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Differential response of tomato accessions to *Meloidogyne arenaria* (Neal) Chitwood infection

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Abstract

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The root-knot nematodes (*Meloidogyne* spp.) are one of the most dangerous and widespread species of nematodes affecting tomatoes. There are few methods for controlling nematodes in tomatoes. Natural resistance is important in conferring resistance against nematodes. A study was conducted to evaluate the reaction of tomato accessions to root-knot nematode *Meloidogyne arenaria* (Neal) Chitwood. Twenty one tomato accessions were subjected for screening. Susceptible tomato variety Ideal was used as control. The tomato plants were evaluated 60 days after inoculation on the basis of the gall index (GI), egg mass index (EMI), final populations (Pf) and reproduction factors (Rf). All the tomato accessions show varying degree of response. Most of the screened accessions were susceptible to *M. arenaria*, six were resistant and two accessions showed hypersensitive reaction.

Keywords: tomato; resistance screening; root-knot nematode

Introduction

Tomato (*Solanum lycopersicum* L.) is an annual crop of the *Solanaceae* family, and the second most widely consumed vegetable after potatoes (Okorley et al., 2018). Tomatoes are a rich source of micronutrients, such as minerals, vitamins, and antioxidants that are essential for the human diet. They also contain high levels of lycopene, an antioxidant that reduces the risks associated with many cancers and neurological diseases (Giovannucci, 1999).

There are many pests and diseases damaging both the quality and quantity of tomato production. Plant-parasitic nematodes are one of them. They represent an important constraint on the delivery of global food security (Nicol et al., 2011).

Root-knot nematodes, *Meloidogyne* spp. are considered to be the most damaging nematode group in the world as they cause high yield losses to most cultivated plant species. Root-knot nematodes are obligate, sedentary endoparasites of many plant species (Seçgin, 2018). Their host range encompasses more than 3000 cultivated and wild plant species (Gharabadiyan et al., 2012).

One of the most important soil-borne diseases affecting tomato crop is root-knot nematode. Damages depend on the nematode species, initial population density, cultivated crop species, and a range of environmental factors, and tomato yield losses may reach up to 100% (Seid et al., 2015). *Meloidogyne incognita*, *M. arenaria, M. javanica* and *M. hapla* that are part of the *Meloidogyne* spp. complex, areamong the top 10 most devastating phytoparasitic nematodes of

economically important crops (Jones et al., 2013). Disease symptoms caused by root-knot nematode on susceptible host plants include damaged root system and the formation of root galls, which affect the uptake of nutrients and water by the plant (Abad et al., 2003). As a consequence of root gall formation, plants can manifest secondary symptoms such as wilting, chlorosis in the oldest leaves, general reduction of plant growth, floral abortions, decrease in both fruit quality and fresh weight, as well as a reduction in the number of fruits. Following senescence and death of the plant in severe infections (Padilla-Hurtado et al., 2021).

Various control methods and management strategies can be applied to avoid economic damage due to root-knot nematode infestation. They can include cultural, physical, biological, cropping-based and chemical methods (Sikora & Fernandez, 2005). The polyphagous nature of the root-knot nematode *Meloidogyne* spp. poses severe constraints for the effectiveness of management practices such as the use of chemical nematicides, integration of cover crops into the cropping system, the use of rootstocks with resistance to root nodulation and biological control (Adam et al., 2014). However, factors such as the toxic effect of chemical nematicides on the environment and the wide range of hosts of *Meloidogyne* spp. as well as the effect of soil properties limit the use of these practices (Barbary et al., 2015).

The use of resistant cultivars is economical and environmentally safe method for controlling *Meloidogyne* species. They are cultivated with a dual purpose: to reduce nematode population levels and to avoid crop damage by nematodes. It is particularly important for organic farming or integrated production since theses systems do not allow, or they restrict, the use of chemical control. Resistant cultivars do not require ignificant changes in farming operations or in market supply (Ornat & Sorribas, 2008). Tomato is one of the few crops in which *Meloidogyne* resistance has been widely used, and commercial resistant cultivars are available for tomato. These tomato cultivars carry the Mi-resistance gene introgressed into cultivated tomato from the wild-type relative of tomato *Lycopersicon peruvianum* (syn. *Solanum peruvianum*). This gene confers resistance to the three most widespread species of root-knot nematodes (*M. incognita*, *M. arenaria* and *M. javanica*) (Rumbos et al., 2011).

The aim of the study was to screen a tomato accessions for resistance against *Meloidogyne arenaria* (Neal) Chitwood.

Material and Methods

The study was conducted during the period 2020–2021 at Maritsa Vegetable Crops Research Institute – Plovdiv.

Preparation of nematode culture

The nematode culture used in the experiments was derived from a single egg mass of *Meloidogyne arenaria* (Neal) Chitwood. Susceptible tomato variety Ideal was used for developing pure culture of root knot nematode. Nematode eggs were extracted from roots with galls using 0.5% sodium hypochlorite solution (Hussey & Barker, 1973). Eggs were allowed to hatch the juveniles (J2) using a modified Baermann tray (Whitehead & Hemming, 1965).

Plant materials and nematode inoculation

A total of 21 tomato accessions were evaluated for resistance to root-knot nematode, *Meloidogyne arenaria* (Neal) Chitwood. Seeds were sown in trays containing sterilized mixture (peat:perlite in 1:1 ratio). Three-weeks old tomato seedlings were transplanted in plastic pots containing 1 kg of sterilized mixture (peat:perlite:sand in 1:1:1 ratio) for artificial inoculation of root-knot nematode. One tomato seedling per pot was used. The susceptible tomato variety Ideal was included as a control. One week after transplanting, tomato plants were inoculated with freshly hatched J2 of *M. arenaria* at a rate of two juveniles (J2)/g of soil. Complete randomized design with six replicates was used.

Nematode analysis

Sixty days after inoculation, plants were uprooted carefully with minimum root damage and washed with tap water to remove the adhering soil particles.

The gall index (GI) based on the number of galls per root system was recorded on 0 – 10 scale, where 0 – no galling and 10 – completely dead plant (Bridge & Page, 1980). The egg mass index (EMI) was assessed through a visual rating based on the six-point rating scale (0–5, where 0 = no egg mass; 1 = 1–2 egg masses; 2 = 3–10; 3 = 11–30; 4 = 31–100 and 5 = 100 or more egg masses (Taylor & Sasser, 1978). The reproductive factor (Rf) was calculated by dividing the final population (Pf) by initial population (Pi).

The gall index (GI) and reproductive factor (Rf) were used as the basis to evaluate the resistance status of the tomato accessions. Tomato accessions were classifiedas resistant when $GI < 2$ and $Rf < 1$, tolerant when $GI < 2$ and $Rf > 1$, susceptible when $GI > 2$ and $Rf > 1$ and hypersensitive when $GI \geq 2$ and $Rf < 1$ (Canto-Saenz, 1983).

Statistical analysis

Data were processed using Duncan's multiple range test at $P < 0.05$ levels (Duncan, 1955) and LSD test using R studio (agricole package). A correlation analysis was performed (Lidanski, 1988).

Results and Discussion

The means of gall and egg mass indexes, nematode populations and reproduction factors of the 21 tomato accessions to the root-knot nematode, *Meloidogyne arenaria* (Neal) Chitwood have been presented in Table 1, along with the means of statistical grouping, based on Duncan test. Scoring the root systems, using the gall index, revealed different levels of susceptibility among the tested tomato accessions. The statistical analysis of the means of gall index showed nine different significant groups. The gall index varied between 0.00 (accession BL469) to 4.92 (control). Accession BL469 had the least root gall index (0.00), with a slight increase for BL443 and BL444 (GI 0.33 and 0.50, respectively) compared with the other accessions, six weeks after transplanting the plants. They have no significant differences with BL469 and fall into the same statistical group. Low root galling on the accession BL823 also was recorded. Accessions BL33 and BL472 had gall indexes of 1.92 and 1.58, respectively. The highest gall indexes over 4 was reported to accessions BL810, BL1360 and BL1987, close to the susceptible control. Control cultivar Ideal had gall index 4.92 (Table 1).

There was a significant difference in production of egg masses by *M. arenaria* among the tested accessions. Mean egg mass index for the accessions ranged from 0.00 (BL469) to 3.58 (BL810). Significantly higher number of egg masses per root system was recorded in BL810 and BL208 (EMI 3.58 and 3.50, respectively) close to the control variety Ideal. In accessions BL337, BL407, BL447, BL1987 and BL1988 also was observed high egg mass production with EMI varied between 2.83 and 3.00. There were no significant differences among these accessions. The lowest egg mass index was recorded in BL443 (0.50) and BL444 (0.67) (Table 1).

The final nematode populations of the tomato accessions were divided into the eight distinct and significant statistical group. Sixty days after inoculation the accessions, lowest final population density ranging from 0.00 to 76.67 were observed on BL469 and BL443. Tomato accessions BL444, BL466, BL472 and BL823 showed lower nematode reproduction than another included in the experiment. Final population densities were found to be maximum in the accessions BL810 and BL1360 (Table 1).

Means of the reproduction factor also showed significant differences among the screened accessions. Tomato accessions BL469, BL443 and BL444 had the least Rf 0.00, 0.04

Table 1. Screening of tomato accessions to root-knot nematode, *Meloidogyne arenaria* (Neal) Chitwood

Accession/cultivar	Gall index (0-10)	Egg mass index (0–5)	Final population (Pf)	Reproduction factor (Rf)	Reaction
BL16	2.67 c-f	3.17 bc	3089.00 c-e	1.55 c-e	S
BL33	1.92 fg	2.25 d-f	1653.83 d-h	0.83 d-h	R
BL 37	2.25 d-g	2.67 c-e	2512.33 c-f	1.26 c-f	S
BL 44	2.08 e-g	2.25 d-f	2136.58 d-h	1.07 d-h	S
BL 208	3.58 bc	3.50 ab	4430.00 c	2.22 c	S
BL 337	3.17 cd	3.00 b-d	4025.00 c	2.01 c	S
BL 407	3.00 c-e	3.00 b-d	3511.75 cd	1.76 cd	S
BL 443	0.33i	0.50 i	76.67 h	0.04 h	R
BL 444	0.50 i	0.67 hi	144.17 gh	0.07 gh	R
BL 447	2.67 c-f	2.83 b-d	4136.00 c	2.07 c	S
BL 465	2.67 c-f	2.50 c-e	3906.00 cd	1.95 cd	S
BL 466	2.33 d-g	1.83 fg	712.33 f-h	0.36 f-h	HS
BL 468	2.33 d-g	2.67 c-e	1018.00 e-h	0.51 e-h	HS
BL 469	0.00 i	0.00 i	0.00 h	0.00 h	R
BL 472	1.58 gh	2.00 ef	794.42 f-h	0.40 f-h	R
BL 753	2.50 d-g	2.67 c-e	2404.00 c-g	1.20 c-f	S
BL 810	4.33 ab	3.58 ab	6687.67 b	3.37 b	S
BL 823	0.92 hi	1.25 gh	507.17 f-h	0.25 f-h	R
BL 1360	4.33 ab	3.25 a-c	7082.42 b	3.54 b	S
BL 1987	4.17 ab	3.00 b-d	3903.00 cd	1.95 cd	S
BL 1988	2.67 c-f	2.83 b-d	2728.50 c-f	1.37 c-f	S
Ideal (control)	4.92 a	3.95 a	14714.00 a	7.36 a	S
LSD	1.05	0.79	2272.13	1.14	-

a,b,c… – Duncan's multiple range test ($p \leq 0.05$); *LSD* ≤ 0.05; R = Resistant, S = Susceptible, HS = Hypersensitive

and 0.07, respectively. Accessions BL33, BL466, BL468 and BL472 also had Rf < 1. The greatest reproduction factors among the screened accessions were observed on the BL810 (Rf 3.37) and BL1360 (Rf 3.54), but statistically different from the susceptible variety Ideal (Rf 7.36) (Table 1).

The reproductive factors (Rf) of the various tomato accessions together with their mean gall indexes (GI) provided an estimate of host suitability, to support nematode reproduction and were used to verify host resistance (Canto-Saenz, 1983).

Six of the tested tomato accessions, BL33, BL443, BL444, BL469, BL472 and BL823 were found to be resistant. In these accessions root damage was minimal (GI < 2) and did not support nematode reproduction (Rf < 1). The following category of tomato reaction included twoaccessions that were found to be hypersensitive, BL466 and BL468. In this accessions GI > 2 and Rf < 1, indicating that, the nematode arrives to the tomato root system, but the resistance of the host prevents the reproduction of the nematode. Remaining all accessions showed susceptible reaction with high plant damage (GI > 2) and nematode reproduction factor (Rf > 1). The smallest significant difference in gall index is 1.05; with egg mass index is 0.79; at Pf is 2272.13 and at Rf 1.14 (Table 1).

Root-knot nematodes (*Meloidogyne* spp.) are sedentary endoparasites with a complex life cycle. The infective stage is the second-stage juveniles. It is attracted to root tips, where it penetrates the zone of elongation and then migrates to thedeveloping vascular tissue. In susceptible genotypes, the nematode initiates a feeding site in vascular tissue, causing the formation of large, multinucleate, metabolically active giant cells. Nearby cells of the cortex, pericycle, and vascular parenchyma enlarge and divide, forming a root knot or gall (Reddy et al., 2016). Susceptible genotypes allowed the juveniles of nematodes to enter the roots, reached maturity and produced many eggs while resistant plants suppressed their development and thus do not allow reproduction (Anupam et al., 2020). Post-infection resistance is often associated with an early hypersensitive reaction (HR), in which rapid localized cell death in root tissue around the nematode prevents the formation of a developed feeding site, leading to resistance (Williamson, 1999).

There is a proven total (functional) positive dependence between reproduction factor (Rf) and final population (Pf). A strong correlation between egg mass index and gall index, between final population (Pf) and gall index, reproduction factor (Rf) and gall index, as well as with egg mass index and final population (Pf) and egg mass index and reproduction factor (Rf) was established (Table 2).

Out of twenty one screened tomato accessions, 62% were susceptible, 29% were resistant and 10% hypersensitive (Figure 1).

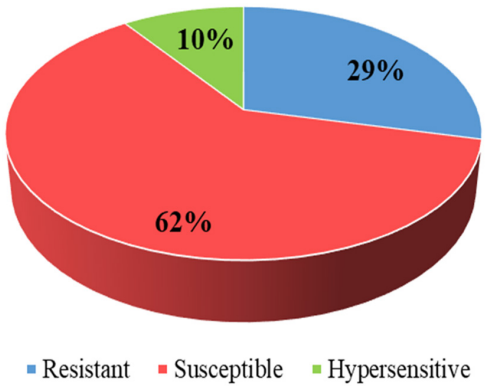

Fig. 1. Distribution of tomato accessions according to their response towards root-knot nematode infestation

Conclusions

This study indicated that significant differences were recorded among the different tomato accessions against the root-knot nematode, *Meloidogyne arenaria* (Neal) Chitwood. Out of a total of 21 tested accessions, 6 showed a resistant reaction to *M. arenaria*. Tomato lines, resistant to *M. arenaria* are extremely valuable and can be used in heterosis breeding as parent lines or in combinatie breeding in tomato as resistant gene carriers.

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Table 2. Correlation coefficient between gall index, egg mass index, final population (Pf) and reproduction factor (Rf) in tomato accessions to infection by root-knot nematode, *Meloidogyne arenaria* (Neal) Chitwood

Signs	Gall index	Egg mass index	Final population (Pf)	Reproduction factor (Rf)
Gall index	1			
Egg mass index	0.824**	1		
Final population (Pf)	0.763**	0.657**	1	
Reproduction factor (Rf)	0.763**	0.658**	1.000**	1

r 0.01

References

Abad, P., Favery, B., Ross, M.-N. & Castagnone-Serena, P. (2003). Root-knot nematode parasitism and host response: molecular basis of a sophisticated interaction. *Molecular Plant Pathology, 4*, 217-224.

Adam, M., Heuer, H. & Hallmann, J. (2014). Bacterial antagonists of fungal pathogens also control root-knot nematodes by induced systemic resistance of tomato plants. *PLOS ONE, 9*(2), e90402.

Anupam, N. K., Kaur, S., Jindal, S. K. & Buttar, H. S. (2020). Phenotypic and genotypic characterization of tomato genotypes for resistance to root-knot nematode. *Meloidogyne incognita*. *Phytoprotection*, *100*(1), 28–33.

Barbary, A., Djian-Caporalino, C., Palloix, A. & Castagnone-Sereno, P. (2015). Host genetic resistance to root-knot nematodes, *Meloidogyne* spp., in Solanaceae: from genes to the field. *Pest Management Science, 71*, 1591-1598.

Bridge, J. & Page, S. L. J. (1980). Estimation of Root-knot Nematode Infestation Levels on Roots Using a Rating Chart. *Int. J. Pest Manag., 26*, 296–298.

Canto-Saenz, M. (1983). The nature of resistance to Meloidogyne incognita (Kofoid and White, 1919) Chitwood 1949. In: C.C. Carter. (ed). *Proc. Third Res. and Plann. Conf. on Root-knot Nematodes Meloidogyne spp.* 22–26 March 1982. International Meloidogyne Project, Lima Peru, 160–165.

Duncan, D. (1955). Multiple range and multiple F-test. *Biometrics, 11,* 1-42.

Gharabadiyan, F., Jamali, S., Yazdi, A. A., Hadizadeh, M. H. & Eskandari, A. (2012). Weed hosts of root-knot nematodes in tomato fields. *Journal of Plant Protection Research 52*(2), 230-234.

Giovannucci, E. (1999). Tomatoes, tomato-based products, lycopene and cancer: review of the epidemiological literature. *Journal of the National Cancer Institute, 91*, 317-331.

Hussey, R. S. & Barker, K. R. (1973). Comparison of methods for collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Disease Reporter, 57*, 1025–1028.

Jones, J. T., Haegemen, A., Danchin, E. G. J., Gaur, H. S., Helder, Jones, M. G. K., Kikuchi, T., Palomares-Rius, J. E., Wesemael, W. M. L. & Perry, R. N. (2013). Top 10 plant-parasitic nematodes in molecular plant pathology. *Mol. Plant Pathol., 14*, 946-961.

Lidanski, T. (1988). Statistical Methods in Biology and Agriculture. *Zemizdat,* Sofia, 374 (Bg).

Nicol, J. M., Turner, S. J., Coyne, D. L., Den Nijs, L., Hockland, S. & Maafi, Z. T. (2011). Current nematode threats to world agriculture. In: Jones, J.T., Gheysen, G. & Fenoll, C. (Eds). *Genomics and molecular genetics of plant-nematode interactions*. Heidelberg, Germany, *Springer,* 21-44.

Okorley, B. A., Agyeman, C., Amissah N. & Nyaku, S. T. (2018). Screening Selected Solanum Plants as Potential Rootstocks for the Management of Root-Knot Nematodes (*Meloidogyne incognita*). *International Journal of Agronomy*, Article ID 6715909, 1-9. https://doi.org/10.1155/2018/6715909.

Ornat, C. & Sorribas, F. J. (2008). Integrated management of root-knot nematodes in Mediterranean horticultural crops. In: Ciancio, A. & Mukerji, K. G. (Eds). *Integrated management and biocontrol of vegetable and grain crops nematodes: integrated management of plant pests and diseases*, Dordrecht, The Netherlands*, Springer, 2,* 295-319.

Padilla-Hurtado, B., Morillo-Coronado, Y., Tarapues, S., Burbano, S., Soto-Suárez, M., Urrea, R. & Ceballos-Aguirre, N. (2021). Evaluation of root-knot nematodes (*Meloidogyne* spp.) population density for disease resistance screening of tomato germplasm carrying the gene Mi-1. *Chilean Journal of Agricultural Research, 82*(1), 157-166.

Reddy, Y. S., Sellaperumal, C., Prasanna, H. C., Yadav, A., Kashyap, S. P., Singh, S., Rai, N., Singh, M. & Singh, B. (2016). Screening of Tomato Genotypes Against Root-Knot Nematode and Validation of Mi 1 Gene Linked Markers. Proc. Natl. Acad. Sci., India*,* Sect. B Biol. Sci. DOI 10.1007/s40011-016-0731-1.

Rumbos, C. I., Khah, E. M. & Sabir, N. (2011). Response of local and commercial tomato cultivars and rootstocks to *Meloidogyne javanica* infestation. *Australian Journal of Crop Science, 5*(11), 1388-1395.

Seçgin, Z., Arvas, Y. E., Ssendawula, S. P. & Kaya, Y. (2018). Selection of Root-Knot Nematod Resistance in Inbred Tomato Lines Using CAPS Molecular Markers. *International Journal of Life Sciences and Biotechnology, 1*(1), 10-16.

Seid, A., Fininsa, C., Mekete, T., Decraemer, W. & Wesemael, W. M. L. (2015). Tomato (*Solanum lycopersicum*) and root-knot nematodes (*Meloidogyne* spp.) – a century-old battle. *Nematology, 17,* 995-1009.

Sikora, R. A. & Fernandez, E. (2005). Nematode parasites of vegetables. In: Luc, M., Sikora, R.A. & Bridge, J. (Eds). *Plant-parasitic nematodes in subtropical and tropical agriculture,* 2nd edition. Wallingford, UK, *CAB International*, 319-391.

Taylor, A. L. & Sasser, J. N. (1978). Biology, identification and control of root – knot nematodes (*Meloidogne* spp.) IMP Publication, Raleigh, North Carolina.

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

Williamson, V. M. (1999). Plant nematode resistance genes. *Current Opinion in Plant Biology, 2*, 327–331.

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