rotation with vegetable crops as a means of reducing root-knot nematode infestations in the Everglades. *Plant Disease Reporter*, 37:187-192.

Thomasson, I.J. and Lear, Bear, B. (1961). Rate of production of *Meloidogyne species* as influenced by soil temperature. *Phytopathology*. 51: 520-524.

Thomas, W.B. (1996). Methyl bromide: effective pest management tool and environmental threat. *Supplement to the Journal of Nematology* 28: 586-590.

Trudgill, D.L.; Bala, G.; Block, V.C.; Daudi, A.; Davies, K.G.; Gowen, S.K.; Fargette, M.; Madulu, J.D.; Mateille, T.; Mwageni, W.; Netscher, C.; Phillips, M.S.; Sawadogo, A.; Trivino, C.G. and Voyoukallou, E. (2000). The importance of tropical root-knot nematodes, *Meloidogyne* species and factors affecting the utility of *Pasteuria penetrans* as a biological agent. *Nematology*. 823-845.

Tyler, J. (1933). Development of the root-knot nematode as affected by temperature. *Hilgaria*. 7: 392-415.

Vargas, R.; Rodriguez, A. and Acosta, N. (1996). Components of nematode suppressive activity of velvet bean, *Mucuna deeringiana*. *Nematropica*. 26: 323 (Abst.).

Veech, J.A. (1978). An apparent relationship between methoxy substituted terpenoid aldehydes and the resistance of cotton to *Meloidogyne incognita*. *Nematologica*. 24: 81-87.

Veech, J.A. (1981). Plant resistance to nematodes. In: *Plant-parasitic nematodes, Volume III*. ed. Zuckerman, R.A. and Rohde, Academic Press. New York, 508. 377- 403.

Vidavsky, F. and Czosnek, H. (1998). Tomato breeding lines immune and tolerant to tomato yellow leaf curl virus (TYLCV) issued from *Lycopersicon hirsutum*. *Phytopathology*. 88: 910-914.

Vrain, T.C.; Barker, K.R. and Holtzman, G.I. (1978). Influence of low temperature on the rate of development of *Meloidogyne incognita* and *M. hapla* larvae. *Journal of Nematology*. 10: 166-171.

Vossen van der. H.AM. ; Nono-Wondin, R and Messiaen, C.M. 2004. *Lycopersicon esculentum* Mill. In Grubben, G.J.H and Denton, O.A (Editors). PROTA 2: Vegetables/Légumes (CD-ROM). PROTA, Wageningen, Netherlands.

Wallace, H.R. (1969). The influence of nematode numbers and particle size, nutrients and temperature on the reproduction of *Meloidogyne javanica*. *Nematologica*. 15: 55-64.

Walter, J.M. (1967). Hereditary resistance to disease in tomato. *Annual Review of Phytopathology*. 5: 131-162.

Whitehead, A.G. and Hemmings, J.R. (1965). A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annual Applied Biology*. 55:25-38.

Williams-Woodward, J.L. and Davis, R.F. (2001). *Meloidogyne incognita* and *M. arenaria* reproduction on Dwarf hollies and Lantana. *Supplementary to the Journal of Nematology*. 33: 332-337.

Williamson, V.M. (1998). Root-knot nematode resistant genes in tomato and their potential for future use. *Annual Review of Phytopathology*. 36: 277-293.

Williamson, V.M.; Ho, H-Y,; Wu,; Miller. N. and Kaloshian, I. (1994). A PCR-based marker tightly linked to the nematode resistance gene *Mi*, in tomato. *Theoretical and Applied Genetics.* 87: 757-763.

Wyatt, J.E.; Fassuliotis, G. and Johnson, A.W. (1980). Efficacy of resistance to root-knot nematodes in snap beans. *Journal of American Society of Horticultural Science*. 105: 923-926.

Zacheo, G.; Lamberti, G.; Anigoni-Liso and Ongoni, O. (1977). Mitochondrial protein hydroxyproline content of susceptible and resistant tomatoes infected by *M. incognita*. *Nematologica*. 23: 471-476.

Zavaleta - Meija, E. and Van Gundy, S. D. (1982). Effects of Rhizobacteria on Meloidogyne infection. *Journal of Nematology*, 15: 475-476.

Zhong, X.; Bodeau J.; Fransz, P.Z.; Williamson, V.M.; Kammen, A.V.; De Jong, J.H. and Zabel, P. (1999). FISH to meiotic pachytene chromosomes of tomato locates the root-knot nematode resistance gene Mi and the acid phosphotase gene Aps-1 near the junction of euchromatin and pericentromeric heterochromatin of chromosome arms 6S and 6L, respectively. *Theoretical and Applied Genetics.* 98: 365-370.

Appendix 1

Preparation of 0.8% Agarose (for genomic DNA).

1X TAE = 300 ml

Agarose = 2.4 g

EtBr = 7.5 µl

Appendix 2

Loading of PCR products**.**

(1) 10 µl of the PCR product and 2 µl dye.
(2) Loading map 200 bp ladder 1, 2, 3, 4, 5, 6, 7, 8, 9, 10.
(3) Stir to dissolve.

Appendix 3

Preparation 1M Tris HCl (500 ml).

(1) Weigh 60.5 of Tris Base and 20-21.25 ml of concentrated HCl.
(2) Add 500 ml of distilled water.
(3) Stir to dissolve.

Appendix 4

Preparation of 5M NaCl (500 ml).

(1) Weigh 146.1 g of sodium chloride.
(2) Add 500 ml of distilled water.
(3) Stir to dissolve.

Appendix 5

Preparation of 5M Potassium acetate.

(1) Weigh 245.5 g 5M potassium acetate (500 ml).
(2) Add 500 ml of distilled water.
(3) Stir to dissolve.

Appendix 6

Preparation of Buffer A (Lysis Buffer) - 50ml

Stock Solution	**Final Conc.**	**Volume required**
1M Tris HCl (pH = 8)	50 mM	1 ml

5M NaCl	300 mM	1.2 ml
0.5M EDTA	20 mM	0.8 ml
PVP (10,000 Mwt.)dry Powder	20 %	0.4 g
20% Sarkocine	1.5 %	1.5 ml
Sodium metabisulphite	1 g/100ml Buffer	0.2 g

Topped up to 50ml with sterile water.

Appendix 7

PCR reaction mix *(reaction vol. 10 µl).*

		1X	27X
(i)	PCR H_2O	6.07 µl	153.9 µl
(ii)	10x Buffer	1.00 µl	27.00 µl

(iii)	$MgCl_2$ (25mM)	0.90 µl	27.50 µl
(iv)	dNTPs	0.40 µl	5.40 µl
(v)	Primer (Mi23F)	0.25 µl	13.5 µl
(vi)	Primer (Mi23F)	0.25 µl	13.5 µl
(v)	Taq polymerase	0.125 µl	2.7 µl
(vi)	DNA Template	1.00 µl	
Total		10. 00 µl.	(*9 µl Rxn mix + 1 µl DNA*)

Appendix 8

DNA EXTRACTION PROTOCOL BY EGNIN *et al*., 1998**.**

Weigh 200mg of tissue into 2ml eppendorf tube.

1. Grind to fine powder with liquid nitrogen.
2. Add 800 µl of Buffer A (lysis powder).
3. Incubate at 90^{o}C for 10mins, vortex every 5mins.
4. Cool at room temperature for 2 mins.
5. Add 400 µl 5M potassium acetate; mix gently by inversion 5 - 6X.
6. Incubate on ice for 30 mins with shaking.
7. Centrifuge at 13, 000 rpm for 10 mins.
8. Transfer the upper phase to a new eppendorf tube.
9. Add 1 volume of cold isopropanol, 1/10th of 3M sodium acetate, mix 10X by inverting.
10. Incubate at - 20^{o}C for 1 hr, centrifuge at 13, 000 rpm for 10 mins.
11. Pour off supernatant, wash pellets with 800µl, 80% ethanol.
12. Centrifuge at 14, 000 rpm for 5 mins.
13. Discard alcohol and dry pellets.
14. Add 500µl 1X TE Buffer to dissolve pellets.
15. Add 4µl RNase A, incubate at 37^{o}C for 30mins.

16. Add 250µl of 7.5M ammonium acetate.
17. Incubate on ice for 3 mins, centrifuge at 13, 000 rpm for 5mins.
18. Transfer supernatant into a new 1.5 ml tube.
19. Add 700µl of isopropanol, mix by inversion (ice inversion) and centrifuge at 13, 000 rpm for 15 mins.
20. Discard supernatant, wash pellets with 1ml 80% ethanol.
21. Centrifuge at 14, 000 rpm for 5mins.
22. Discard supernatant, dry pellets at room temperature.
23. Dissolved DNA pellets in 200µl 1X TE Buffer.
24. Check DNA quality on 0.8% agarose gel.

Appendix 9

Resistance rating chart

Highly resistant	0 – 0.4
Resistant	0.5 – 1.0 Moderately
resistant	1.1 – 1.5
Tolerant	1.6 – 2.5 Moderately
tolerant	2.6 – 2.8
Susceptible	2.9 – 3.5
Highly susceptible	3.6 – 5.0

Appendix 10

Gall score chart on a scale of 0-5

0 = 0……No galling

1 – 20 = 1

21 – 40 = 2……Light galling

41 - 60 = 3……Moderate galling

61– 80 = 4

> 80 = 5 ……Heavy galling