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REPRODUCTION OF ROOT KNOT NEMATODE (Meloidogyne incognita) ON POTENTIAL TOMATO (Lycopersicum esculentum) CULTIVARS AND BREEDING LINES

ABSTRACT

*Two tomato cultivars and five (5) potential breeding lines from the Institute of Plant Breeding (IPB), UP at Los Banos, College, Laguna were evaluated under greenhouse conditions to determine the reproduction of root knot nematode, Meloidogyne incognita (Kofoid and White,1919). Reproductive factor (Oostenbrink, 1966) (Rf=Pf/Pi) was calculated for each cultivar/ breeding line. The breeding lines S602 and S610 supported lower population densities of **M. incognita** but they did not differ significantly with S611 and S608. The highest reproductive value was recorded in Apollo with 19.30; however, it was comparable to Anath and 20-05, with 15.30 and 15.17, respectively. Based on the criteria proposed by Robinson (1980) all the tomato cultivars and breeding lines tested are considered efficient hosts of **M. incognita**. However, additional trials are recommended to have more conclusive results.*

INTRODUCTION

Root-knot nematodes of the genus Meloidogyne are among the main pathogens of tomato crops worldwide. Infected plants show an aberrant development of the root system characterized by the formation of typical galls that alter the uptake of water and nutrients and interfere with the translocation of minerals and photosynthates (Williamson and Hussey, 1996). As a result, above ground deficiency symptoms appear which may lead to severe yield decreases, depending on the severity of infestation.

Because of the adverse effects associated with the use of chemi-cal nematicides, plant resistance is currently considered as the method of

choice for controlling root knot nematodes. Resistance to Meloidogyne spp. was observed originally in some accessions of the wild tomato species Lycopersicon peruvianum (Bailey, 1941), and subsequently shown to be due to a single dominant gene named Mi (Gilbert and McGuire, 1956). Further studies demonstrated that this gene controls the three major species: Meloidogyne arenaria, M. incognita and M. javanica (Barham and Win-stead, 1957). The Mi gene was transferred from L. peruvianum PI128657 into L. esculentum using embryo rescue (Smith, 1944). According to Jac-quet et al (2005) reproduction of the above nematodes was similar to or more often significantly higher on heterozygous tomato genotypes than on homozygous ones, suggesting a possible dosage effect of the Mi gene. Data also indicated that the tomato genetic background had a major effect on the variations observed in nematode reproduction, especially when tomato genotypes were heterozygous for the Mi gene. These results have important consequences in terms of breeding strategies and durability of the resistance conferred by the Mi gene.

In the Philippines, precise information on yield loss due to root knot nematode is still scanty. An initial infestation of 100-5000 M. incognita larvae per hill has been found to cause 24.1 % to 38.7% reduction in tomato yield (Ducusin and Davides, 1971). Likewise, studies on the pathologic reaction of tomato varieties to Meloidogyne spp. are very few. Davide and Deanon (1967) reported that out of 13 tomato lines tested, only eight (8) showed resistance to M. incognita. On the other hand, of the 17 tomato lines evaluated for resistance to the above nematode, 4 showed intermediate reaction while 13 were susceptible (Reyes and Villanueva, 1981).

This study was conducted at the Biological Control Laboratory and Horticulture Research and Training Institute (HORTI) Greenhouse, Benguet State University, La Trinidad, Benguet from June 2006 to January 2007 to determine the reproduction of Meloidogyne incognita on potential tomato cultivars and breeding lines.

MATERIALS AND METHODS

The study was conducted at Biocon Laboratory and Greenhouse, Horticulture Research and Training Institute, Benguet State University, La

Trinidad, Benguet from September, 2006 to February, 2007. Two toma-to cultivars and five potential breeding lines from the Institute of Plant Breeding (IPB) UP at Los Baños, College, Laguna were used in the study namely, Anath, Apollo, 20-05 S602, S608, S610 and S611. For each variety there were three (3) replicates with 3 sample plants per replication. The pots were arranged randomly in the greenhouse using the Randomized Complete Block Design (RCBD) with factorial arrangement.

Establishment of *M. incognita* in Culture

M. incognita was established from a single egg mass derived galled roots of tomato grown in sterile soil. The culture plants were arranged with 15-cm spacing in the greenhouse. When the culture plants matured, the nematode was again re-established in new plants in sterile soil to provide inocula for the succeeding experiments.

Preparation of Nematode Inoculum

Infected roots were gently washed to remove adhering soil particles and shaked vigorously in a stoppered flask containing 1% NaOCl solution (commercial bleach) The liquid was poured over nested sieves and the collected eggs were thoroughly washed with water to remove the bleach. Nematodes in water suspension were standardized by determining the number of eggs in a known volume. The suspension was agitated by stirring and blowing of pipette to make air bubbles while an aliquot was being withdrawn to ensure the even distribution of nematodes. When the tip of the pipette was midway between the top and the bottom of the liquid, an aliquot was immediately drawn and released into a counting dish. The number of eggs in a 1 ml aliquot was counted under a stereomicroscope and multiplied by the to-tal volume of the suspension (Shepherd, 1986). One thousand five hundred juveniles were used for inoculation per pot of the inoculated treatments.

Inoculation

Plants were inoculated two weeks after transplanting to allow sufficient development of the roots and were maintained until harvest. Inocula of 1500 juveniles per plant were used.

The soil used in the experiment was obtained from Benguet State

University Experimental area located at Balili, La Trinidad, Benguet, containing a mixture of sand and rice hull. It was heat-sterilized in an autoclave at 15 psi to get rid of the soil borne pathogens that might contaminate and affect the development of the nematode. After sterilization the soil was placed in plastic pots with 20-cm diameter.

Data Gathered

To determine whether the population of root knot nematode decreased or increased, 200 cm3 of soil samples were obtained from each pot and processed using the modified Baermann-tray technique. The nema-todes were collected separately in 150-ml capacity Erlenmeyer flasks. The suspensions were allowed to stand at least 3 hours at room temperature and the excess water in the flasks was reduced to 50 ml by suction using a pipette. The nematodes were transferred in 50-ml capacity test tubes and were again allowed to stand at least one hour for nematodes to settle at the bottom. Nematodes were counted under stereomicroscopic. Likewise, population counts of nematodes in feeding position were made from 1-gram root samples taken at random per treatment. The roots were stained in acid fuchsin glycerol to facilitate counting. A reproductive factor (Oosten-brink, 1966) (Rf=Pf/Pi) was calculated for each replicate. All count data were transformed to log10 (x + 1) before calculation of Rf values and statis-tical analysis. Transformed nematode counts and Rf values were analyzed statistically using Analysis of Variance (ANOVA) of the MSTAT-C Package Software. Significant differences between means were partitioned using Duncans Multiple Range Test (DMRT).

RESULTS AND

DISCUSSION Nematodes in the Roots

Figure 1 shows the effect of tomato cultivars and potential breeding lines on the number of nematodes in the roots. Apparently, the highest num-ber of advanced second stage juveniles was noted in cultivar Apollo (7,066) however, it was not significantly different from cultivar Anath (3,255), and the breeding lines 20-05 (3,094) and S602 (3,261) S610 (2,658) and S608 (2,548). The lowest nematode count was recorded in breeding line S611; this was significantly different from cv. Apollo.

Significantly more number of second stage juveniles developed into third and fourth stage juveniles in cultivars Apollo and Anath, and breeding lines 20-05, S608, however, they were comparable to S610 and S611 (Fig. 2). On the other hand, the lowest number of third and fourth stage juveniles was recorded in breeding line S602. This was significantly different from Apollo, Anath, 20-05 and S608, but at par with S610 and S611.

The highest number of adult female nematodes was recorded in cultivar Anath (5328). This was followed closely by cv. Apollo (4,348), 20-05 (3,914), and S611(3,668). The breeding lines S602 and S608 gave significantly lower number of mature females than cv. Anath but comparable to cv. Apollo, 20-05 and S611. The lowest number of juveniles that devel-oped into adult nematode was obtained from S610 with 1,190 (Fig. 3).

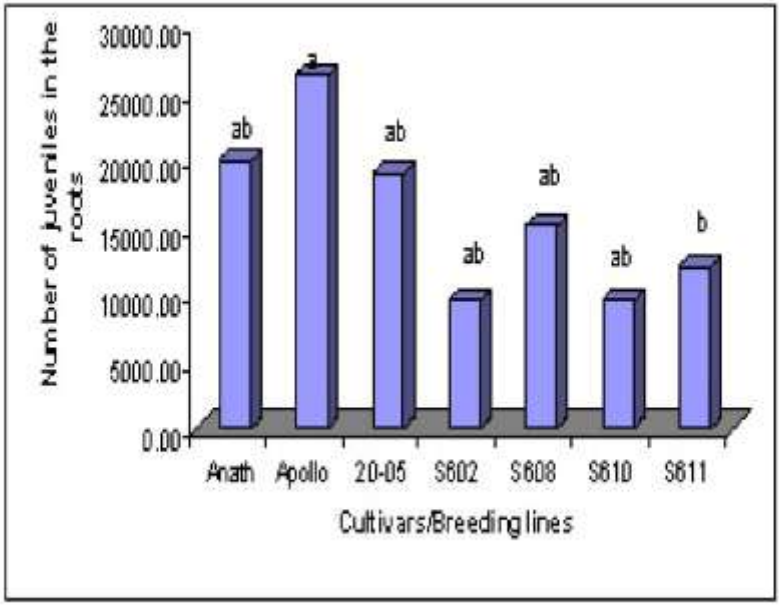

Fig. 1. *Number of J2 juveniles of M. incognita in the roots of tomato cultivars/ breeding lines*

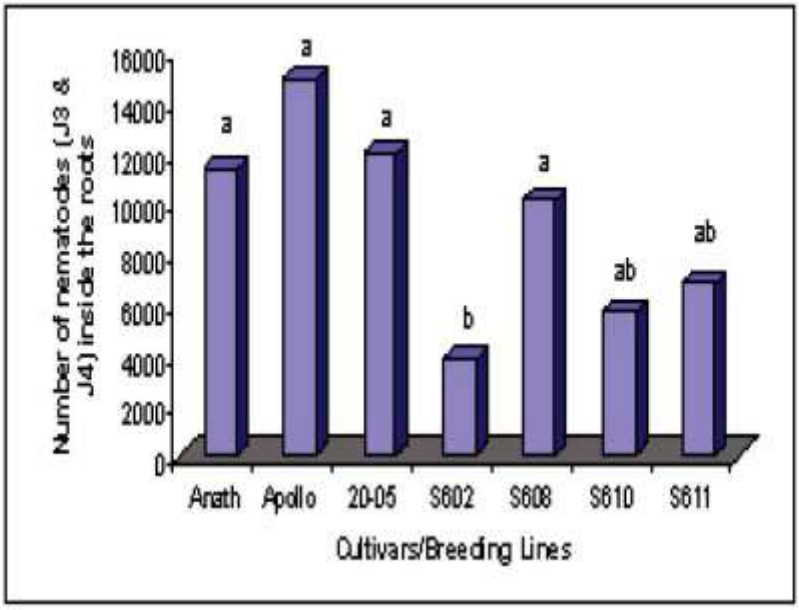

Fig. 2. *Number of J3 and J4 juveniles of M. incognita in the roots of tomato cultivars/ breeding lines*

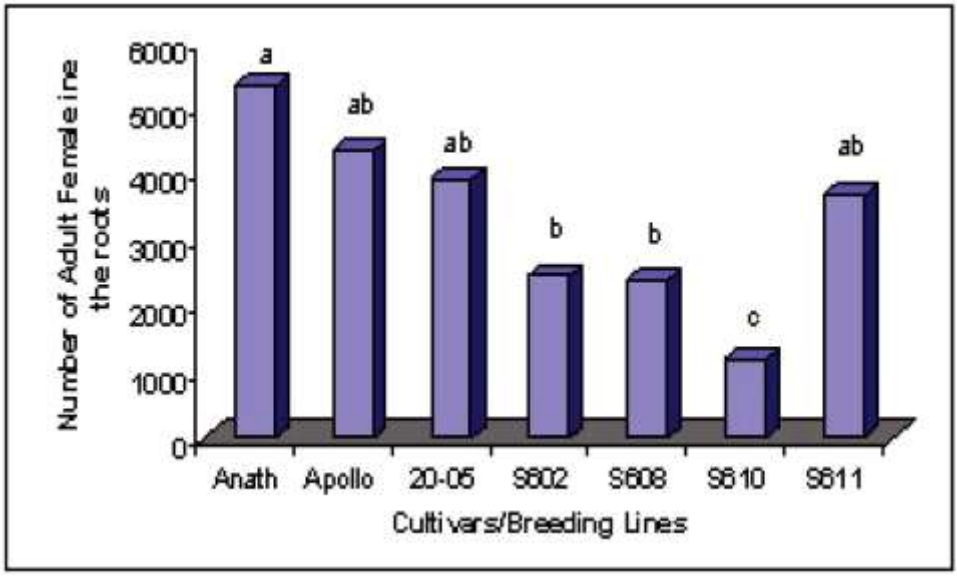

Fig. 3. *Number of adult female of M. incognita in the roots of tomato cultivars/ breeding lines*

When the total number of nematodes that penetrated the roots was computed, the highest was recorded in cv. Apollo but this was comparable to cv. Anath, 20-05 and S608. The lowest nematode count was noted in breeding lines S610 and S602, however, this was comparable to S611.

Nematodes in the Soil

The highest nematode count in the soil was obtained from the breed-ing line 20-05, but this was comparable to cv. Anath, S608, cv. Apollo, S602 and S611 (Fig. 4). On the other hand, S610 gave significantly the lowest nematode count although it was at par with S611. When the total number of nematodes in the roots and soil were combined, the same trend of host suitability was observed (Fig. 5).

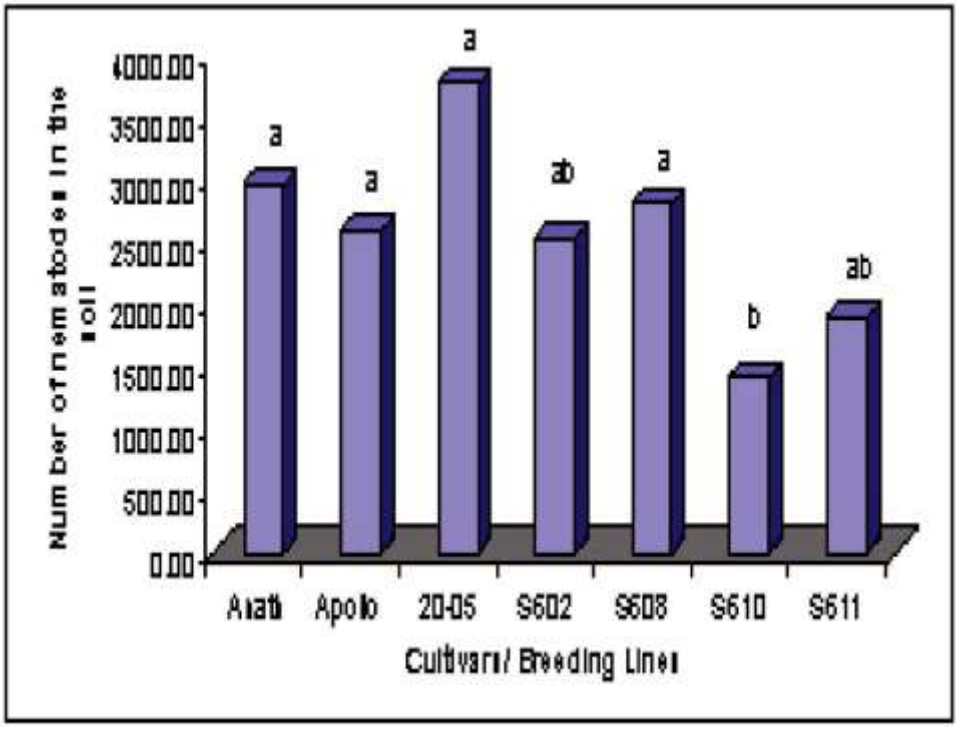

Fig. 4. *Number of J2 juveniles of M. incognita in the soil*

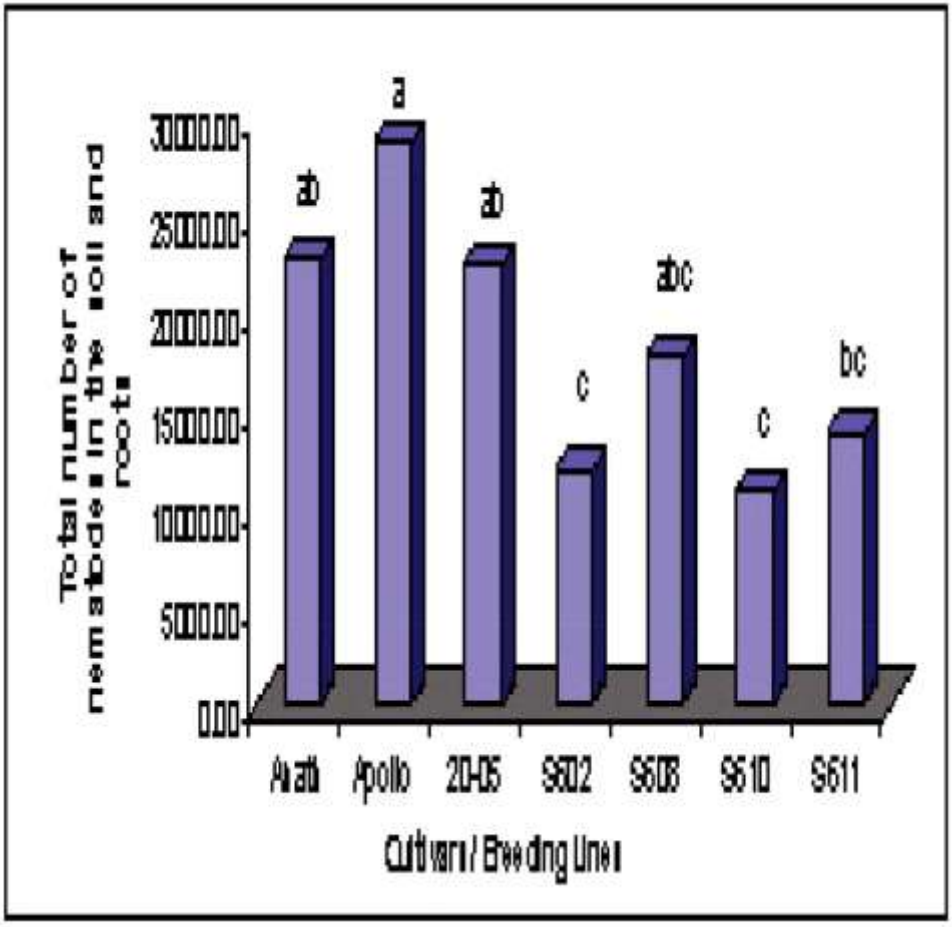

Fig. 5. *Total number of M. incognita in the soil and roots of tomato cultivars/breeding lines*

Nematode Reproduction

The reproduction of *M. incognita* in two tomato cultivars and five breeding lines is shown in Table 1. Apparently, the breeding lines S610 and S602 demonstrated the lowest reproductive factor with 7.31 and 8.04, respectively. They were significantly different from cvs. Apollo and Anath and breeding line 20-05, but comparable to S608 and S611 with reproductive values of 19.30, 15.30, 15.17, 11.92 and 9.32 respectively. According

to Robinson (1980) when the reproductive value is less than 1, the plant is considered non-host or non-efficient host; but when the reproductive factor is more than 1, it is considered efficient host. Based on the above criteria, the two cultivars and five potential breeding lines evaluated are considered efficient hosts of *M. incognita*.

Table 1. Reproduction of M. incognita on the potential tomato cultivars/ breeding lines

CULTIVARS/ ACCESSIONS	Pi_a	Pf_b	Rf_c
1– Anath	1500	22943.70	15.30_{ab}
2– Apollo	1500	28946.00	19.30_{a}
3– 20-05	1500	22749.00	15.17_{ab}
4– S602	1500	12056.00	8.04_{c}
5– S608	1500	17882.70	11.92_{abc}
6– S610	1500	10963.30	7.31_{c}
7– S611	1500	13979.30	9.32_{bc}

In a column, means followed by the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT).

a Initial population density
b Final population density
c Reproductive factor
(Rf=final population density / initial population density)

For a complete evaluation of plant response to nematode, two parameters should be measured: nematode reproduction and damage caused by the nematode (Jones, 1956). Host efficiency is usually not correlated with damage (Madamba et al., 1965) and a low level of parasite damage may be due either to a high level of resistance in the host or to a low level of parasitic ability in the parasite. Host efficiency is measured by gall index, number of egg masses/g of roots, females/g of root, eggs/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, 1979).

CONCLUSION AND RECOMMENDATION

Based on the criteria proposed by Robinson (1980) the two tomato cultivars and five breeding lines are considered efficient hosts of *M. incognita.* Additional trials however, are necessary in order to have more conclusive results. In addition, the effect of root knot nematodes on the growth and yield of tomato cultivars/breeding lines should also be determined.

LITERATURE CITED

BAILEY, D.M. 1941. The seedling test method for root knot nematode résistance. Proceedings of the American society for horticultural science 38:73-5.

BARHAM, W.S. N.N. WINSTEAD. 1957. Inheritance of resistance to root knot nematodes in tomatoes. Proceedings of the American Society for Horticultural Sciences 69: 372-377.

DAVIDE, R.G., & J.R.DEANON,JR. 1967. Reactions of various breeding lines of tomato and ampalaya to Meloidogyne incognita. Philippine Phytopathol. 3:4-5.

DUCUSIN, A.R. & R.G. DAVIDE. 1971. Meloidogyne incognita: Its effects on tomato yield and some methods of control. Philippine Agr. 55: 261-281.

FASSULIOTIS, G. 1979. Plant breeding for root knot nematode (*Meloidogyne Spp.*) systemics, biology and control, ed. F. Lamberti and C. E. Taylor. 477 pp.

GILBERT J.C, & D.C. MCGUIRE, 1957. Inheritance of resistance to severe root knot from *Meloidogyne incognita* in commercial type tomatoes. Proceedings of the American Horticultural Sciences 68:437-42.

JACQUET, M., M. BONGIOVANNI, M. MARTINEZ, P. VERSCHAVE, E. WAJNBERG &P.CASTAGNONE-SERENO. 2005. Variation in resistance to the root knot nematode, *Meloidogyne incognita* in to-mato genotypes bearing the Mi gene. Plant Pathology 54: 93-99.

JONES, F. G. W. 1956. Soil populations of beet eelworm (H*eterodera schacctii, Schm.*) in relation to cropping microplot and field plot re-sults. Ann Appl. Biol.44:25-56

MADAMBA, C.P., J. N. Sasser, and L. A. Nelson. 1965. Some characteris-tics of the effect of *Meloidogyne spp*. on unsuitable host crop. N. C. Agric. Exp. Stn. Tech. Bull. 169:1-3.

REYES,T.T. AND L.M.VILLANUEVA.1981. Screening of crop varieties for resistance to *Meloidogyne incognita:* Vegetable Varieities. Proceedings of the Third Research Planning Conference on Root-Knot Nematodes, Meloidogyne spp. International Meloidogyne Project . Contract No. AID/ta-c-1234. 76-79.

ROBINSON, R. A. 1980. New Concepts in Breeding for diseases resistance. Annu. Rev. Phytopathology18:189-210 .

SMITH, P.G. 1944. Embryo culture of a tomato species hybrid. Proceedings of the American Society for Horticultural Sciences 44: 413-416.

WILLIAMSON, V. M. and R. S. HUSSEY. 1996. Nematode pathogenesis and resistance in plants. Plant Cell 8:1735-45.

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