Research Article

Species of Root-Knot Nematodes (*Meloidogyne* spp.) Infecting Soybean in Different Localities of Egypt and Rating of its Local Genotypes for their Resistance to *Meloidogyne incognita*

Ahamed Mohamed Korayem, Moawad Mohamed Mohamed* and Mohamed and Mostafa Mohamed Attia Hamman

Plant Pathology Department, National Research Centre, El-Tahrir Str., Dokki, Giza, Egypt.

Abstract | Survey and identification of root-knot nematode species infecting soybean grown in six Egyptian governorates; Beni-Suef and Menia representing middle Egypt and Alexandria, El-Beheira, Kafr-Elsheikh and Dakahlia representing northern Egypt, was conducted during the growing season 2020. A total of 100 root samples were collected and processed for identification of root-knot nematode species. Data revealed the presence of three root-knot nematode species, they were *Meloidogyne incognita*, *M. javanica* and *M. arenaria*. The former species was the most frequent (58.8%) followed by *M. javanica* (31.9%) and *M. arenaria* (9.4%). Six Egyptian soybean cultivars; Giza-21, Giza-22, Giza-35, Giza-82, Giza-83 and Giza-111 were evaluated for their resistance to *M. incognita*. Data indicated that all cultivars were susceptible to the nematodes based on the nematode damage (root-gall index) and the rate of nematode reproduction (Rf).

Received | September 05, 2022; **Accepted** | February 15, 2023; **Published** | May 04, 2023
***Correspondence** | Mohamed M.M.M, Plant Pathology Department, National Research Centre, El-Tahrir Str., Dokki, Giza, Egypt; **Email:** moawad_bondok@yahoo.co.uk
Citation | Korayem, A.M., Mohamed, M.M.M., and Hammam, M.M.A., 2023. Species of root-knot nematodes (*Meloidogyne* spp.) infecting soybean in different localities of Egypt and rating of its local genotypes for their resistance to *Meloidogyne incognita*. *Pakistan Journal of Nematology*, 41(1): 18-23.
DOI | https://dx.doi.org/10.17582/journal.pjn/2023/41.1.18.23
Keywords | Root knot nematodes, Soybean and yield

Introduction

Soybean (*Glycine max* L.) is considered one of the greatest oilseed crops on the international level, as its seed have an oil content of 19-23% and protein content of 30-40% (Sikora *et al.*, 2018). So, its seeds are extensively used in the manufacture of animal and human food-stuff and other purposes such as the manufacture of plastics, lubricans, candles, varnishes, soap, biodiesal and lecithin (Fontana, 2011). Soybean was first cultivated for food in the temperate zone of northern China (Hymowitz, 1970). Currently it is grown in many tropical and subtropical regions, especially in South America, Brazil, the Far East and recently Africa (Hymowitz *et al.*, 2015).

The root-knot nematodes (*Meloidogyne* spp.) are considered serious pathogens of soybean causing considerable losses in its yield worldwide (Wrather and Koenning, 2009; Sikora *et al.*, 2010; Fourie *et al.*, 2015; Koenning, 2015; Li *et al.*, 2016; Lima *et al.*, 2017; Korayem and Mohamed, 2018).

Currently, there are more than 90 described root-knot nematodes species parasiting more than 3000 plant species worldwide (Trudgill and Blok, 2001; Karssen and Moens, 2006; Hunt and Handoo, 2009). Four species of root-knot nematodes; *Meloidogyne incognita, M. javanica, M. arenaria* and *M. hapla* are the major species recovered from soybean fields. The former three species are prevalent in the warmer climates causing significant reductions in soybean yield, while *M. hapla* occurs in colder climates and rarely causing economic loss (Schmitt and Noel, 1984; Noel, 2008; Fourie *et al*., 2015). *M. javanica* was reported as the most frequently species in Brazil with 64% frequency (Castro *et al*., 2003), while in the USA *M.incognita* was the most prevalent species (70%) followed by *M. javanica* (24%) and *M. arenaria* (6%) (Noel, 2008). *M. incognita and M. javanica* were the most species frequent detected in Argentina (Doucet and Pinochet, 1992).

Soybean was introduced to Egypt more than 50 years ago, however its cultivation as a economic crop has been began in 1970 (Kella *et al*., 2011). Presently it is successfully grown as a summer crop in different localities of the country. Soybean plants in Egypt also were infected with the root-knot nematodes (El-Sherif and Ismail, 2011), loss in soybean yield due to *M. arenaria* was estimated by 44.7% at severe infection (Korayem and Mohamed, 2018).

Over the last ten years (2010-2019), the production of soybean has not changed with average of 40 thousand tones annually, while the consumption has rapidly increased than production with average of 735 thousand tones annually (Anonymous, 2021), so Egypt imports more than 90% from its soybean seeds requirements (FAO, 2017). So, planting soybean varieties of high productivity and applying effective tactics for controlling the root knot nematodes damaging it is obligatory to increase soybean domestic production, and to minimize the current imports for reducing the depletion of hard currently.

Recently the Ministry of Agriculture has planned to increase the distribution of six certified soybean cultivars of high productivity and grow well in different soil types. These cultivars are subjected to infect with the root-knot nematodes, thus study of their response to the nematode infection is urgent. Therefore, the objectives of the present study were to survey of root-knot nematode species which infect soybean grown in different localities of Middle and Northern Egypt and to screen the local soybean cultivars for their resistance to the root-knot nematode, *Meloidogyne incognita*.

Materials and Methods

Survey study

During the growing season 2020, root samples of soybean infected with the root-knot nematodes were collected from eight locations of six governorates; Beni-Suef and Menia representing Middle Egypt, Alexandria, El-Beheira, Kafr-Elsheikh and Dakahlia, representing Northern Egypt (Table 1).

Table 1: *Surveyed of locations from which soybean samples were collected.*

Governorates	Locations	Number of root samples
Beni-Suef	Beba	15
	Somsta	10
Menia	Maghagha	10
Alexandria	Mariout	15
	Amria	10
El-Beheira	Nobaria	20
Kafr-Elsheikh	Desuque	10
Dakahlia	Meet-Ghamr	10
Total samples		100

Collecting and identification of Meloidogyne species

The soybean roots infected with root-knot nematodes (*Meloidogyne* spp.) collected from different locations were washed well from adhering soil and cut into small pieces in a plastic dish with sufficient amount of water. Root galls with mature females were then teased with dissecting needles to collect adult females necessary for species identification (Hooper, 1970). Females were processed for preparing perineal patterns (Taylor *et al*., 1955; Charcher and Eisenback, 2000), and were identified according to their perineal patterns morphological shape (Eisenback *et al*., 1981).

Maintence of root-knot nematode stock culture and preparation of nematode inoculum

Pure stock culture of the root-knot nematode originally obtained from galled soybean roots was established. Single egg mass of female (identified as *Meloidogyne incognita*) was used to inoculate healthy eggplants grown in 30 cm diameter plastic pots filled with sterilized sandy loam soil. Two months after inoculation, plants were examined for nematode infection and reproduction. The culture was propagated and maintained on eggplants using infected roots with enough egg masses for massive pure culture. For preparation of nematode inoculum, infected roots of eggplants were cut into small pieces and placed in a 1000ml container with 200 ml of 0.5% sodium hypochlorite (NaOCI), then container was shaken vigorously for 90 sec. The liquid suspension of eggs was poured through a 100-mesh sieve nested upon a 500-mesh sieve. Eggs collected on the 500-mesh sieve was washed under a slow stream of distilled water (Barker, 1985). The egg suspension was adjusted to a known volume, and eggs were counted under research microscope by using the eel-worm counting slide (Hawksley slide), the number of eggs per one ml was estimated.

Soybean genotypes

Seeds of six certified local soybean cultivars i.e., Giza-21, Giza-22, Giza-35, Giza-82, Giza-83 and Giza-111 were obtained from the Crop Research Institute, Ministry of Agriculture, Egypt.

Host susceptibility test

In the beginning of May, 2021, seeds from above mentioned soybean cultivars were planted in plastic pots 15cm diam, filled with sterilized sandy loam soil. About two weeks after seed germination, plants were thinned to one plant per pot. Each plant was inoculated with *Meloidegyne incognita* eggs as initial population (Pi) at the rate of 3000 eggs per plant. The nematode inoculum was poured in holes around the plant. Each cultivar was replicated ten times. Pots were arranged in a greenhouse of 30°C by day and 20°C nightly and watered as needed.

About fifty days from inoculation, the tops of the tested plants were cut off and the roots were gently washed and cleaned from the adhering soil particles. The roots were processed for nematode root gall indices as follows:

0= no galls, 1=1-2 galls, 2= 3-10 galls, 3=11-30 galls, 4=31-100 galls and 5= 100+galls (Taylor and Sasser, 1978). For estimating the final nematode population (Pf), the infected roots were processed for nematode eggs extraction as previously mentioned in inoculum preparation. Eggs number per plant was counted and averaged with the other replicates. Nematode reproduction factor (Rf)= Pf/Pi, was then calculated, with Pf being the average final egg count among replicated and Pi in this case, being the 3000 eggs used as original inoculum. Host suitability was designated based on the root -gall index (GI) as an indicator of plant damage and on the reproduction factor (Rf) as an indicator of nematode reproduction or host efficiency. Cultivars with GI greater than 2 are designated as either susceptible (efficient host with Rf >1) or hyper-susceptible (poor host with Rf ≤ 1), cultivars with GI ≤ 2 were designated as resistant (poor host with Rf≤1), while cultivars with GI ≤ 2 and Rf >1 were designated as tolerant (Table 2).

Table 2: *Quantitative scheme for host suitability (resistance) designation.*

Plant damage (Gall index: GI)	Host efficiency (Rf)	Degree of resistance designation
≤ 2	≤ 1	Resistant
≤ 2	> 1	Tolerant
> 2	≤ 1	Hyper susceptible
> 2	> 1	Susceptible

Sasser *et al*., 1984

Results and Discussion

Survey of root-knot nematodes infected soybean

Identification of root-knot nematode, *Meloidogyne* species infecting soybean roots collected from different locations showed the occurrence of three *Meloidogyne* species; *M. incognita*, *M. javanica* and *M. arenaria* (Table 3). The former species was the most frequent with average of (58.75%) followed by *M. javanica* with (31.88%), and *M. arenaria* with 9.37%. Also, it was observed that both *M. incognita* and *M. javanica* were found in samples of all locations, while *M. arenaria* was recovered from samples of only Nobaria and Mariout locations. Highest frequency percent of *M. incognita* occurred in Maghagha location (90%) followed by Beba location (80%), and the lowest one occurred in Mariout location (40%). For *M. javanica* its highest frequency occurred in Meet-Ghamr and Amria locations.

Host suitability of soybean genotypes to Meloidogyne incognita

Data on host suitability of the tested soybean cultivars to *M. incognita* are presented in Table 4. Data revealed that all of cultivars; Giza-21, 22, 35, 82, 83 and 111 were susceptible to nematodes based on the root gall index (GI) and nematode reproduction factor (Rf). As all cultivars had GI greater than 2 with Rf > 1 (Table 4).

Table 3: *Species of Meloidogyne isolated from infected roots of soybean collected from different locations of Egypt.*

Gover-norates	Locations	% frequency of occurrence of *Meloidogyne* species		
		M. incognita	***M. javanica***	***M. arenaria***
Beni-Suef	Beba	80	20	-
	Somsta	70	30	-
Menia	Maghagha	90	10	-
Beheira	Nobaria	30	25	45
Kafr-El-Elsheikh	Desuque	60	40	-
Dakahlia	Meet-Ghamr	50	50	-
Alexan-dria	Mariout	40	30	30
	Amria	50	50	-
Average		58.75	31.875	9.375

Table 4: *The root gall index (GI), and nematode reproduction factor (Rf) of Meloidogyne incognita on roots of six soybean cultivars and their susceptibilities to nematode infection.*

Soybean cultivars	P_i	GI	P_f	Rf (P_f/P_i)	Host category
Giza-21	3000	3.5	6000	2.0	Susceptible
Giza-22	3000	4.0	7500	2.5	Susceptible
Giza-35	3000	3.5	7500	2.5	Susceptible
Giza-82	3000	4.5	8400	2.8	Susceptible
Giza-83	3000	5.0	9000	3.0	Susceptible
Giza-111	3000	4.5	8700	2.9	Susceptible

GI (root gall index): 0 = no galls, 1= 1-2 galls, 2= 3-10 galls, 3=11-30 galls, 4= 31-100 galls and 5=100+gall (Sasser et al., 1984). Pi= Initial population; Pf= Final population; Rf (Reproduction factor) = P_f/P_i

The success of root-knot nematode (RKN) control basically depends on the identification of prevalent RKN species in the regions in which the control will be applied, either on susceptible or resistant cultivars. Failure of growth of some plant cultivars resistant to root-knot nematodes has observed in some regions. This was attributed to the lack of knowledge what species and races of RKN is prevalent in these regions. So, an accurate identification of RKN species is crucial for obtaining the effective nematode control.

Survey of the root-knot nematodes, *Meloidogyne* species infecting soybeans in different ecological localities of Egypt indicated the presence of three nematode species, they were *M. incognita, M. javanica* and *M. arenaria. M. incognita* was the most frequent with 58.8% followed by *M. javanica* (31.8%), while *M. arenaria* was the lesser frequent (9.4%). All of these species have been prevalent in the warmer climates such as tropical and subtropical regions and reported to infect soybean plants (Schmitt and Noel, 1984; Koenning, 2015), causing damage to soybean (Sikora *et al.*, 2010). In a survey of large soybean fields in USA, it was found that *M.incognita* was the most frequent (70%), followed by *M. javanica* (24%) and *M. arenaria* (6%) (Garcia and Rich, 1985). On the contrary, *M. javanica* was the most prevalent with 77% frequency followed by *M. incognita*, 31% on soybean fields in Brazil (Castro *et al.*, 2003). Studies carried out in Argentina, South Africa and Senegal reported that *M.incognita* and *M.javanica* were also the most species frequently detected in soybean fields (Doucet and Pinochet, 1992; Fourie *et al.*, 2001; Trudgill *et al.*, 2000).

We tested six Egyptian soybean genotypes for their resistance to the root-knot nematode *M. incognita*. Data revealed that all genotypes were susceptible to the nematode, thus applying choosing an effective and sustainable control method should be considered. The root-knot nematodes (*Meloidogyne* spp.) are sedentary-endo-parasites, have overlapping in their life cycle and have a wide host range, so their control is extremely difficult even with using polluting chemical nematicides. Using resistant plants is considered one of the best options for nematode management, because its cost effective and it is typically compatible with other management tactics and environmentally harmless (Starr *et al.*, 2002). So, searching for soybean genotypes of high productivity resistant to the root knot nematodes and adapted to Egyptian conditions may be the best way to increases soybean domestic production and to minimize the current imports for reducing the depletion currently.

Conclusions and Recommendations

The most effective way to improve local soybean production and to minimize current imports in order to stop the present depletion may be to look for high productivity soybean genotypes that are resistant to the root knot nematodes and suitable to Egyptian conditions.

Novelty Statement

The current study showed that root knot nematode species are distributed in Egypt soil and infected soybean infected plant. All certified soybean cultivars grown in Egypt are suscepticle to the root knot nematodes.

Author's Contribution

AMK conducted the study, identified the RKN species and writing the manuscript; MMMM Survey and identification of RKN and MMAH Preparation of materials and data analysis.

Conflict of interest

The authors have declared no conflict of interest.

References

Anonymous, 2021. Ministry of Agric. and Land Rec., Central Administration of Agric. Economics and Statistics (different volumes), Egypt.

Barker, K.R., 1985. Nematode extraction and bioassay. In: An advanced treaties on *Meloidogyne*, Vol. II) Methodology. K.R. Barker, C.C. Carter and J.N. Sasser (eds). North Carolina State University. pp. 19-35.

Castro, J.M.C., Lima, R. and Carneiro, R.M.D.G., 2003. Variabilidade isoenzimatica de poplucoes de *Meloidogyne* spp. Provenientes de regioes brasilieras de soja. Nematol. Brasil., 27(1): 1-12.

Charcher, J.M. and Eisenback, J.D., 2000. An improved technique to prepare perineal patterns of root-knot nematodes for SEM. Nematol. Brasil., 24: 245-247.

Doucet, M.E., and Pinochet, J., 1992. Occurrence of *Meloidogyne* spp. in Argentina. Supplement. J. Nematol., 24: 765-770.

Eisenback, J.D., Hirschmann, H., Sasser, J.N., and Triantaphyllou, A.C., 1981. A guide to the four most common species of root-knot nematodes (*Meloidogyne* species) with a pictorial key. The Department of Plant Pathology and Genetics, North Carolina State Univ., Raleight NC. USA.

El-Sherif, A.G., and Ismail, A.F.A., 2011. Role of certain biological measures in comparison with oxamyl for controlling *Meloidogyne incognita* infecting soybean. Egypt. J. Agronematol., 10(1): 49-63.

FAO, 2017. Promoting sustainable investment in Egypt's food security, market snapshots, Egyptian grain production and trade. Egypt-Agrinvest-Fourum@fao.or, http://www.fao.org.

Fontana, J.D., 2011. Biodiesel para leitores de 9 a 90 anos. I[st] ed. Curitiba, Brazil: UFPR. pp. 253.

Fourie, H., De Waele, D., Mc Donald, A.N., Mienie, C., Marais, M. and De Beer, A., 2015. Nematode pests threatening soybean production in South Africa, with reference to *Meloidogyne*. S. Afr. J. Sci., 111(9110): 9. https://doi.org/10.17159/sajs.2015/20140212

Fourie, H., McDonald, A.H. and Loots, G.C., 2001. Plant parasitic nematodes in filed crops in South Africa 6: soybean. Nematology, 3: 447-454. https://doi.org/10.1163/156854101753250773

Garcia, M.R. and Rich, R., 1985. Root-knot nematodes in north-central florida soybean fields. Nematropica, 15(1): 43-46.

Hooper, D.J., 1970. Extraction of nematodes from plant material. In: Laboratory methods for work with plant and soil nematodes (Southey J.F, ed). Ministry of Agric., Fisheries and Food. Technical Bulletin 2. Her Majesty's Stationery Office, London. pp. 34-38.

Hunt, D.J., and Handoo, Z.A., 2009. Taxonomy, identification and principal species. Chapter 3 in: Root-knot Nematodes (R.N. Perry, M. Moens and J.L. Starr, eds). CABI, pp. 55-97. https://doi.org/10.1079/9781845934927.0055

Hymowitz, R.L., Nelson, R.L., Sinclair, J.B. and Hartman, G.L., 2015. Introduction: History and growth of the soybean plant. In: (Hartman, G.L., Rupe, J.C., Sikora, E.J., Domier, L.L., Davis, J.A. and Steffey, K.L., (eds). Compendium of soybean diseases and pests. Am. Phytopathol. Soc., St Paul, Minnesota, pp. 1-4. https://doi.org/10.1094/9780890544754.001

Hymowitz, T., 1970. On the domestication of the soybean. Econ. Bot., 24(4): 408-421. https://doi.org/10.1007/BF02860745

Sikora, R.A., Claudius-Cole, B., and Sikora, E.J., 2018. Nematode parasites of food legumes. In: Sikora, R.A., Coyne, D., Hallman, J. and Timper, P. (Eds.). Plant parasitic nematodes in subtropical and tropical agriculture, 3[rd] Eds. CABI Publishing, Wallingford, UK., pp. 290-345.

Karssen, G., and Moens, M., 2006. Root-knot nematodes. In: Plant nematology (Perry R.N. and Moens M., eds). Wllingford, UK CABI Publishing. pp. 59-90. https://doi.org/10.1079/9781845930561.0059

Kella, A.M., El-Sayed, S.A. and El-Shennawy, R.Z., 2011. Survey, identification and control of nematodes and fungi infecting soybean plant at Minufiya governorate, Egypt. J. Agronematol., 10(1): 22-36.

Koenning, S.R., 2015. Root-knot nematodes. In: (Hartman, G.L., Rupe, J.C., Sikora, E.J., Domier, L.L., Davis, J.A. and Steffey, K.L., eds). Compendium of soybean Diseases and pests. The American Phytopathological Society, St Paul, Minnesota, pp. 98-100.

Korayem, A.M. and Mohamed, M.M.M., 2018. Potential loss in soybean yields due to root knot nematode *Meloidogyne arenaria* in sandy soil. Egypt. J. Agronemotol., 17(1): 43-50. https://doi.org/10.21608/ejaj.2018.53868

Lic, C., Hua, C., Hu, Y., You, J., Mao, Y., Li, J., Tian, Z., and Wang, C., 2016. Response of soybean genotypes to *Meloidogyne* incoginta and M. hapla in Heilongjiang province in China. Russ. J. Nematol., 24(2): 89-98.

Lima, F.S.O., Correa, V.R., Nogueira, S.R. and Santos, P.R.R., 2017. Nematodes affecting soybean and sustainable practices for their management. In: Kasai, M. (Ed.), Soyabean-The basis of yield, biomass and productivity. IntechOpen London, UK, pp. 95-110.

Noel, G.R., 2008. IPM of soybean cyst nematode in the USA. Pp. 119-126. In: Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes (A. Cianco and K.G. Mukerji). Springer, Dordrecht, The Netherlands. https://doi.org/10.1007/978-1-4020-6063-2_6

Sasser, J.N., Carter, C.C., and Hartman, K.M., 1984. Standardization of host suitability studies and reporting of resistance to root-knot nematodes. A Cooperative Publication of the Department of Plant Pathology, North Carolina State University and the US Agency for Inter. Devel., Raleigh, NC, USA.

Schmitt, D.P., and Noel, G.R., 1984. Nematode parasite of soybean. In: Plant and insect nematodes (W.R. Nickle, ed). New York: Marcel Dekker. pp. 13-59.

Sikora, E.J., Lawrance, K.S., and Murphy, J., 2010. Survey of plant parasitic nematodes in soybean fields in Alabama. Available from http://www.aaes.auburn.edu/comm/pubs/researchreports/ogsoubearnrrpdf.

Starr, J.L., Bridge, J., and Cooke, R., 2002. Resistance to plant parasitic nematodes: History, current use future potential. In: (Starr, J.L., Cook, R., and Bridge, J., eds). Plant resistance to parasitic nematodes. CAB International, Walling ford, UK, pp. 1-22. https://doi.org/10.1079/9780851994666.0001

Taylor, A.L., and Sasser, J.N., 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne* spp.). Coop. Pub. Dept. Plant Pathol., North Carolina State Univ., and U.S. Agency Int. Deu. Raleigh, N.C., pp. 111.

Taylor, A.L., Dropkin, V.H., and Martin, G.O., 1955. Perineal pattern of root-knot nematodes. Phytopathology, 45: 26-34.

Trudgill, D.L., Bala, G., Vivian, C. B., Andrew, D., Keith, G. D., Simon, R., Gowen, M.A. , Joseph, D., Madulu, T., Werner, M. , Casp,R., Ketchh, M.S. , Abdoussalam, S., Carmen, G.T., and Effie, V. 2000. The importance of tropical root knot nematodes (*Meloidogyne* spp.) and factors affecting the utility of *Pasteuria pentrans* as a biocontrol agent. Nematologia, 2: 823-845. https://doi.org/10.1163/156854100750112789

Trudgill, D.L., and Blok, V.C., 2001. Apomictic, polyphagous root-knot nematodes: exceptionally successful and damaging bio-trophic root pathogens. Ann. Rev. Phytopathol., 39: 53-77. https://doi.org/10.1146/annurev.phyto.39.1.53

Wrather, J.A. and Koenning, S.R., 2009. Effect of diseases on soybean yield in the United States 1996-2007. Plant health progress. https://doi.org/10.1094/PHP-2009-0401-01-RS