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Screening of Mutant Lines and Varieties/Hybrids of Tomato (*Solanum lycopersicum*) for Resistance to the Northern Root-Knot Nematode *Meloidogyne hapla*

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Abstract

Root-knot nematodes, *Meloidogyne* spp., are widespread phytoparasites that cause a significant reduction in the yield of tomato *Solanum lycopersicum*. In the Russian Federation, where the use of chemical nematicides is limited due to environmental and toxicological risks, the cultivation of resistant varieties and hybrids remains the most effective and environmentally safe method to control *Meloidogyne*. In the course of this study, the resistance screening of 20 tomato varieties/hybrids and 21 mutant lines from the collection of the FSBSI FRCBPP to *M. hapla* was carried out using a comprehensive approach that included morphological and biochemical analysis methods. Resistance was assessed by calculating the gall formation index, the degree of root system damage, and biochemical parameters of fruits—vitamin C content and titratable acidity. In addition, molecular screening was carried out using the SCAR marker Mi23 to identify the *Mi-1.2* gene, known as a key factor in resistance to a number of *Meloidogyne* spp. Although *Mi-1.2* is not typically associated with resistance to *M. hapla*, all genotypes carrying this gene showed phenotypic resistance. This unexpected correlation suggests the possible involvement of *Mi*-associated or parallel mechanisms and highlights the need for further investigation into noncanonical resistance pathways. It was found that when susceptible genotypes were infected with *M. hapla*, there was a tendency for the vitamin C content to decrease, while resistant lines retained values close to the control. The presence of the *Mi-1.2* gene was confirmed in 9.5% of samples. However, the phenotypic resistance of some lines, such as Volgogradets, which do not contain a marker for the *Mi-1.2* gene, indicates a polygenic nature of resistance, alternative genetic mechanisms, or the possible influence of epigenetic mechanisms. The obtained data highlight the potential of using the identified resistant genotypes in breeding programs and the need for further studies of the molecular mechanisms of resistance, including the search for new markers specific to *M. hapla*, to develop effective strategies for tomato protection in sustainable agriculture.

Keywords: *Solanum lycopersicum*; root-knot nematodes; *Meloidogyne hapla*; resistance; *Mi-1.2* gene; biochemical markers; molecular screening; gall formation; breeding

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1. Introduction

The high biological value of tomato (*Solanum lycopersicum*) contributes to the annual increase in its production and consumption. *Meloidogyne* spp. root-knot nematodes are

obligate phytoparasites, causing the meloidogynosis disease, which leads to a significant decrease in the viability and productivity of tomato plants.

Currently, more than 90 species of *Meloidogyne* have been described, the most widespread and aggressive of which are *M. incognita* Kofoid & White, 1919, Chitwood, 1949, *M. javanica* Treub, 1885, Chitwood, 1949, *M. arenaria* Neal, 1889, Chitwood, 1949, and *M. hapla Chitwood*, 1949 [1,2]. The penetration of nematodes into the root system leads to the formation of hypertrophied cellular structures (galls), which is accompanied by a change in the anatomy of the roots and a violation of their functionality, the inhibition of physiological processes, and a decrease in the efficiency of water and minerals absorption. As a result of infection, the photosynthetic activity of plants is suppressed, and as a consequence, their immune status is weakened and the possibility of infestation with secondary infections increases. The root system suffers to the greatest extent [3]. The susceptibility of tomato to phytoparasite infection may vary depending on its genetic potential. Moreover, the degree of virulence in the same *Meloidogyne* species also depends on the damaged plant variety [4]. This genus of nematodes can infect more than 2000 plant species. They have a wide range of hosts and a high ability to survive, adapting to various environmental conditions. Their eggs can survive in the soil for several years, remaining viable even under unfavorable conditions [5].

The infection of tomato plants with the northern root-knot nematode *Meloidogyne hapla* leads to changes in the biochemical composition of the fruit, including the total acid and vitamin C content. *Meloidogyne* infection reduces the content of vitamin C, which is actively spent on the antioxidant protection of the crop during the regulation of the redox balance of cells [6]. Vitamin C is involved in the regulation of the hormonal status of plants and can affect the level of ethylene [7,8]. These indicators are associated with metabolic processes responsible for the resistance of plants and their ability to withstand the negative physiological effects caused by the infection [9].

Organic acids play an important role in regulating plant metabolism [10]. When infected with root-knot nematodes, the activity of the enzymes of the Krebs cycle, malate dehydrogenase and citrate synthase, changes, and the concentration of total acids, including malic, citric, and succinic acids, increases [6]. This is associated with the intensification of respiratory processes and energy spending on tissue protection and restoration, while a decrease may indicate the suppression of metabolism under infection conditions [9,11].

The resistance of tomato to root-knot nematodes was first discovered by Bailey in the wild species *Lycopersicon peruvianum* L. [12]. Smith then integrated this characteristic into the domesticated *Lycopersicon esculentum* Mill [13]. It was later found that the resistance of *Solanum lycopersicum* to root-knot nematodes is due to the action of the dominant *Mi-1.2* gene, which is part of the *Mi-1* cluster, localized on the short arm of chromosome 6 [14,15]. The *Mi-1.2* gene encodes an NBS-LRR-type protein and activates the innate immune response via the ETI (Effector-triggered immunity) mechanism, providing protection against 13 *Meloidogyne* species, including *M. incognita*, *M. javanica*, and *M. arenaria* [16]. A histopathological examination revealed that resistant *Mi-1.2* plants exhibit suppressed giant cell formation and developed a hypersensitive response (HR), while susceptible genotypes exhibited gall development and vascular tissue destruction [17]. However, *Mi-1.2*-mediated resistance was not demonstrated upon infection with *M. hapla* and *M. enterolobii* [16], and the loss of gene functionality was observed at temperatures above 28 °C [12,18]. This is due to the fact that *Mi-1.2* functions together with the chaperone HSP90, which is necessary for its stability. Under heat stress, HSP90 is redistributed to stabilize other proteins, which disrupts the formation of an active complex with *Mi-1.2* and leads to a loss of resistance [19]. In addition to *Mi-1.2*, independent resistance loci have been described in wild tomato forms, including *Mi-3* and *Mi-5*, localized on chromosome 12, and *Mi-9*, located in a different

position on the short arm of chromosome 6 outside the *Mi-1* cluster [20,21]. Mi-9 encodes an NBS-LRR-type protein and provides resistance to *M. incognita* at temperatures up to 30 °C, and is therefore considered a promising source of heat-stable resistance [20]. Given the widespread use of *Mi-1.2* in breeding programs and its availability as a molecular marker, a parallel screening of the tested genotypes was carried out to identify potential carriers of this gene. *Mi-1.2* is traditionally considered ineffective against *Meloidogyne hapla* and *M. enterolobii*, and only partially effective against *M. hispanica*, as reported in previous studies [22,23].

Since *Mi-1.2* is considered ineffective against *M. hapla*, phenotypic testing with this species was conducted to identify sources of resistance independent of *Mi-1.2*. However, when the two independent experimental datasets were compared, a correlation was observed between the presence of *Mi-1.2* and the phenotypic resistance to *M. hapla*.

High fertility and a rapid life cycle contribute to the accumulation of the phytoparasite in soil, especially in a greenhouse. The process of infestation with root-knot nematodes begins with the penetration of invasive second-stage larvae (J2) into the root system of the host plant. Having reached the vascular cylinder, they induce the formation of giant cells—syncytium, which provides nutrition to the parasite. At this stage, the larvae lose mobility and move on to the next phase of development—J3, and then J4. After the completion of molting, adult individuals are formed: females that remain attached to the root, and males that leave the plant tissues after insemination. Mature females lay eggs in an egg sac on the root surface. There first-stage larvae (J1) hatch, which develop in the rhizosphere to the invasive stage J2, capable of a new infestation process, completes the life cycle (Figure 1) [24,25].

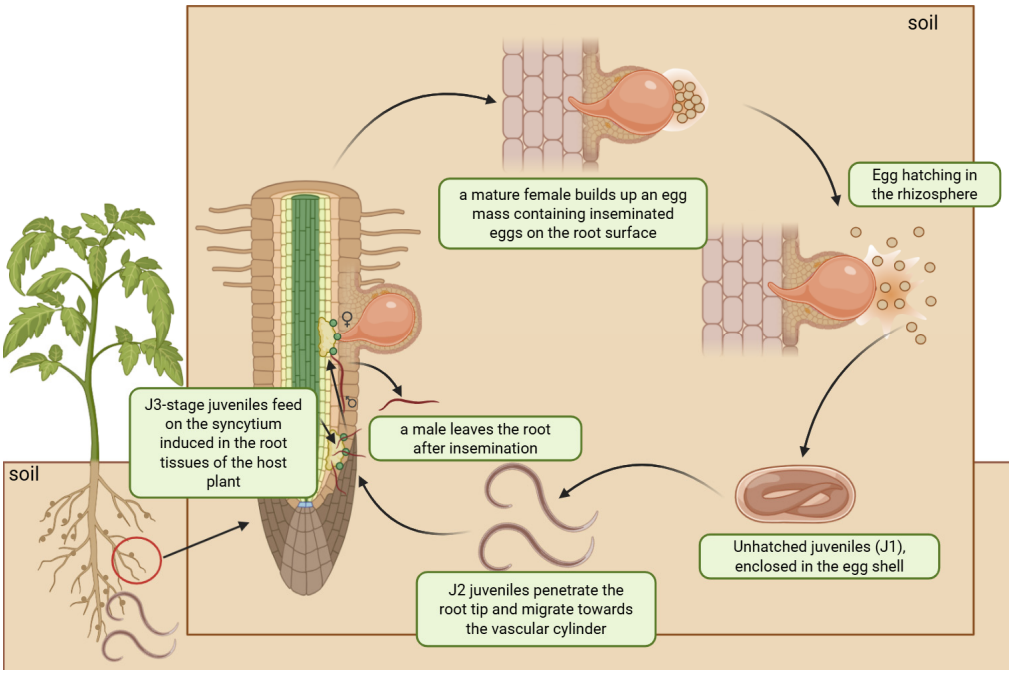


Figure 1. Life cycle of a root-knot nematode (*Meloidogyne* spp.) in tomato root tissues: J2 larvae penetrate the root, initiate syncytium formation, develop through J3–J4 stages, and develop into adult forms. Females lay eggs, which hatch into J1 larvae, which develop into J2 in the soil. Figure created using BioRender.com. Publication license: Tukhuzheva, Zh. (2025), https://BioRender.com/5q1aqe5.

When infected with root-knot nematodes, plants activate two levels of immune defense: innate (PAMP-triggered immunity, PTI) and induced (Effector-triggered immunity, ETI). Both levels of defense are regulated by receptor proteins and a cascade of intracellular signaling pathways that activate plant defense responses [26].

The primary line of plant defense (PTI) is based on the recognition of conserved pathogen structures—PAMPs (pathogen-associated molecular patterns), including nematode effector proteins. This mechanism is activated upon the contact of PAMPs with tomato membrane receptors, receptor-like kinases (RLKs) and receptor-like proteins (RLPs). The activation of RLKs and RLPs triggers a cascade of defense responses involving both early and late adaptive mechanisms. At the initial stages of infection, there is an accumulation of reactive oxygen species (ROS), including H_2O_2 [27], and regulation by phytohormones: salicylic acid (SA), ethylene (ET), and jasmonic acid (JA) [19]. A change in ion homeostasis occurs, accompanied by an influx of calcium into the cytosol, which serves as a signal for the further transmission of the stress response [28,29]. At later stages, mitogen-activated protein kinase (MAPK) cascades are activated, leading to the activation of transcription factors of the WRKY family and, as a consequence, to the increased expression of genes associated with the plant's defense response [30,31]. SlWRKY3 has been found to activate the expression of defense genes, contributing to resistance to *M. javanica*, while SlWRKY16 and SlWRKY31 function as negative regulators of the immune response, increasing plant susceptibility to infection [32,33].

The synthesis of phytoalexins and other antimicrobial compounds that prevent the spread of the pathogen increases in cells [34]. An important aspect of protection is the strengthening of the cell wall due to the deposition of callose and lignin, which creates a mechanical barrier for the phytoparasite and reduces the likelihood of its penetration into plant tissues [35,36]. A set of protective signaling cascades is aimed at preventing the penetration of nematodes, but in some cases they can be suppressed by the effector proteins of the pathogen [3].

If nematodes bypass the primary line of defense PTI, a second line of immunity is triggered, based on the recognition of pathogenic effectors by intracellular proteins of the NBS-LRR (nucleotide-binding site leucine-rich repeat) family. The central role in the defense of tomatoes against nematodes is played by the *Mi-1.2* gene encoding the NBS-LRR receptor protein, which is responsible for resistance to *Meloidogyne* spp. ETI can be divided into two molecular mechanisms of the tomato defense response—direct and indirect. The direct pathway is characterized by gene-for-gene interactions [37]. In this mode, the tomato NBS-LRR receptor protein complex directly interacts with nematode effectors [38]. After nematodes penetrate the plant roots, effectors encoded by the nematode virulence genes (Avr) trigger the production and expression of *Mi* genes responsible for resistance in plants [39]. The Mi-1.2 protein (NBS-LRR) directly recognizes nematode effector proteins, activating a cascade of defense reactions [40]. According to this theory, the formation of giant cells is blocked, which makes further nutrition and the vital functioning of the nematode impossible. However, for *Meloidogyne* spp., the direct binding of *Mi-1.2* to effectors has not been reliably demonstrated, so most studies support an indirect mechanism known as the "guard hypothesis" [41]. Within this pathway, *Mi-1.2* does not recognize the effector directly, but responds to modifications of cellular targets caused by the action of nematode effectors. The activation of *Mi-1.2* in this case requires the formation of a stabilizing protein complex, including the chaperone HSP90 and its regulatory co-factors SGT1, which ensure the folding, stability, and functionality of the receptor [42,43].

In the absence of the *Mi-1.2* gene, nematodes freely penetrate the root system and form galls inside which the nematodes complete their life cycle [40]. The identification of genotypes exhibiting resistance to *M. hapla* in the absence of *Mi-1.2* indicates the involvement of alternative molecular mechanisms which can be promising for targeted selection under thermal and biotic stress conditions.

Currently, there are no registered and approved nematicides for use in the Russian Federation to protect tomatoes [1]. At the same time, on crops such as potatoes, carrots, and sugar beets, the use of preparations with the active ingredients oxamil and fluopyram (2024) against cyst-forming, stem, and root-knot nematodes is approved [44]. Oxamil is classified by the WHO as an extremely hazardous (class I) substance, and fluopyram as a moderately hazardous (class II) substance [45]. Fluopyram has mutagenic and carcinogenic properties and is toxicologically hazardous to humans [46].

An alternative method of control may be biological nematicides based on predatory nematodes, nematophagous fungi (*Arthrobotrys oligospora*), or antagonistic bacteria [25]. Only one biological product is registered on the Russian market—Nematophagin-Mycopro (*Arthrobotrys oligospora* F-1303) [44], so the possibilities of using biological methods in practice are limited. The problem of meloidogynosis remains relevant and it is necessary to search for and study alternative methods of controlling root-knot nematodes [47].

The widespread introduction of varieties and hybrids into production that carry genes for resistance to certain diseases (including meloidogynosis) significantly curbs the spread and negative impact of harmful objects on tomatoes, ensuring the production of environmentally friendly products in sufficient quantities [48]. We believe that the cultivation of resistant varieties and hybrids of tomatoes is the only effective way to protect tomatoes against root-knot nematodes.

The Federal State Budgetary Scientific Institution "Federal Research Center of Biological Plant Protection" (FSBSI FRCBPP) maintains, studies, replenishes, and renews a unique working collection of tomato genetic resources, which is part of the gene pool of the crop [49]. The collection contains lines with economically valuable traits. The detection, search, and identification of genotypes with the presence of *Mi* resistance genes are relevant and will provide promising breeding material for the development of varieties and hybrids resistant to meloidogynosis.

To identify genotypes of tomato *Solanum lycopersicum* resistant to *Meloidogyne hapla*, 20 varieties/hybrids and 21 mutant lines of tomato of the FSBSI FRCBPP were inoculated with a laboratory population of *M. hapla* [1]. To determine genotypes resistant to root-knot nematodes *Meloidogyne* spp., molecular genetic screening was carried out using the SCAR marker Mi23, developed to identify the resistance gene *Mi-1.2*.

2. Materials and Methods

2.1. Research Location

Experimental studies were carried out in the laboratory of biorational means and technologies of plant protection for environmentally friendly, resource-saving, and organic farming, and in the greenhouse of the FSBSI FRCBPP, Krasnodar, Russia.

2.2. Plant Material

To determine the degree of damage and resistance of tomato to the northern root-knot nematode *M.hapla*, 20 varieties/hybrids and 21 mutant lines of tomato from the genetic collection of tomato of the FSBSI FRCBPP were planted in five-liter pots. All genotypes were divided into two experimental options: with and without nematode infection. Both groups included control lines—resistant mutant line Mo 147 and susceptible line Mo 463.

Experimental scheme:

Control (susceptible)—Mo 463;

Control (resistant)—Mo 147;

Varieties and hybrids: Beliy naliv 241, Volgogradets, Hyperbola, Evpator, Zolotoy grebeshok, Klubnichniy dessert, Malinka, Medoviy naliv, Oranzhevoye solnyshko,

Oranzheviy slon, Polosatiy reis, Torbey, Chelnok, Prima donna F1, Rumyaniy shar F1, Ranniy-83, Persey, Lyubimets Podmoskoviya, Rio Grande, and Titan Rozoviy;

Mutant lines with economically valuable properties: Mo 74, Mo 304, Mo 353, Mo 500, Mo 556, Mo 572, Mo 588, Mo 623, Mo 741, Mo 117, Mo 311, Mo 342, Mo 393, Mo 406, Mo 566, Mo 600, Mo 726, Mo 748, and Mo 871.

Amount of work: 21 mutant lines, including control lines × 2 (infected/not infected) × 3 replicates = 126 plants, 20 hybrids × 2 (infected/not infected) × 3 replicates = 120 plants. Total plants: 246 plants. Pot volume—5 L.

2.3. Artificial Infestation of Tomato Plants with Northern Root-Knot Nematode

Tomato plants were inoculated with a laboratory population of *M. hapla* (Figure 2), maintained in the laboratory of biorational means and technologies of plant protection for environmentally friendly, resource-saving, and organic farming of the FSBSI FRCBPP. To confirm the species identity of the root-knot nematode population (Meloidogyne hapla), the mitochondrial COI region was amplified and Sanger-sequenced using the universal primers JB3 (5′–TTTTTTGGGCATCCTGAGTTTAT–3′) and JB5 (5′–AGCACCTAAACTTAAAACATAATGAAAATG–3′), following the protocol described by Perry et al. [50]. The detailed sequences obtained, along with their BLAST (version 1.4.0) confirmation results, are provided in Supplementary Table S1. Egg sacs of the northern root-knot nematode (*M. hapla*) were extracted from the tomato stock culture. Under a binocular magnifying glass, the egg sacs were separated from the roots with dissecting needles and transferred to Petri dishes with water, stored at room temperature (25 ± 3 °C), and the water was regularly aerated with a pipette. Three days later, the larvae that hatched from the eggs were collected with a pipette into a test tube for further studies in a greenhouse [13]. The inoculation of tomato plants was carried out using an aqueous suspension of nematode larvae at a rate of ~500 larvae/plant, 10 days after planting in pots [50].

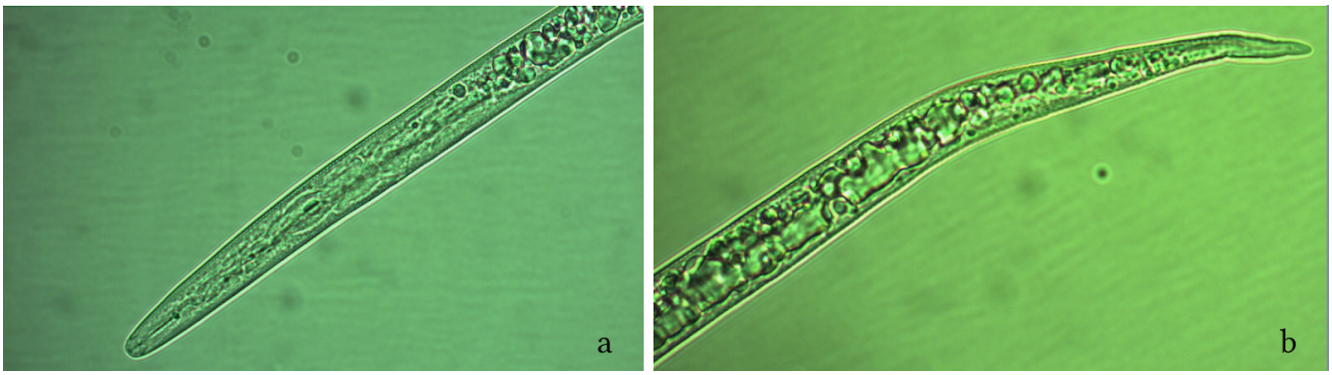


Figure 2. Anterior (**a**) and posterior (**b**) ends of the second-stage infective juvenile (*Meloidogyne hapla* apud), (microscope magnification 40×/0.65).

2.4. Assessment of the Plant Damage Based on Physiological Indicators

In the laboratory, 45 days after the infestation of plants with *M. hapla*, tomato mutant lines, varieties, and hybrids were visually analyzed. Afterwards, the gall formation index was calculated to assess the development of meloidogynosis and determine the degree of resistance. The experiment included 21 mutant lines (including two controls) and 20 commercial hybrids and varieties. Each genotype was tested under two conditions: *Meloidogyne hapla*-inoculated and non-inoculated (control). For each condition, three biological replicates were used, with one plant per replicate. In total, 246 individual plants were evaluated.

The damage to tomato plants by root-knot nematode was assessed using a scale [51]:

1—no galls;

2—single galls;

3—galls on 50% of roots;

4—galls on 75% of roots;

5—galls on the entire root system.

Based on the damage assessment, the gall formation index (GI) was calculated using the formula in [52,53]:

$$GI = \frac{1 \times n1 + 2 \times n2 + 3 \times n3 + 4 \times n4 + 5 \times n5}{N}$$

where N is the number of plants;

n1, n2 . . . n5 is the number of plants assessed with scores of 1, 2 . . . 5.

Plant infestation was considered weak with a GI score of less than 3, and strong with a score of 4 and 5.

0—GI = 0%—immunity;

1—GI = 1–10%—very weak damage;

2—GI = 11–25%—weak damage;

3—GI = 26–50%—moderate damage;

4—GI = 51–75%—strong damage;

5—GI = >76%—very strong damage.

Based on the data obtained, the degree of resistance of the experimental samples to root-knot nematodes was determined according to the Taylor and Sasser (1978) scale [54]:

0—no galls or no infection (immunity);

1—1–2 galls (highly resistant);

2—3–10 galls (resistant);

3—11–30 galls (moderately resistant);

4—31–100 galls (susceptible);

5—100 or more galls (highly susceptible).

2.5. Assessment of the Impact of M. hapla Infection on Fruit Quality and Tomato Crop Structure Based on Biochemical Parameters

The quality of fruits and the structure of the tomato crop, under the influence of *M. hapla*, were determined in accordance with Russian GOSTs (national standards) [55–57].

2.6. Statistical Data Processing

All statistical analyses were performed using RStudio (v.4.3.2) with dplyr, ggplot2, agricolae, and stats packages [58]. Changes in concentrations of fruit biochemical parameters (vitamin C content and titratable acidity), depending on the degree of genotype resistance (susceptible, moderately resistant, highly resistant, immune) and infection status (infected/uninfected), were assessed using a two-way analysis of variance, followed by the post hoc test using Tukey's significant difference (HSD) method [58,59]. Interaction effects (resistance × infection) were also assessed. Results are presented as average ± standard deviation (SD). The significance threshold of $p < 0.05$ was used in all tests.

2.7. Molecular Screening

To identify the Mi23 marker, previously developed and validated by Padilla-Hurtado (Table 1), the molecular screening of mutant tomato lines of the FSBSI FRCBPP was carried out [60].

PCR was performed in a 20 µL reaction mixture containing 40 ng of genomic DNA, 1× reaction buffer (Dialat, Moscow, Russia), 2 mM $MgCl_2$, 0.5 mM dNTPs, 0.20 µM forward and reverse primers, and 1 unit of Taq polymerase (Dialat, Moscow, Russia). To increase the efficiency of the PCR, it was performed in plates to which 4 µL of DNA solution diluted with water to a concentration of 10 ng/µL was preliminarily added to each well. When preparing PCR for one plate, a master mix was prepared containing all components except DNA.

Table 1. Primers for molecular screening.

Phytopathogen	Gene	Marker	Primer Sequence 5′-3′	Temperature	Size of Diagnostic Fragment
Meloidogyne spp.	*Mi-1.2*	Mi23SCAR	F: tggaaaatgttgaatttcttttg R: gcatactatatggcttgttttaccc	54 °C	380 bp

The DNA of the mutant tomato line resistant to *Meloidogyne* spp., Mo 147, was used as a standard. Water was used instead of DNA as a negative control to detect possible contamination.

The following program was used for PCR: initial denaturation 94 °C—5 min; 36 cycles [denaturation 99 °C—5 s, primer annealing 55 °C—40 s, elongation 72 °C—1 min]; and final elongation 72 °C—7 min.

The PCR products were separated by electrophoresis in 3% agarose gels. Just before pouring, ethidium bromide was added to the gel (50 µL/L at a standard solution concentration of 10 mg/mL); after pouring, the gel was left to polymerize for 1.5–2 h at room temperature.

To determine the size of DNA fragments, the Step100 Long molecular weight marker from Biolabmix (Russia, Novosibirsk) was used.

3. Results

3.1. Assessment of the Degree of Plant Damage Based on Physiological Indicators

To assess the degree of resistance, a phenotypic analysis of the inoculated mutant lines, varieties, and hybrids was carried out based on the number of galls per plant. The highest number of galls was observed in susceptible mutant lines Mo 463 (82.2 pcs.) (Figure 3a), Mo 871 (88.7 pcs.), and Mo 463 (67.54 pcs.). Resistance to *M. hapla* was shown by the mutant line Mo 600 (6.83 pcs.) and the following varieties: Rio Grande, Hyperbola, Torbay, and Zolotoy grebeshok, on which the number of galls did not exceed 10 pcs. Moderate resistance to meloidogynia was demonstrated by most of the samples: Mo 353, Mo 572, Mo 588, Mo 117, Mo 311, Mo 342 (Figure 3b), Mo 556, Mo 623, Mo 741, Mo 406, Mo 726, and the following varieties: Lyubimets Podmoskoviya, Oranzhevoe solnyshko, Polosatiy reis, Prima Donna F1, Rumyaniy Shar F1, Ranniy-83, Klubnichniy dessert, Medoviy Naliv, and Oranzheviy slon. Mutant lines Mo 147 (control), Mo 566, Mo 748, and Mo 500 are characterized by a complete absence of galls on the plant and are resistant to meloidogynosis (Figure 3c,d).

The analysis of each plant showed that no galls were formed on the mutant lines Mo 147 (control), Mo 566, Mo 748, and Mo 500, and the following varieties: Volgogradets and Evpator (GI = 0.0). The ratio of gall number to root weight of 0.7–1.6 (on a scale of 1–10%) was obtained for the Rio Grande, Hyperbola, Torbay, and Zolotoy grebeshok varieties, as well as for Mo 600.

The tomato varieties Lyubimets Podmoskoviya, Oranzhevoe solnyshko, Polosatiy reis, Prima Donna F1, Rumyaniy shar F1, Ranniy-83, Klubnichniy dessert, Medoviy Naliv, and Oranzzheviy slon, and the mutant lines Mo 353, Mo 572, Mo 588, Mo 117, Mo 311, Mo 342,

Mo 556, Mo 623, Mo 741, Mo 406, and Mo 726 formed galls within the GI of 2.0–2.9 (on a scale of 11–25%).



Figure 3. Root system of mutant lines with different degrees of resistance to meloidogynosis: (**a**)—Mo 463 (susceptible); (**b**)—Mo 342 (moderately resistant); (**c**)—Mo 147 (immune); (**d**)—Mo 500 (immune).

The GI varied within 3.2–3.3 points (on a scale of 26–50%) for most of the studied tomato plants: Lyubimets Podmoskoviya, Oranzhevoe solnyshko, Polosatiy reis, Prima Donna F1, Rumyaniy shar F1, Ranniy-83, Klubnichniy dessert, Medoviy Naliv, and Oranzzheviy slon, and mutant lines Mo 353, Mo 572, Mo 588, Mo 117, Mo 311, Mo 342, Mo 556, Mo 623, Mo 741, Mo 406, and Mo 726. The greatest number of galls were formed by Mo 304, Mo 463, and Mo 871, and the Persey variety (Figure 4), where the GI was 3.5–4.8 points (on a scale of 51–75%).



Figure 4. Root system of the susceptible Persey tomato plants, damaged with *M. hapla*.

When analyzing the root system to determine the damage caused with meloidogynosis, the lowest damage score was noted in the varieties Volgogradets and Evpator and mutant lines Mo 147, Mo 500, Mo 748, and Mo 566. It was noted that the plants of the hybrids Prima donna F1 and Rumyaniy shar F1, and varieties Ranniy-83, Perseys, and Mo 304, Mo

556, Mo 623, and Mo 741 formed a root mass that was most active deep in the soil. As a result, there was a different susceptibility of tomato plants to meloidogynosis (the average damage score was from 3.0 to 4.8).

Based on the data obtained, the degree of resistance of the experimental samples to the northern root-knot nematode was determined (Table 2).

Table 2. Assessment of the resistance degree of varieties, hybrids, and mutant lines of tomato by the number of galls.

Resistance Level	Mutant Line	Variety/Hybrid
Immune (no galls)	Mo 147, Mo 500, Mo 748, Mo 566	Volgogradets, Evpator
Highly resistant (1–2 galls)	Mo 74, Mo 393	Beliy naliv 241, Titan rozoviy, Malinka, Chelnok
Resistant (3–10 galls)	Mo 600	Rio Grande, Hyperbola, Torbay, Zolotoy grebeshok
Moderately resistant (11–30 galls)	Mo 353, Mo 572, Mo 588, Mo 117, Mo 311, Mo 342, Mo 556, Mo 623, Mo 741, Mo 406, Mo 726	Lyubimets Podmoskoviya, Oranzhevoe solnyshko, Polosatiy reis, Prima Donna F1, Rumyaniy shar F1, Ranniy-83, Klubnichniy dessert, Medoviy Naliv, Oranzzheviy slon
Susceptible (31–100 galls)	Mo 304, Mo 463, Mo 871	Persey
Highly susceptible (100 and more galls)	—	—

3.2. Changes in Vitamin C and Titratable Acid Content Depending on the Resistance Degree of Tomatoes to Meloidogyne hapla

This experiment studied the impact of *M. hapla* infection on the ascorbic acid (vitamin C) content and total acidity of fruits. These parameters are considered as indicators of the physiological state of the plant and possible deterioration in crop quality.

The experiment involved the quantitative determination of vitamin C and titratable acid concentrations in mature tomato fruits grown under controlled conditions with the subsequent inoculation with *M. hapla*. A comparative analysis was performed between resistant and susceptible genotypes previously characterized by the gall formation index. Measurements were performed using standardized biochemical methods that allow an objective assessment of the pathogen's effect on fruit metabolism.

The two-way ANOVA revealed significant effects of the resistance level ($p < 0.001$), infection status ($p < 0.001$), and their interaction ($p < 0.001$) on the vitamin C concentration. The lowest average values were observed in infected susceptible plants (8.52 $\pm$ 0.29 mg/100 g), while infected immune genotypes maintained high levels (11.21 $\pm$ 0.18 mg/100 g), close to the uninfected control (11.94 $\pm$ 0.23 mg/100 g). According to Tukey's HSD, infected susceptible plants were significantly different from all other groups ($p < 0.001$) (Figure 5).

For titratable acidity, infection status had a significant effect ($p = 0.028$), while resistance level did not ($p = 0.687$), and the interaction ratio was also insignificant ($p = 0.79$). Infected plants, regardless of resistance level, showed a slight increase in acidity (Figure 6), but Tukey's test did not reveal significant differences between pairs.

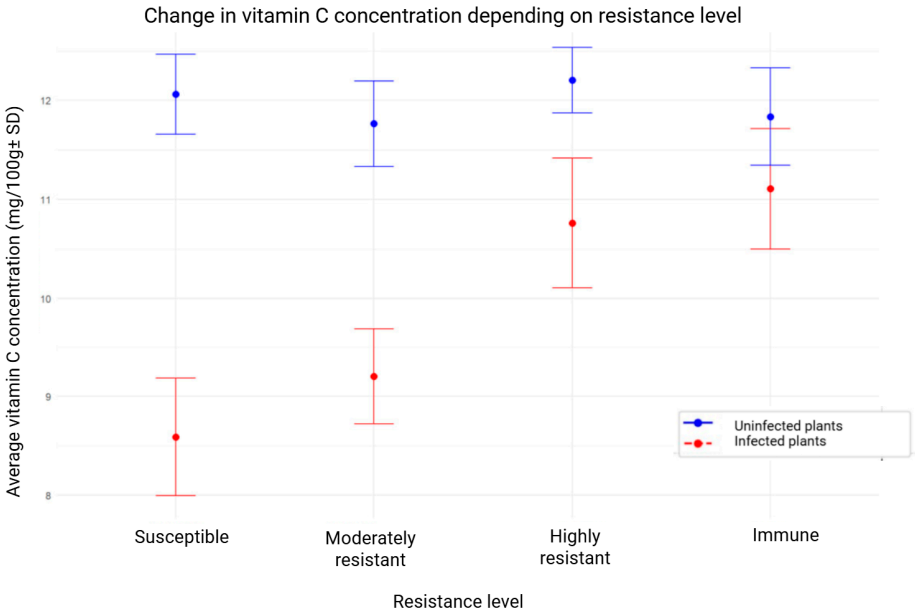


Figure 5. Average vitamin C content (mg/100 g ± SD) in tomato fruits depending on the resistance level of the genotype and infection status.

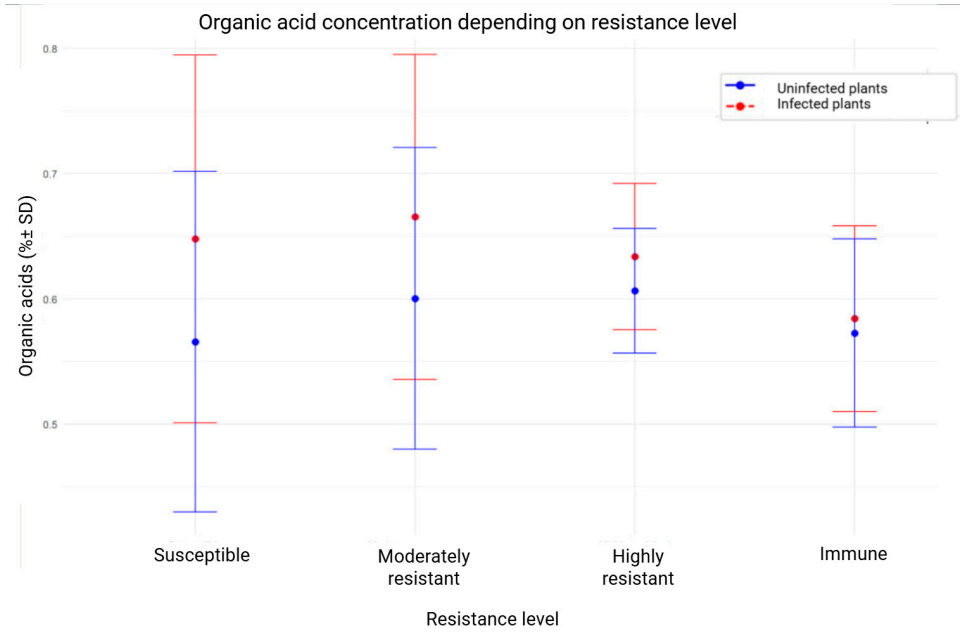


Figure 6. Average titratable acidity (% ± SD) in tomato fruits depending on the resistance level of the genotype and infection status.

3.3. Molecular Screening with the Resistance Gene Marker Mi-1.2

To identify tomato genotypes resistant to *Meloidogyne* spp. root-knot nematodes, the molecular genetic screening of 41 tomato samples was performed using the SCAR marker Mi23, previously developed to identify the resistance gene *Mi-1.2* [61]. The size of DNA fragments was analyzed using the molecular weight marker Step100 Long from Biolabmix (Figure 7A). As a control, DNA from the mutant line Mo 147 was used, in which the presence of this marker was previously identified by the scientists from the Institute of Genetics and Cytology of the National Academy of Sciences of Belarus [62].

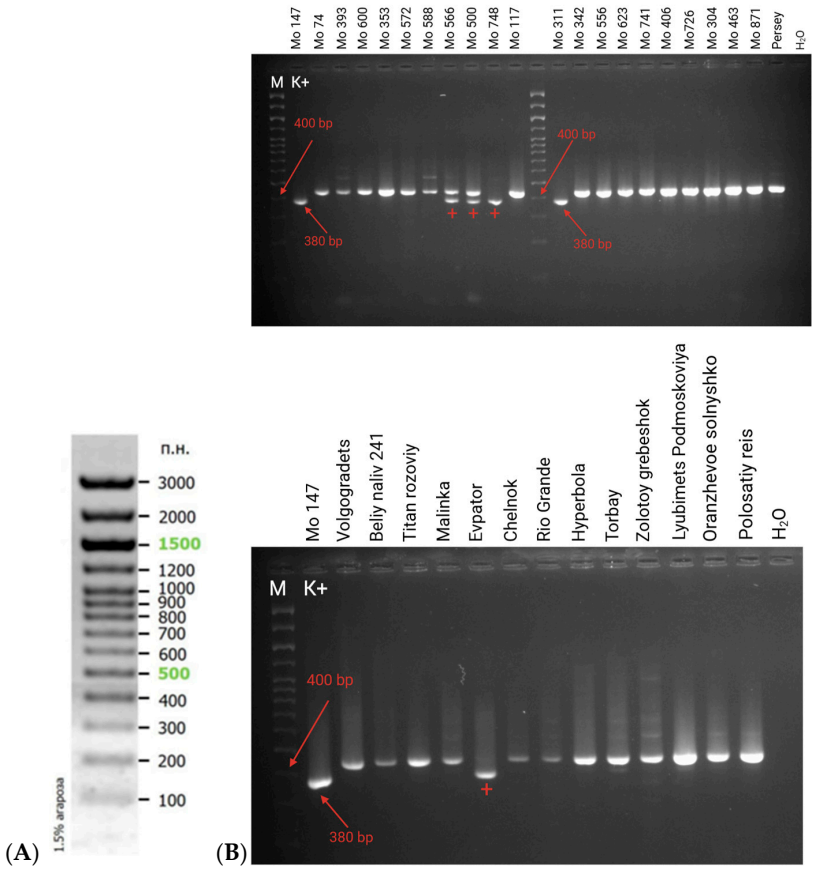


Figure 7. Visualization of PCR products in 3% agarose gel. SCAR marker of the *Mi-1.2* gene has a size of 380 bp. (**A**)—molecular weight marker Step100 Long by Biolabmix; (**B**)—PCR products: K—DNA standard of the *Mi-1.2* gene; H—H_2O (water, as a contamination control).

As a result, the presence of the *Mi-1.2* gene marker was detected in five of the analyzed samples: Mo 566, Mo 500, Mo 748, and Mo 147 (standard), and the Evpator variety (Figure 7B). The frequency of occurrence of samples with this marker in the sample is 9.5%, that is, such samples are rare and are of great practical interest.

The Mo 566 and Mo 500 lines had two PCR products, a 380 bp fragment associated with resistance and a 430 bp fragment present in susceptible forms. Since the studied DNA was isolated using the bulk sample method, the presence of two PCR products indicated the heterogeneity of the seeds in the sample.

4. Discussion

Changes in the biochemical composition of tomato fruits infected with *Meloidogyne hapla* reflect characteristic metabolic changes, which is consistent with previously published data [9,63]. Ascorbic acid, the main antioxidant, is involved in the suppression of oxidative stress and the maintenance of cellular homeostasis [64].

Our study showed that the vitamin C level significantly decreased in susceptible plants (8.6 ± 0.29 mg/100 g), while it remained stable in immune lines (up to 11.1 ± 0.18 mg/100 g in immune infected and 11.8–12.2 mg/100 g in uninfected). The two-way analysis of variance revealed a significant effect of both resistance ($p < 0.001$) and infection ($p < 0.001$), as well as their interaction ($p < 0.001$). Tukey's test confirmed the differences between the main groups. These data indicate the role of vitamin C as a sensitive marker of resistance, which is consistent with the results obtained for infection with *Meloidogyne incognita* [6], as well as the general patterns described for *Meloidogyne* spp. [8].

In contrast to vitamin C, titratable acidity demonstrated a less pronounced relationship with resistance. Infection resulted in a moderate increase in acidity (p = 0.028), but the effect of genotype (p = 0.687) and the interaction of factors (p = 0.79) were not statistically significant. Tukey's test also did not reveal significant differences between the groups. In susceptible plants, acidity reached 0.651 $\pm$ 0.011%, and in resistant plants it reached 0.585 $\pm$ 0.011%, but the fluctuations were high. Such variability may be associated with the complex composition of the indicator: titratable acidity reflects the content of organic acids (citric, malic, succinic) involved in respiration, buffering reactions, osmoregulation, and signaling. According to Etienne and Tian, the composition of the acid profile can vary under the influence of various factors and does not always correlate with resistance [10,65]. Therefore, unlike vitamin C, titratable acidity reflects a non-specific metabolic response to stress rather than resistance per se.

Thus, vitamin C can be used as an informative biomarker of resistance to *M. hapla*. Titratable acidity demonstrates challenge-related metabolic changes but does not differentiate resistant and susceptible genotypes.

Molecular screening using the SCAR marker Mi23 revealed the presence of the *Mi-1.2* gene in five samples (Mo 147, Mo 500, Mo 566, Mo 748, and the Evpator variety), which is 9.5% of the total sample. All samples showed phenotypic immunity to *M. hapla*, which confirms the relationship between the presence of the *Mi-1.2* gene and resistance. However, in the immune variety Volgogradets, the Mi23 marker was not identified, which confirms the hypothesis of the polygenic nature of resistance to this type of nematode [3].

In the course of the phenotypic assessment, the Volgogradets variety demonstrated complete resistance to *Meloidogyne hapla* (GI = 0). However, the amplification product of the SCAR marker Mi23, specific for the *Mi-1.2* locus, was not detected. Thus, the resistance of the Volgogradets variety is formed in the absence of *Mi-1.2*, which indicates the involvement of alternative genetic or signaling mechanisms.

Interestingly, all genotypes that carried the *Mi-1.2* marker—Mo 147, Mo 500, Mo 566, Mo 748, and Evpator—demonstrated complete phenotypic resistance to *M. hapla*. Conversely, only one genotype without *Mi-1.2* (Volgogradets) exhibited immunity. Although *Mi-1.2* is traditionally considered ineffective against *M. hapla* and *M. enterolobii*, and only partially effective against *M. Hispanica* [22,23], our results revealed an unexpected correlation between the presence of *Mi-1.2* and resistance to *M. hapla* under our experimental conditions. This raises the possibility that *Mi-1.2* may contribute indirectly to *M. hapla* resistance through unknown mechanisms or that its presence is linked to other resistance loci or regulatory elements. Alternatively, these results may reflect a context-dependent activation of *Mi*-mediated pathways influenced by genetic background, epigenetic factors, or specific effector profiles in the nematode population used. While this study does not provide definitive evidence of functional *Mi-1.2*-mediated resistance to *M. hapla*, it highlights the need for further molecular and histological investigations to clarify the mechanisms involved. These findings underline the importance of re-evaluating current assumptions about *Mi-1.2* specificity and exploring the broader spectrum of its potential interactions in tomato–nematode systems.

It should be emphasized that in this study, the term "resistance" is used in the context of a reduced level of damage, and not absolute immunity. For example, the Rio Grande variety, known for its susceptibility to *Meloidogyne* spp., showed a moderately resistant reaction under our experimental conditions, corresponding to GI $\leq$ 2. This does not mean the absence of infection, but only indicates partial resistance compared to the control susceptible lines.

The comparative analysis of phenotypic resistance and molecular data in this study suggests that resistance to *M. hapla* may be mediated by a polygenic architecture involving one or more loci unrelated to the *Mi-1* cluster. Similar phenomena have been previously described

for *Mi-3*, *Mi-5*, and *Mi-9*, as well as in the studies demonstrating the involvement of epigenetic regulators and interacting immune signaling pathways [9]. In addition, the lack of a complete correlation between the presence of the *Mi-1.2* marker and phenotypic resistance to *M. hapla* may be due to the presence of specific effectors in this nematode species that disrupt the functioning of *Mi*-mediated defense, as previously noted for *M. incognita* [3].

Along with the hypothesis of genetic independence of resistance, the involvement of innate immune response mechanisms, including MAPK cascades, WRKY transcription factors, the ROS system, as well as hormone-mediated pathways regulated by salicylic and jasmonic acids and ethylene, is not excluded [9,40].

The obtained data highlight the need to develop new molecular markers specific for *M. hapla*, as well as the further characterization of resistance mechanisms not associated with *Mi-1.2*. The breeding significance of the Volgogradets variety is that it can be considered as a promising donor of resistance independent of *Mi-1.2*, with the potential to develop tomato genotypes with increased stability and a broad spectrum of action against root-knot nematodes.

The analysis of morphological, molecular, and biochemical parameters allowed us to make a comprehensive interpretation of the obtained data and identify clear patterns in the response of tomato genotypes to *Meloidogyne hapla* infection. Resistant samples that had the *Mi-1.2* gene marker demonstrated a low gall formation index (GI) and stable vitamin C content, which reflects both the effectiveness of Mi-mediated protection and the maintenance of metabolic homeostasis under infection conditions. At the same time, the immune variety Volgogradets, which did not have the *Mi-1.2* marker, was also characterized by zero GI values and the absence of biochemical deviations, indicating other resistance mechanisms not associated with the *Mi-1* cluster. In contrast, susceptible genotypes, as a rule, combined the absence of the SCAR marker with pronounced gall formation and a reliable decrease in vitamin C content, which may indicate the development of oxidative stress in response to the pathogen. Thus, the integration of phenotypic, molecular, and physiological–biochemical data allowed not only to increase the reliability of the interpretation of the results, but also to clarify the nature of the resistance of the studied genotypes, which is absolutely important for the formation of a selection strategy.

The obtained results have both theoretical and practical importance. However, the limited frequency of *Mi-1.2* in the experimental sample (9.5%) and its incomplete effectiveness against *M. hapla* indicate the need to expand research to identify other resistance genes and their interactions. We believe that further searches and studies of resistant lines, such as Volgogradets, which do not contain *Mi-1.2*, but demonstrate phenotypic resistance, will reveal new genetic mechanisms regulating the tomato response to *M. hapla*.

Our data highlight the complexity of tomato resistance mechanisms to *M. hapla* and the need for long-term research to develop effective breeding strategies. The resistant genotypes identified in this work can serve as valuable material for further genetic and agronomic studies aimed at reducing yield losses and improving the environmental safety of tomato production.

5. Conclusions

The conducted study allowed us to assess the resistance of 20 tomato varieties/hybrids and 21 mutant lines from the collection of the FSBSI FRCBPP to the northern root-knot nematode using phenotypic, biochemical, and molecular genetic methods. The results showed that resistance to *M. hapla* varies widely: mutant lines Mo147, Mo500, Mo566, and Mo748 and varieties Volgogradets and Evpator demonstrated immunity (absence of galls).

The biochemical analysis revealed that infection with *M. hapla* leads to a decrease in vitamin C content (up to 8.6 mg/100 g in susceptible plants versus 11.1–12.2 mg/100 g in

resistant and uninfected ones). This fact confirms metabolic stress and deterioration of fruit quality in susceptible genotypes.

Molecular screening using the Mi23 marker revealed the presence of the *Mi-1.2* gene in five samples (Mo147, Mo500, Mo566, Mo748, and Evpator), but the resistance of some lines, such as Volgogradets, which do not contain *Mi-1.2*, indicates the existence of alternative genetic mechanisms that require further study.

Notably, all genotypes carrying the *Mi-1.2* gene in our study—despite its traditional classification as ineffective against *M. hapla*—demonstrated complete phenotypic resistance, while only one *Mi-1.2*-negative genotype ('Volgogradets') exhibited immunity. This consistent pattern contradicts previous assumptions regarding the specificity of *Mi-1.2* and suggests the possible involvement of modifier genes, linked resistance loci, or indirect molecular interactions contributing to the observed resistance. These findings warrant further investigation into the functional role of Mi-1.2 in noncanonical resistance pathways and emphasize the complexity of tomato–nematode interactions.

The data obtained emphasize the value of the identified resistant genotypes as promising pre-breeding material. However, the complexity of the genetic basis of resistance and the lack of a complete correlation between the *Mi-1.2* marker and phenotypic resistance indicate the need to search for additional molecular markers and a detailed analysis of signaling pathways and epigenetic factors. Further studies will clarify the contribution of various genes to the resistance to *M. hapla* and develop effective strategies for the biological protection of tomato, which is especially important in the absence of nematicides approved for use in the Russian Federation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae11070798/s1, Table S1: Details of species confirmation by COI sequencing.

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Abbreviations

RKN	Root-knot nematode
SCAR	Sequence Characterized Amplified Region
FSBSI FRCBPP	Federal Research Center of Biological Plant Protection
Mo	Mutant tomato line from the FSBSI FRCBPP collection (e.g., Mo 147, Mo 500, etc.)
CAPS	Cleaved Amplified Polymorphic Sequences
PCR	Polymerase Chain Reaction
MS	Murashige and Skoog medium
ANOVA	Analysis of Variance
HSD	Honestly Significant Difference (Tukey's test)
PTI	Pattern-Triggered Immunity
ETI	Effector-Triggered Immunity
ROS	Reactive Oxygen Species
HR	hypersensitive response

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