EVALUATION OF DIFFERENT GENES FOR RESISTANCE TO ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA* IN TOMATO

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INTRODUCTION

Tomato (Lycopersicon esculentum Mill.) is a major crop throughout the tropical, subtropical and temperate growing regions of the world. In many tropical and subtropical regions, the root-knot nematode, Meloidogyne incognita (Koford and White, 1919) Chitwood, 1949, is a major problem of crop production. With high populations of nematodes infecting the roots, complete failure of the tomato crop may result (Dhillon et al., 1975). Certain nematicides have proven to be useful in the control of root-knot nematodes, but they are expensive, labor consuming, and not always available. Thus, the cost of applying nematicides to field plantings may outweigh the economic returns, especially in the developing countries.

A number of workers have reported resistance to M. incognita in tomato (Smith, 1944; Gilbert, 1975; Sidhu and Webster, 1975). Smith first crossed an L. esculentum tomato with the nematode resistant L. peruvianum, P.I. 128657. Gilbert (1955) reported this resistance to be controlled by a single dominant gene, which he designated Mi. Sidhu and Webster (1975) reported three more genes that confer resistance, which they have designated $LMiR_1$, $LMiR_2$ and $LMir_3$.

Over the years, biotypes or races of M. incognita have been reported that are able to infect plants which have the Mi gene for resistance (Southart et al., 1975; Riggs et al., 1959; Dropkin, 1959; Viglierchio, 1978; Singh, 1974). These biotypes present problems in effectively utilizing the resistance in tomato.

It is the scope of this study to compare the performance of the $LMiR_1$, $LMiR_2$ and $LMir_3$ genes under Hawaiian conditions and to test them for allelism with the Mi gene. Hopefully, it might be possible to discover resistance to a pathotype at Poamoho Experimental Farm, Oahu, Hawaii which can infect lines with the Mi gene.

REVIEW OF LITERATURE

The Host:

Tomato is a perennial fruit crop that is universally treated as an annual vegetable. A member of the nightshade family, Solanaceae, it has as relatives Solanum melongena, eggplant; Capsicum annuum, bellpepper; and Solanum tuberosum, white potato. In the United States, tomato is the leading vegetable crop with a value of $827.7 million in 1978 (United States Department of Agricultural Crops Statistics).

The center of origin is located in the new world with Mexico as the probable site of domestication. L. esculentum is one of nine species in the genus. The others, L. peruvianum (L.) Mill, L. pimpinellifolium Mill., L. glandulosum, L. chilense Dun, L. cheesmanii Riley, L. hirsutum Humb., L. parviflorum, and L. chmielewskii are mostly inedible, but have been used as sources of genes in breeding.

The Pathogen:

Nomenclature and Identification

The first report of root-knot nematode was by Berkley (1855) in England who reported what he called "vibrios" coming from galls on cucumber roots. Over the years, various names were given to root-knot nematode, including Anguillula marioni (Cornu, 1879), Meloidogyne exiqua (Goeldi, 1887), Anguillula arnaria (Lavergne, 1901), Hederodera vialac (Kofoid and White, 1919), Oxyuris incognita (Sandground, 1923), and Caconema spp. (Cobb, 1924). All were included in Hederodera marioni by Goody (1932).

Chitwood in 1949 revised the taxonomy of the root-knot nematodes, separating them from the cyst nematodes, Hederodera, into a separate genus, Meloidogyne, with five species and one sub species distinguished by differences in the circular striations or perineal patterns around the vulva (and on the basis of several general characteristics of each genus). Meloidogyne has a thinner, softer cuticle than Hederodera with the anus and vulva terminus close together, while the female retains no eggs within the body cavity.

The five species and one subspecies divided by Chitwood (1949) are M. incognita, M. arenaria, M. javanica, M. hapla, M. exiqua, and M. incognita acrita. Up to the end of 1976, at least 36 species of the nematode genus Meloidogyne have been named (Taylor and Sasser, 1978).

Of these 36 species, four, M. incognita, M. arenaria, M. javanica, and M. hapla are the most widespread and very probably cause more damage to crops than all the other Meloidogyne species combined (Sasser, 1977).

The five species described by Chitwood (1949) are listed below giving common name, geographic range, and host range.

1) M. incognita (Kofoid and White) Chitwood is of major economic significance throughout the tropics and warmer regions of the world. It is now considered to include M. incognita acrita. Its distribution includes Africa, Australia, Central and South America, India, Japan, Malaysia, USA, and the glasshouses of Northern Europe, Canada and USSR. Often called the Southern root-knot nematode, it attacks over 700 species and varieties from the families Leguminosae, Compositae, Solanaceae,

Crucifereae, Gramineae, Rosaceae, Musaceae, Vitaceae, Convolvulaceae, Caryophyllaceae, Euphorbiaceae, and Liliaceae. Four races or pathotypes are reported to occur (Sasser, 1976). Crops in which resistance has been reported are clover, cotton, Lespedeza stipulacea, peach, peanut, pineapple, maize, sweet potato, tobacco, tomato, bean, lima bean, and soybean (Williams, 1973; Singh, 1974; Malo, 1964; Hartmann, 1976).

2) M. javanica (Treub) Chitwood is also widely distributed in warm and tropical climates and is often the dominant root-knot nematode at higher altitudes. Its range includes Africa, Australia, Brazil, Ceylong, Colombia, Cyprus, India, Israel, Malaysia, Pakistan, Spain, Trinidad, USA, and the glasshouses of northern Europe. Often called Javanese root-knot nematode, its host range is very wide with over 770 species attacked including many economic crops such as tea, tobacco, potato, tomato, many other vegetables, fruit trees, ornamentals, and cereals. Pathotypes have been reported.

3) M. arenaria (Neal) Chitwood is found in most parts of Canada, South Africa, Middle East, India, Malaysia to Japan, Australia, and countries bordering on the Mediterranean. Although found in glasshouses in cooler climates, it is not as common as the first two species. About 330 species of plants are reported as hosts, including many vegetables and other food crops along with ornamentals and cash crops. Commonly called the peanut root-knot nematode. Resistance has been reported in some Nicotiana spp. (Graham, 1952), Rhododendron spp., strawberry

(Sasser, 1954), Tagetes erecta and T. patula (Suatinadji, 1968) and several grasses (McGlohon et al., 1961). Biotypes have been reported.

4) M. hapla Chitwood, the Northern root-knot nematode, is one of the few known Meloidogyne species capable of surviving in temperate climates. It is cosmopolitan in distribution, being found at higher altitudes in tropical and subtropical regions. Nearly all vegetables of economic importance in cooler climates are liable to attack as well as strawberry, rose, peanut, soybean, and pyrethrum. Resistant plants include coffe, cotton, maize, watermelon, and most grasses and cereals. Pathotypes have been reported (Williams, 1974).

5) M. exiqua (Goeldi) Chitwood, commonly called coffee root-knot nematode, also attacks such important crops as tea, pepper, and watermelon. Its geographic range is limited to Brazil, Peru, Matinique, and the New York Botanical Garden.

In Hawaii, M. incognita is the most commonly found root-knot nematode except on pineapple where M. javanica is a major problem (Apt, 1980, personal communication). M. incognita is found on all major islands in the Hawaiian chain, attacking a wide range of plants including many vegetable, ornamental, and fruit crops. M. hapla has also been reported on plants at higher elevation on the island of Maui.

Host Susceptible Reaction:

Response of the Whole Plant

The most prominant symptom of root-knot nematode infection is the formation of galls (hyperplasia) on the roots. Callus formation on the stems and stunting of the root system are also symptoms. These are usually followed by the formation of adventitious roots.

The above ground symptoms of infection mimic symptoms of other diseases, especially physiological disorders. These include chlorosis, symptoms of mineral deficiency, stunting, defoliation, wilting, and reduced yield. Under heavy infection and stress conditions, complete crop failure can occur due to stunting, lack of fruiting, or, in some cases, death of the host.

The major cause of mortality, however, is not the nematode itself, but secondary microbial invaders (Mayol and Bergeson, 1969, 1970). M. incognita and the secondary invaders in many instances form disease complexes in which the nematode plays various roles such as vector, wounding agent, host modifier, rhizosphere modifier, or resistance breaker (Bergeson, 1972).

Response at Cellular level

At the cellular level, the most prominent symptom of root-knot nematode infection is the development of syncytia or giant cells, which are multinuclear and induced and maintained by the feeding nematode. They are areas of high metabolic activity, and contain increased amounts of chemical components such as hemicelluloses, organic acids, free amino acids, proteins, nucleotides, RNA, DNA, and

lipids (Endo, 1971). It is thought that giant cells are formed from single cells by the failure of cell wall development after mitosis or by the breakdown of cell walls and subsequent fusion of adjacent cells (Bird, 1972).

A number of chemical inducers exuded from the nematode have been proposed as the stimulus of giant cell development, such as proteins and glycoprotein from the esophageal glands of the nematode (Bird, 1974), indolyl derivatives (Bird, 1962), and auxin (Viglierchio, and Yu, 1965). Sayre (1971) proposed an interaction of plant hormones with nematode secretions to be the stimulus.

Host Resistant Reactions:

A resistant reaction to a nematode by the host is defined as an active and dynamic response by the host in resisting the attack of the pathogen (Nelson, 1973). The reaction may range anywhere between tolerance and hypersensitivity. Tolerant plants are susceptible to the parasite, but resist the impact of the disease by desensitization of the plant whereas hypersensitive plants exhibit premature necrosis of the infected tissue causing inactivation and localization of the pathogen (Nelson, 1973).

Most reports indicated that hypersensitivity is the mechanism of host resistance to M. incognita (Dropkin, 1969; Rohde, 1972; Hung and Rohde, 1973; Rich and Keen, 1975; Zancheo et al., 1978; Sawhney and Webster, 1979). On the microscopic level the resistant reaction is seen as a shrinking and browning of cells that the nematode has fed on, with subsequent necrosis of the cells surrounding the nematode. The

nematode larva then remains quiescent in the root until it presumably starves to death (Dropkin, 1969).

Hypersensitivity has been observed beginning as early as 12 hours after infection and is genetically controlled by one or a few genes.

It is generally agreed that the oxidation products of phenolic compounds are responsible for the browning of infected cells. Chlorogenic acid has been identified as the major phenolic compound in tomato roots (Dropkin, 1969).

Most phenols in plants are bound in the form of glycosides of low physiological and chemical activity. Oxidation of glycosides by nematode secretions of polyphenol oxidase (Hung and Rohde, 1973) and Beta-glycosidases (Giebel, 1974) releases free phenols which kill the cells, isolating the nematode. When the necrotic reaction is prevented (by suppression of the action of free phenols), resistance is lost (Rohde, 1972; Dropkin et al., 1969; Dropkin, 1969; Sawhney et al., 1979). Recent reports have indicated that when the necrotic response was inhibited by cycloheximide (a protein synthesis inhibitor), the cells did not die and the neamtodes were free to move away, suggesting that the mere inhibition of the hypersensitive response does not make resistant plants susceptible. Therefore, it is suggested that there is more than one metabolic switch in determining the susceptible/resistant response. Findings also indicate that active protein synthesis seems necessary for triggering hypersensitivity.

The absence of secretions of polyphenol oxidase and Beta-glycosidases may be indicative of pathotypes of resistant breaking strains of nematodes. It was found that Hederodera rostochiensis pathotype A has

a very active Beta-glucosidase, while pathotype B has a less active Beta-galactosidase (no hypersensitivity and breaks resistance) (Giebel, 1974). From this it is assumed that the plants resistant to pathotype A are not resistant to pathotype B because pathotype B is not able to release "the resistant factor" (free active phenols) from the nonactive glycoside.

In recent findings, a proposed defense mechanism of plants to nematodes is the synthesis of hydroxyproline in the mitochondria of tomato roots (Giebel, 1974; Zancheo et al., 1978). Hydroxyproline concentration increased in resistant varieties and decreased in susceptible varieties infected with root-knot nematode. According to this hypothesis, an increase in hydroxyproline induces conditions in which auxin is destroyed resulting in a diminution of cell elongation and suppression of hypertrophy (Giebel and Krenz, 1975). It is believed that greater synthesis of mitochondrial proteins containing hydroxyproline occurring in resistant varieties enables the nematode damaged cells to develop a more cyanide-insensitive respiration able to counter the pathogen. They believe developing cyanide-insensitive respiration following infection is the basis of resistance, where by producing a poisoning cytochrome oxidase which has little effect on the host but is detrimental to the attacking nematode.

Factors Affecting Root-knot Nematode Infection:

Investigators working with root-knot nematodes sometimes find that the level of infection on a susceptible host is less than expected. Generally, in these cases, it has been found that the nematode population

number had declined. Sayre (1971) and Wallace (1971) have reviewed the factors which affect root-knot nematode infection and classified them into abiotic and biotic factors.

Abiotic Factors

The major abiotic factors that affect nematode infection are temperature, moisture, soil structure, aeration and soil chemistry (Wallace, 1971).

Temperature is the environmental factor that has the greatest influence on the development of the nematode. Temperature effects can be divided into five arbitrary phases: 1) lethal low temperatures, 2) non-lethal low temperatures at which activities are inhibited, 3) optimum temperatures, 4) non-lethal high temperatures at which activities are inhibited, and 5) lethal high temperatures (Wallace, 1963). The optimum temperatures for M. incognita are 18-32°C. Lethal low temperatures are below 12°C, while 12-18°C are non-lethal low temperatures. 32-40°C are non-lethal high temperatures while above 40°C is considered to be lethal.

Temperature also affects the expression of resistance in several hosts. At soil temperatures above 32°C, M. incognita is able to infect root-knot resistant tomatoes with the Mi gene (Holtzmann, 1965; Dropkin, 1969). A similar temperature effect on nematode resistant snap beans, and lima beans was reported by Santoso (1973). Vanderplank (1978) proposed that enzyme functions are responsible for expression of resistance and at temperatures of 32°C and above, these enzyme systems are disfunctional.

Soil moisture also influences nematode activity. Under dry conditions, the eggs may hatch but the larvae will be inhibited in moving to a host. Water is also important in causing the gelatinous matrix in which the eggs are embedded to swell, thus aiding larval expulsion (Wallace, 1968B).

Soil structure was reported to be the primary factor affecting nematode infection (Jones, 1932; Kincaid, 1946; O'Bannon and Reynolds, 1961; Sleeth and Reynolds, 1955). Infection was found to be greater in coarse-textured soil than in fine-textured soil. The reason for this is that nematodes move in the soil pore space because they lack the mechanical strength to distort soil particles as earthworms do. Since finer soil texture means smaller pore space, movement of the nematode to the host would be restricted. Sleeth and Reynolds (1955) proposed that a map of soil types may determine the potential areas hazardous to crops susceptible to root-knot nematodes.

Adequate aeration of the soil is also important. Bird and Jenkins (1965) reported reduced nematode populations with low soil O_2. Wallace (1968A) attributed nematode inactivation to soil O_2 stress which increases with higher soil moisture levels.

The chemistry of the soil solution also affects root-knot nematodes. It was reported that the absence of calcium chloride, magnesium sulphate, or chelated iron in soil solution resulted in a reduction in survival of Meloidogyne larvae, while the presence of sodium or potassium chloride depressed emergence (Loewenberg, 1960). pH was reported to affect nematodes indirectly through the host plants. At too high or too low a pH, the host plants do not grow well or fail to grow and in turn

provides a poor source of food and reduce the reproduction of the nematodes.

Biotic Factors

Several authors have reported they were able to decrease root-knot nematode pathogenicity by adding organic matter amendments to the soil (Linford et al., 1938; Johnson, 1959; Singh and Sitaramaidh, 1966; Singh, 1967; Johnson et al., 1967). However, the addition of organic amendments has not generally been accepted as a nematode control measure due to the excessively large quantities of organic material needed and because nematicides give far better control than the organic residues used.

The mode of action of organic amendments varies. First, there is toxicity from the amendments directly or from decomposition products such as butyric acid from decomposing rye and timothy (Sayre, 1971) or hydrogen sulfide. Second, the decomposing organic matter causes a build up of a large population of micro-organisms that are predatory or parasitic on root-knot nematodes. Fungi, nematodes, tardigrades, turbellarians, enchytraeids, insects, and mites have been shown to be predators, while viruses, protozoa, bacteria have been shown to be parasitic or antagonistic (Sayre, 1971).

Predators:

1) Fungi - most predatory fungi come from the subclasses, Moniliales and Zoopagales. Predatory fungi are of two types, trappers and endozoic parasites. Currently, there are over 100 species of predatory fungi reported.

2) Nematodes - usually characterized by a large bucal opening armed with a "tooth" to rip prey apart or swallow them whole or armed with a stylet. Generas with predatory nematodes are Monochus, Butlerius, Anatonchus, Diplogaster, Tripyla, Seinura, Derylaimus, Discolaimus, and Actinolaimus.

3) Tardigrades - very small arthropods with two stylets for feeding, very slow moving.

4) Turbellarians - mostly carnivorous minute flatworms. Found in rich woodland soil. Adenoplea sp. found to prey on M. incognita in greenhouse soil (Sayre and Powers, 1966).

5) Collembola and Mites - a small group of insects and arthropods, respectively. Collembola are found near roots in highly organic soils. Soil mites have been observed feeding on root-knot nematodes, which are believed to be part of their normal diet (Linford and Oliveira, 1938).

6) Enchytraeids - suggested antagonists or nematodes, but not truly known to control them.

7) Protozoa - ameboid organisms found to prey on nematode larvae, engulfing them in 20 minutes to 2 hours.

Parasites:

1) Virus - Loewenberg (1960) reported a virus disease of M. incognita that caused immobilization and death of the larvae.

2) Bacteria - believed to infect nematodes, more tests are needed to confirm this.

3) Protozoa - accumulate within the body, especially around the reporductive organs, sterilizing and killing the host. Have

been observed parasitizing Meloidogyne sp. (Prasad and Mankan, 1969).

The selection of certain crops for rotation may also decrease the nematode population. Asparagus, Asparagus officinalis, (Rohde, 1972) and French Marigolds, Tagetes patula (Motsinger, 1979) have been reported to inhibit the reproduction of root-knot nematodes. Asparagus is thought to contain a toxic substance which kills or inhibits the nematodes in the root zone. Marigolds work as a trap crop in which the larval nematodes enter the roots, but fail to develop and reproduce.

Control of Root-knot Nematode:

Root-knot nematodes may be controlled in three general ways, by the use of resistant varieties, by chemicals, and by cultural practices. Control may also be obtained by sanitation to prevent the establishment of nematodes in a clean field.

Resistant varieties, when available, are the most effective way to control root-knot nematodes. The method is easy to use, safe, and has no adverse effects on the environment. But resistant varieties have not been developed in all susceptible crops and new races of nematodes have been found which can infect previously resistant varieties.

When resistant varieties are not available, chemical control is the next most effective method, especially when used in combination with crop rotation. But the cost of this control method can be uneconomical for low value crops. Also, every year more and more nematicides are being banned due to side effects on the environment. Thus, the agrichemical companies are reluctant to develop and register

new nematicides. In turn, farmers find it increasingly difficult to use this method of control.

Adoption of appropriate cultural practices is the third method of nematode control. While less effective than resistance or chemicals, this is often the only practical choice available. This method will not eliminate the nematodes in the soil, but it can lower the population to a tolerable level so it is possible to grow a susceptible crop for a season or two. Cultural practices would include fallowing, crop rotation, and trap cropping. Trap cropping is especially valuable for the home gardener because it is safe to use, the instructions are simple, and it works well under many conditions (Motsinger, 1979).

Genetics of the Host:

Nematode resistance conferred by the Mi gene was first discovered in L. peruvianum P.I. No. 128657. Smith (1944) transferred the gene to L. esculentum by using embryo culture to get around the problem of cross incompatibility. Cuttings of this progeny were obtained by Watts (1947) who then back-crossed the hybrid to L. esculentum. He reported an 8.76 to 7.24 resistant to susceptible ratio and indicated resistance was controlled by two dominant factors. Frazier and Dennett (1949) obtained this source of nematode resistance from Watts and reported that no more than two major genes were involved. Also, some 3:1 fits had been obtained and they concluded that this still could be a 2 gene system with one gene pair homozygous.

Gilbert and McGuire (1952), working with the same material, isolated true-breeding resistant lines. They obtained both 3:1 and a

few 13:3 F_2 ratios and concluded resistance was controlled by one major gene and a modifier. In 1955, Gilbert and McGuire concluded there was one dominant gene in linkage group IV. Barham and Windstead (1957) suggested that the Mi gene was incompletely dominant or dominant whereas Harrison (1960) suggested that the resistance was controlled by one dominant gene or a block of genes acting as a unit.

At the present time, it is agreed that the Mi gene for root-knot nematode resistance is a dominant gene with no modifiers, located on chromosome #6, 6-35 map units from the centromere (Rick, 1978; Sidhu and Webster, 1973, 1975; Gilbert, personal communication, 1978).

Over 100 cultivars of tomato have been introduced up to the present time that are resistant to root-knot nematodes (Singh et al., 1974; Hartmann, 1978; Tanaka, personal communication, 1979).

However, there are reports that cultivars with the Mi gene do not always respond to nematodes in the same manner (Sikora, et al., 1973; Khan et al., 1975; Dhillon et al., 1975). Healani and Kalohi both possess the Mi gene (Gilbert, personal communication, 1979), yet sometimes show a difference in root galling indicies (Khan, 1975). When 'Kolohi' was crossed with a susceptible line 24/2 the hybrid had 7.0 galls per plant, but when 'Healani' was crossed with 24/2 the hybrids were free of galls.

Sidhu and Webster (1973) reported three possible genes other than the Mi gene. They designated the three genes as $LMiR_1$, $LMiR_2$, and $LMir_3$ in the cultivars 'Nematex', 'Small Fry', and 'Cold Set', respectively. In linkage and allelic relationship experiments (Sidhu and Webster, 1975), they found that Mi, $LMiR_1$, and $LMiR_2$ are dominant,

while $LMir_2$ is recessive. $LMiR_1$ and $LMiR_2$ were closely linked, about 5.65 morgan unites apart with the Mi gene similar or allelic to $LMiR_1$. They also reported that they could not establish the relationship of $LMir_3$ to the other resistant genes. No subsequent publication on these genes have appeared.

Variability of the Pathogen:

Throughout history, several investigators have reported the occurrence of races, biotypes, host races, etc. of M. incognita as with other pathogenic organisms (Allen, 1952; Christie, 1946; Dropkin, 1969A, Goodey, 1932; Goplen et al., 1959; Netscher, 1976, 1977; Riggs and Winstead, 1959; Santoso, 1973; Southards et al., 1973; Viglierchio, 1978). Biotype has been the choice of nomenclature in defining these occurrences where nematodes are involved (Sturham, 1971). The populations show no or little morphological differences, but very definite differences in biology such as food preference.

Netscher (1976). reported two races of M. incognita in Senegal, a natural population unable to infect resistant tomato which he called A-race and a resistant breaking type he designated B-race which was able to infect the cultivar Hawaii 5229. Riggs and Winstead (1969), working with cultivars 5229 and STEP 174, found differences in the galling index from 2 populations of nematodes. The galling indexes were 0.06 and 3.69, respectively, form the 2 populations on 5229, but 4.00 and 3.50 on STEP 174.

Southards et al. (1973), working with isolates from 17 locations, and Viglierchio (1978) with 10 locations, found populations to vary in

pathogenicity. Southards used only the susceptible 'Rutgers' tomato and found galling ranging from 6.5-9.9 with 1 = no galls and 10 = severe galling. Viglierchio also found galling to vary from location to location, from zero plants with galls to all plants with galls.

Many investigators have reported variation in root-knot nematodes when working with only a single egg mass culture (Allen, 1952; Martin, 1954; Riggs and Winstead, 1959; Sturhan, 1966A; Triantaphllou and Sasser, 1966).

Riggs and Winstead (1959) reported that a few offspring of cultures from a single egg mass from a single larva could parasitize and develop on resistant plants. They then postulated that the resistant plants are not the cause of a new strain, but merely selected those which are able to overcome the resistance and survive.

Nematode biotypes may be 'bred' or selected for the resistance-breaking character in a number of ways. Many investigators agree that the genetic variations to form new biotypes are already present in the population. Isoenzyme studies show that Meloidogyne populations, in spite of their parthenocarpic reproduction, exhibit genetic variability (Dalmasso and Berge, 1978).

Pehnotypic variations that reflect both genetic differences as well as environmentally induced modifications can be found within populations of nematodes, hence, the development of new biotypes may be nothing more than the selection of the best adapted gene combination (Sturhan, 1971; Kehr, 1966; Brun, 1966; Sasser and Nusbaun, 1955). Sasser and Nusbaun, (1955) reported that in a 2-year rotation in which tabacco was rotated with corn, M. incognita at first reproduced well on tobacco but not on

corn. After 4 or 5 cycles, M. incognita reproduced well on both crops, showing equal pathogenicity.

In an unusual case, a resistant breaking biotype was reported from Senegal (Netscher, 1976) that developed without any inducement, and caused heavy galling of resistant tomatoes on newly cleared land never known to be cultivated.

At the present time when reference is made to 'races' of nematode which attack tomato, the race which attacks only susceptible lines is designated A, the race able to attack a resistant as well as the susceptible lines is designated B, the next pathotype C, etc. (Sturhan, 1971). Sasser (1978) has developed a method of race differentiation using selected host plants and has reported 4 races of M. incognita. He does not indicate if any of these races will attack root-knot resistant tomatoes.

MATERIALS AND METHODS

Parental Lines:

For this study, six Lycopersicon esculentum cultivars were chosen on the basis of their reported genotypes for resistance to root-knot nematode, M. incognita (Table 1). Also, two L. peruvianum and one L. glandulosum lines were chosen because of their reported (Santoso, 1973) low gall number due to a B-race of M. incognita. 'Lanai' is an old cultivar selected in Hawaii and was chosen to serve as a susceptible check. 'Healani' is another selection made in Hawaii in the early 1960's and has the Mi gene for nematode resistance. 'Nemared' and 'VFN-8' are two other lines which also have the Mi gene. 'Nemared' is a home/market type adapted to the Southeastern United States. 'VFN-8' is a market/cannery type with adaptation to the Western United States. 'Small Fry' is a cherry type tomato which contains the resistant gene $LMiR_2$ (Sidhu and Webster, 1973). 'Cold Set' is a market/cannery type tomato adapted to Canada which carries a recessive gene for resistance, $LMir_3$ (Sidhu and Webster, 1973).

Sidhu and Webster (1973) also described another source of resistance to M. incognita, $LMiR_1$, found in the cultivar 'Nematex'. This was not obtained in time to include it in the experiment.

The parental lines were tested for their galling response in the same manner and at the same time as the F_2's.

Crossing Method:

All crosses were made between 8:00 a.m. to 12:00 noon in the months of August and September, 1978 on two plants of each cultivar.

Table 1

List of Parental Lines

Parent	Scientific Name	Source of Seed	Reported Genotype	Author
Lanai	L. esculentum	U.H. Horticulture Department	mi	-
Cold Set	"	Stokes Seed Inc.	$LMir_3$	Sidhu, Webster, 1973
Healani	"	U.H. Horticulture Department	Mi	Gilbert, 1966
Nemared	"	Oklahoma Foundation Seed Stocks Inc.	Mi	
Small Fry	"	Vaughan Jaklin Corp.	$LMiR_2$	Sidhu, Webster, 1973
VFN-8	"	Ferry Morse Seed Comp.	Mi	
PI 126431	L. peruvianum	Regional Plant Introduction Station Ames, Iowa	unknown	Santoso, 1973
PI 126443	L. glandulosum	Regional Plant Introduction Station Ames, Iowa	unknown	Santoso, 1973
PI 129152	L. peruvianum	Regional Plant Introduction Station Ames, Iowa	unknown	Santoso, 1973

Crosses were made in all combinations among the L. esculentum lines to establish F_2 populations to compare the genetic ratios from the different genes for resistance. Crosses were not successful with the L. peruvianum and L. glandulosum lines.

F_1 seeds were planted in flats and transplanted into fumigated fields at Poamoho Experimental Farm. F_2 seeds were collected from open pollinated fruits from 5 plants of each cross.

Testing of F_2 Progeny:

All nematode tests were done at the Poamoho Experimental Farm in central Oahu, which is 200m above sea level and has a Wahiawa silty clay soil type. F_2 seeds were planted in speedling trays and then transplanted at 5-6 weeks of age into two fields at Poamoho Experimental Farm which have given different results with root-knot nematode resistant tomatoes having the Mi gene (Holtzmann and Gilbert, 1969; Santoso, 1973). Field J has a biotype of nematode which does not produce galls on tomato cultivars with the Mi gene. Field Q_1, however, has a biotype of nematode which can produce galls on tomato cultivars including those with the Mi gene.

Before running a nematode test, the nematode populations in the fields were increased by planting a susceptible crop. In field J, the susceptible crop used was cantaloupe, while in field Q_1, the susceptible crop was 'Healani' tomato, which carries the Mi gene, but is galled in field Q_1. In order to further guard against escapes, each F_2 test plant was planted together with a check plant; 'Lanai' tomato in field J, 'Healani' tomato in field Q_1. Only test plants which were paired

with a galled check plant were included in the data. Test plants with either missing or low galling index number (1 or 2) check plants were eliminated from results.

The field was arranged as a randomized complete block with three replications. Each plot consisted of 20 plants spaced 60 cm apart in rows spaced 120 cm apart. Plants were dug 80-90 days after transplanting and the roots of each test plant and check plant evaluated for galling.

Method of Evaluation:

F_2 plants were scored in the following manner:

Class 1 - Shows no visible galls of any size

Class 2 - Has one or a very few tiny galls (10 galls less than 1/16" in diameter)

Class 3 - Has a large number of small galls and/or a few medium size galls

Class 4 - Has larger galls than those in Class 2 or 3, but not as extensively galled as class 5)

Class 5 - Extensively galled (galls coalesce into one large gall the length of the root)

Note: A single fairly large gall is sufficient to place a plant in class 4. Classes 1 and 2 are generally considered genetically resistant, while classes 3, 4, 5 are considered genetically susceptible (modified from Gilbert and McGuire, 1955).

Statistical evaluations were done with the Chi-square Goodness of fit test (Snedecor and Cockran, 1967) to determine the probability that observed qualitative genetic ratios fit those expected on the basis of the parental responses.

RESULTS AND DISCUSSION

Field Q_1:

Field Q_1 is the field in which a biotype of M. incognita can infect tomato cultivars with the Mi gene for resistance. No apparent resistance to this biotype was detected among the tomato lines tested in this study (Table 2). The only significant difference detected was between Healani, the most susceptible line and Cold Set, the least susceptible.

'Cold Set' is the only cultivar which was statistically more resistant than any of the others. It had 5 out of 53 plants which were rated '2'. 'Cold Set' has been reported to have a gene for resistance which is both different from the Mi gene and recessive in action (Sidhu and Webster, 1973), so it would not be surprising if it reacts differently than other cultivars. However, very few plants in this cultivar show the resistance, which suggests either that the gene is very susceptible to environmental influences, confers only partial resistance, or only some of the plants carry the gene.

The F_2 progeny were also tested in Field Q_1 (Table 3). No differences in resistance were observed. As with the parental lines, nearly all plants were definitely galled, but a very few plants were classified as '2'. The one exception was in the cross 'Cold Set' x 'Nemared', in which 10 out of 52 plants were rated either '1' or '2' (resistant). This ratio fits a 1 resistant: 3 susceptible ratio ($X^2 = 1.10$, $p = .50-.10$), which would be expected if 'Cold Set' carries a recessive gene for resistance as reported by Sidhu and Webster (1973). However, this was the only cross in which 'Cold Set' was involved that

Table 2

Galling Observed in Parents in Field Q_1

Parents	No. Plants	Galling class 1	2	3	4	5	$\bar{x}^z$	Observed R:S
Lanai	57	0	1	6	24	26	4.31 ab	1:56
Cold Set	58	0	5	23	15	15	3.68 b	5:53
Healani	60	0	0	1	7	52	4.85 a	0:50
Nemared	59	0	1	8	20	30	4.35 ab	1:58
Small Fry	55	0	2	6	8	41	4.71 ab	2:53
VFN-8	54	0	0	13	21	20	4.07 ab	0:54
PI 126443	60	0	1	5	10	38	4.12 ab	1:59
PI 129152	53	0	2	15	16	20	4.02 ab	2:51
PI 126431	59	0	0	4	10	45	4.70 ab	0:59

[z]Duncans multiple range test, 5% significance level. Means followed by the same letter are not significantly different.

Table 3

Galling Observed on F_2 Progeny in Field Q_1

Parents	No. Plants	Galling class 1	2	3	4	5	$\bar{x}^z$	Observed R:S
Lanai x Healani	58		2	20	14	22	3.97	2:56
Lanai x Cold Set	59		1	16	16	23	4.09	2:57
Lanai x Small Fry	52		2	21	11	18	3.83	2:52
Lanai x VFN-8	55			7	16	32	4.45	0:55
Lanai x Nemared	56		3	19	15	19	3.89	3:53
Healani x Cold Set	51			10	22	19	4.18	1:51
Healani x Small Fry	54			7	9	38	4.57	1:53
Healani x VFN-8	59			5	25	29	4.58	0:59
Healani x Nemared	53		2	25	13	13	4.53	2:51
Cold Set x Small Fry	53		4	17	17	15	4.75	4:49
Cold Set x VFN-8	51		2	5	17	30	4.41	2:49
Cold Set x Nemared	52	3	7	18	14	10	3.40	10:42
Small Fry x VFN-8	46	1	1	24	16	4	3.34	2:44
Small Fry x Nemared	59		1	15	17	26	4.14	1:59
VFN-8 x Nemared	57			12	13	32	4.35	0:57

[z]Duncans multiple range test, 5% level; no significant differences.

showed any resistance in this field, so it cannot be concluded that Sidhu and Webster's report is confirmed. However, since the 'Cold Set' parent was not uniform for its response to the Q_1 strain of nematode (Table 2), it is possible that one of the plants of 'Cold Set' that were used to make crosses, the one crossed with Nemared, carried the gene reported by Sidhu and Webster, while the second was not resistant and was used for the remaining crosses. Further work is necessary to identify the cause of the resistance observed here in 'Cold Set'.

Field J:

The nematode population in field J has previously been observed to be the normal type generally called Race 'A', for which the resistance conferred by the Mi gene is effective. The galling observed for the parental lines shows clear differences between different lines (Table 4). Lanai was significantly more susceptible than all other lines except Nemared. Healani, VFN-8, P.I. 126443, P.I. 126431, and P.I. 129152 were significantly more resistant than all other lines except Small Fry. Nemared, Cold Set, and Small Fry all included a range of responses. Since Healani and VFN-8, which carry the Mi gene were nearly free of galls, while Lanai was heavily galled, the Mi gene if effective in this field. The L. peruvianum and L. glandulosum lines were also almost completely free of galls, which suggests that perhaps Santoso (1973) inadvertently tested these lines against this nematode strain, rather than the field Q_1 strain as he reported.

Nemared is also supposed to carry the Mi gene, but its response does not indicate that it is uniform in this respect. Obviously, a

Table 4

Galling Observed in Parents in Field J

Parents	No. Plants	Galling class 1	2	3	4	5	$\bar{x}^z$	Observed R:S
Lanai	60					60	5.00 a	0:60
Cold Set	52	8	11	16	21	5	2.86 b	19:33
Healani	55	55					1.00 c	55:0
Nemared	53	17	2	1	4	29	3.52 ab	19:34
Small Fry	55	27	2	7	8	11	2.51 bc	29:26
VFN-8	60	58	1	1			1.05 c	59:1
PI 126443	58	56	2				1.05 c	58:0
PI 129152	48	48					1.00 c	48:0
PI 126431	56	56					1.00 c	56:0

[z]Duncans multiple range test, 5% significance level. Means followed by the same letter are not significantly different.

considerable portion of the Nemared plants do not have any resistance and it must be concluded that a mixed lot of seed was received for this cultivar.

Cold Set and Small Fry, which are supposed to have the $LMir_3$ and $LMiR_2$ genes, respectively, also seem not be uniform for their nematode response. Small Fry generally responds similarly to Nemared, with some clearly resistant plants and other clearly susceptible plants. Cold Set, however, has mostly intermediate types. Since Cold Set is supposed to have a recessive resistant gene instead of a dominant one as the other cultivars, and it is a cultivar which is more adapted to Canadian conditions, it is possible that the higher temperatures and shorter daylengths under which it was grown have affected its response to M. incognita. It also seems likely that it, too, like Nemared and Small Fry, is a mixed lot of seed.

F_2 Segregations:

The three uniform parents, Lanai, Healani, and VFN-8 behaved predictably in their crosses (Table 5). When Healani or VFN-8 were crossed to the susceptible Lanai, a 3 resistant: 1 susceptible ratio was observed in the F_2. When the two parents with the Mi gene were crossed with each other, all the F_2 progeny were resistant.

Nemared is also supposed to have the Mi gene. Although the parental line was not apparently uniform for this gene, the results of the F_2 crosses between Nemared and Lanai, Healani, and VFN-8 (Table 6) indicate that the two Nemared plants used to make the crosses both carried the Mi gene, because a 3:1 ratio was observed in the cross with Lanai as

Table 5

Galling Observed of F_2 Progeny of Crosses Between Lanai, Healani, and VFN-8 in field J

Parents	No. Plants	Galling class 1	2	3	4	5	Observed R:S	Expected ratio	X^2 Value	Prob.
Lanai x Healani	56	34	7	6	2	7	41:15	3:1	0.0718	.90-.50
Lanai x VFN-8	56	43	1	1	5	6	44:12	3:1	0.4995	.50-.10
Healani x VFN-8	50	37	11	1	1		48:2	1:0		

Table 6

Galling Observed of F_2 Progeny of Crosses Between Nemared and Lanai, Healani, and VFN-8 in field J

Parents	No. Plants	Galling class 1	2	3	4	5	Observed R:S	Expected ratio	X^2 Value	Prob.
Lanai x Nemared	53	33	3	3	5	9	36:17	3:1	1.216	.50-.10
Healani x Nemared	50	38	5	4		3	43:7	1:0		
VFN-8 x Nemared	47	40	3		1	3	43:4	1:0		

expected and 1:0 ratios were observed in the crosses with Healani and VFN-8, also as expected. It can be concluded, then, that Healani, VFN-8, and Nemared all carry the same gene for resistance, Mi.

The results of the crosses involving Small Fry are given in Table 7. According to Sidhu and Webster, Small Fry carries a dominant gene, $LMiR_2$, which is closely linked to a different dominant gene carried by the Nematex cultivar, which is similar or allelic to Mi. The F_2 results observed are not as would be expected if Small Fry carries a dominant gene for resistance, either the same or different from the Mi gene in Healani, VFN-8, and Nemared. When crossed with Lanai, which has no resistance, there were more susceptible than resistant plants in the F_2 and the observed ratio actually fit a 1 resistant: 3 susceptible ratio (X^2 = .23, p = .90-.50), suggesting that Small Fry carries a recessive gene for resistance. A more likely explanation, however, is that one of the plants of Small Fry used to make crosses was resistant, but the other was not, and the F_2 progeny includes a mixture of plants from the two parents. The ratios of the F_2 progenies of the crosses between Small Fry and the three cultivars known to carry the Mi gene all gave ratios of 3 resistant: 1 susceptible, which suggests that the Small Fry parent contributed no resistance at all. Apparently, the plant of Small Fry used in all 3 of these crosses was one which had no resistance.

The results of the crosses involving Cold Set are given in Table 8. Cold Set carries a recessive gene for resistance, according to Sidhu and Webster (1973). Again, as in the crosses with Small Fry, the F_2 segregations observed in the crosses with Cold Set are not what would be expected if Cold Set is uniform for the gene reported. When crossed

Table 7

Galling Observed on F_2 Progeny of Crosses Between 'Small Fry', and Lanai, Healani, VFN-8, and Nemared in field J

Parents	No. Plants	Galling class 1	2	3	4	5	Observed R:S
Small Fry x Lanai	57	11	5	5	10	26	16:41
Small Fry x Healani	56	38	3	3	6	6	41:15
Small Fry x VFN-8	56	32	5	7	6	1	40:16
Small Fry x Nemared	53	35	4	1	8	4	39:14

Table 8

Galling Observed on F_2 Progeny of Crosses Between 'Cold Set' in field J

Parents	No. Plants	Galling class 1	2	3	4	5	Observed R:S
Lanai x Cold Set	58		1	2	18	37	1:57
Healani x Cold Set	56	38	4	5	6	3	42:14
VFN-8 x Cold Set	49	34	3	3	6	2	38:11
Nemared x Cold Set	56	35	5	5	5	6	40:16
Small Fry x Cold Set	53	20	5	5	9	14	25:28

with Lanai, no resistance was observed in the F_2, indicating that the Cold Set plants used to make this cross had no resistance. In the F_2's of the crosses with Healani, VFN-8, and Nemared, the results fit a 3 resistant: 1 susceptible ratio as would be expected if only the Mi gene for the latter cultivars was involved. However, if Cold Set did carry a separate recessive gene for resistance, the ratio expected would be 13 resistant: 3 susceptible. The observed numbers also fit this ratio and it is not possible to distinguish between the two ratios with the small number of plants observed. This leaves the cross between Cold Set and Small Fry, in which a ratio of 25 resistant: 28 susceptible was observed. This ratio is not one that would be expected from any combination of dominant and recessive genes in Small Fry and Cold Set, so the only conclusion is that it has again resulted from using a mixture of parental plants that were not uniform in their genetic constitution.

SUMMARY AND CONCLUSION

Two different nematode races were observed in fields Q_1 and J. In field Q_1, all cultivars were heavily galled (susceptible) with the possible exception of 'Cold Set' which produced a few resistant plants. In field J, however, the cultivars 'Healani', 'VFN-8', P.I. 126443, P.I. 126431, and P.I. 129152 were completely resistant, Nemared, Small Fry, and Cold Set exhibited a range of resistance, and Lanai was completely susceptible. It is concluded, therefore, that the nematode race in field J is the normal or 'A' race, while the race in field Q_1 is a different or 'B' race which can infect resistant tomato plants.

It appears possible that plants with the Mi gene (Healani, VFN-8, Nemared) and the $LMiR_2$ gene (Small Fry), the dominant genes, may react in one way to the B-race nematodes, whereas plants with the recessive gene, $LMir_3$ (Cold Set) respond differently and are able to withstand attack.

Intercrossing Healani, VFN-8, and Nemared, as well as crossing them to the susceptible Lanai, confirmed that all three cultivars carry the same gene for resistance, Mi. Since the three P.I. lines behaved similarly to the cultivars with the Mi gene in both fields, it is likely that they also carry the Mi gene, although this could not be confirmed by progeny segregations. The Mi gene originally came from L. peruvianum, so it is likely that other lines of this species may carry the gene also.

The 'Small Fry' and 'Cold Set' seed sources used are suspected to have not been uniform. The consequent variable responses in crosses made with these 2 parents prevent any conclusion on allelism of their

genes to each other and the Mi gene from being made, although transmission of resistance was observed in some crosses.

It was not possible to accomplish the objectives of this study to compare the genes Mi, $LMiR_1$, $LMiR_2$, and $LMir_3$ for root-knot nematode resistance because of the unexpected variability found in the parents. To overcome this problem, either the parents must be selected for homogeneity or each individual plant must be separately identified in the crosses for which it is used. Also, more F_2 plants must be grown so it is possible to distinguish between 3:1 and 13:3 genetic ratios in crosses where 1 dominant and 1 recessive gene is segregating. Little and Hill (1977) suggest 700 plants for the 5% level of significance and 1184 plants for the 1% level, using the X^2 goodness of fit test. Testing such large numbers of plants might be done in pots in a greenhouse with controlled temperature.

Further studies are necessary to determine the nature of the resistance of 'Cold Set'. The resistance observed to the nematode race in field Q_1 should be investigated to see if it is due to genes or other factors. The nematodes in fields Q_1 and J should also be tested with Sasser's (1976) multiple host range test to identify the species and races to allow comparison with similar studies elsewhere.

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