THE SCREENING OF SEVERAL MOLDAVIAN TOMATO CULTIVARS FOR IDENTIFICATION OF MI-NEMATODE RESISTANCE GENE

Maria DUCA1*, Angela PORT1, Antonina CROITORU1, Nadejda VRANCEAN2, Isgouhi KALOSHIAN3

Abstract: Twenty two tomato cultivars grown in the Republic of Moldova were screened for resistance to root-knot nematode by molecular marker analysis and nematode bioassay. DNA based test and nematode bioassay were used to determine the presence of the root-knot nematode resistance gene Mi in these tomato cultivars. PCR product digestion with Taq I enzyme was performed to distinguish the homozygous and the heterozygous state of a particular allele at the Mi locus. One tomato line (348-III-2) was identified as homozygous resistant for the Mi gene, one variety (Djina) and two lines (35-IX-1, 351-IV-1) as heterozygous. The rest of the tomato genotypes tested were homozygous for the susceptible allele. Nematode bioassays were in agreement with the molecular marker analysis. This screening represents the first study of resistance to root-knot nematodes of tomatoes cultivated in the Republic of Moldova.

Keywords: Root-knot nematode - GI- Meloidogyne - Solanum lycopersicon - Mi

Introduction

Tomato (Solanum lycopersicon L.) (Peralta and Spooner, 2001) is the second most consumed vegetable after potato (FAOSTAT, 2010), widely grown around the world, and constitutes a major agricultural industry especially for agricultural countries such as the Republic of Moldova. The tomato crop is seriously affected by over 200 diseases caused by pathogenic fungi, bacteria, viruses and nematodes (Abad Z. G. and Abad J. A., 1997; Koch et al. 1992; Lukyanenko, 1991; Moriones and Navas-Castillo, 2000); therefore a major goal of modern tomato breeding programs is the development of cultivars with improved disease resistance.

The tomato resistance gene Mi encodes a protein with nucleotide-binding and leucine-rich repeat domains and is located near the centromere of the short arm of chromosome 6 (Mehlenbacher, 1995; Milligan et al., 1998). It was introgressed into cultivated tomato from its wild relative Solanum peruvianum, using traditional breeding and embryo rescue (Smith, 1944). The gene confers resistance against three major Meloidogyne species that infect tomato, M. arenaria, M. incognita and M. javanica (Holliday, 1989; Martinez de Ilarduya al., 2001; Nombela et al., 2003). Root-knot nematodes are endoparasites considered to be the most damaging pests of tomato worldwide (Trudgill and Blok, 2001). Disease symptoms caused by root-knot nematode on susceptible host plants include damaged root system and the formation of root galls, wilting, chlorosis, and stunted growth resulting in poor yield (Abad et al., 2003). Mi mediated resistance is characterized by a rapid (within 12 hours of infection), localized necrosis or hypersensitive response near the infecting head of the juvenile that limits its...
feeding and establishment of a feeding site known as giant cells (Ho et al., 1992; Hwang et al., 2000). Breeding root knot nematode resistant tomato varieties constitutes an economically feasible option for nematode management (Cook, 2000). Although nematode infection sometimes occurs on resistant tomato cultivars, the pest generally fails to develop and reproduce to high levels on these resistant tomato genotypes grown under greenhouse or field conditions (Terrell et al., 1983).

Marker assisted selection represent a powerful tool to overcome some limitations of traditional breeding methods and is successfully adopted by tomato breeding programs. Marker assisted selection for disease resistance offer the additional advantage of permitting selection for resistance in the absence of the pathogen(s) (Kelly, 1995; Mehlenbacher, 1995). Among crop species, tomato is very rich in the number of available molecular markers. Therefore, it is possible to identify and develop an efficient DNA PCR marker that is tightly linked to the trait of interest.

Rex-1 is a co-dominant PCR–based marker that has been shown to be tightly linked to the Mi locus and is frequently used as a diagnostic marker to differentiate root-knot nematode resistant and susceptible tomato cultivars (Williamson et al., 1994). The objective of this study was to screen several tomato cultivars grown in Moldova, for resistance to root-knot nematode using molecular marker assays with perspective to use them in the development of new nematode resistance hybrid cultivars.

Materials and methods

Plant material. In this study eleven tomato varieties were used: Fakel, Djina, Missouri, Shakira, Onix, Zagadca, Peto-86, Laguna, Nadejda, Novicioc, Leana. In addition, two hybrids Dacia and Lira, and nine tomato lines: 528, 13, 8, 346-I-1, 347-IX-1, 348-III-2, 349I-2, 35-I-1, 351-IV-1 kindly provided by the Scientific-Practical Institute of Phytotechnology and the Institute of Genetics (Republic of Moldova). Two tomato lines were used as controls. Cultivar VFNT (Mi/Mi) that is homozygous for the nematode resistant allele Mi and cultivar UC82B (mi/mi), homozygous for the susceptible allele mi.

The plants were grown in plastic pots of 500 ml volume filled with a mix of steam sterilized sand and soil (9:1). Every two weeks plants were watered with a mix of organic and mineral fertilizer of Biohumus Raduga trademark (1 litter solution contains: NH₄NO₃-10g; K₂O-10g; P₂O₅-10g; huminic acids-2 g).

Nematode isolation and inoculation. Root-knot nematode infected tomato plants, grown in a greenhouse where nematode susceptible tomatoes are grown for several years, were used as the source of the nematode inoculum. Tomato roots were cut to pieces and blended in a 0.5% NaOCl solution to extract eggs (Hussey and Barker, 1973). The solution was poured directly through a 200-mesh (75 µm) sieve nested on a 1 l beaker, and then eggs were collected on a 500-mesh (26 µm) sieve. Eggs were allowed to hatch using a modified Bearmann funnel (Martinez de Iñárruigo et al., 2001).

Two plants per genotype were infected at the stage of three true leaves. For the nematode infection, 1 ml of inoculum was added at the base of each plant in small holes. Three months after infection, the tomato plants were carefully removed from the pots, and the root systems washed free of soil. Then, the roots were stained with a 0.05% erioglaucine (Sigma, Saint Louis, MO, USA), egg masses and galls counted, and analyzed microscopically (light microscope МБС-9, Russia).
The degree of root knot nematode infection was evaluated by the gall index (GI) using a scale of 0-5 as follows: 0 - no galls or egg masses on the plant root; 1 - 1 to 2 galls or egg masses; 2 - 3 to 10 galls or egg masses; 3 - from 11 to 30 galls or egg masses; 4 - 31 to 100 galls or egg masses, 5 - more than 100 galls or egg masses per root system.

**Analysis of tomato DNA for the REX-1 marker by PCR and digestion with TaqI.** Genomic DNA was isolated from leaves using CTAB method (Doyle J. J. and Doyle J. L., 1987). DNA quality and yield was evaluated by 0.8% agarose gel electrophoresis and UV absorbance (A260/A280) using PG instruments UV.VIS NC 60 spectrophotometer (PG instruments, Great Britain).

PCR amplification was carried out with the following reaction mix: 50 ng of genomic DNA template, 5 pmol of primers: REX-F1 (5’-TCGGAGCCTTGGTCTGAATT-3’) and REX-R2 (5’-GCCAGAGATGATTCGTGAGA-3’) (Williamson et al., 1994); 200 µM dNTP, 2.5 mM MgCl₂, 1x PCR buffer provided by the manufacturer, 1.25 units Go Taq DNA polymerase (Promega, Madison, WI, USA) in a total volume of 25 µl. The PCR reaction was performed in an automated thermal cycler (CG1-96, Corbett, Austria) programmed: 94°C for 3 min, followed by 35 cycles: 94 °C for 1 min, 55° C for 2 min, 72 °C for 2 min and a final extension cycle at 72°C for 5 min.

Amplified products (7 µL) were digested overnight at 65°C using 5 units of TaqI (Sigma, USA) per reaction (10 µL). Digested products were resolved on a 1.5% agarose gel and 50 bp DNA ladder (Fermentas, USA) was used to estimate the fragment size.

**Results and discussion**

**Root-knot nematode resistance bioassay.** The nematode bioassay revealed typical disease symptoms caused by root-knot nematodes characterized by the presence of small galls, 1 to 10 mm in size, on most infected tomato roots (Fig. 1A, B and C). Scoring the root systems, using the galling index, revealed different levels of susceptibility among the tomato genotypes tested. Since the root systems of the tomato plants were not large, the nematode infection rate was low on the susceptible control as well as on the susceptible genotypes (Fig. 1D). The evaluation of the degree of root galling showed that eleven varieties and eight lines had GI between 1 and 4 displaying galls with different sizes. Peto-86 was scored as the most susceptible genotype. Five genotypes varieties Shakira, Peto-86, Laguna and line 349-I-2 as well as the susceptible control UC82-B had the highest GI scores between 3 and 4 (Fig. 1D). The hybrid plants Dacia and Lira had GI of 2 and 1, respectively, most galls being small in size, <1mm. The tomato genotypes 348-III-2, 35-IX-1, as well as the resistant control VFNT showed no root galls.

The susceptible plants displayed also some symptoms of wilting and chlorosis. Since the vascular alterations caused by the formation of the giant cells restrict water and nutrients uptake, and interfere with the translocation of minerals and photosynthates in the host, most likely the plants manifested a reduced capacity for nutrient uptake with a general reduction in vigor typical symptoms observed on root-knot nematode infested hosts.
Molecular screening of tomato genotypes for the presence of the Mi gene. DNA based test with the PCR marker Rex-1 and post-PCR digestion with the restriction enzyme Taq I was performed to identify the presence or absence and the zygosity of a particular allele at the Mi locus.

A single amplified product of 750 base pair (bp) was obtained with all samples using PCR and the Rex-1 primers (data not shown). Digestion of the PCR products with the Taq I restriction enzyme results in two fragments of 570 bp and 160 pb, if Mi allele is present in the homozygous condition (Mi/Mi) as it is shown in the control VFNT tomato sample (Fig. 2 lane 4). If the Mi allele is in the homozygous susceptible condition, then the Rex-1 PCR product doesn’t contain a Taq I cleavage site and is not restricted as it is in the susceptible control cultivar UC82B sample (Fig. 2 lane 3).

The molecular test of studied eleven varieties, nine lines and two F₁ hybrids showed that the Mi allele is present in the heterozygous state (Mi/mi) in one variety (Djina) and two lines (35-IX-1, 351-IV-1) because when resolved electrophoretically these samples yielded three fragments of 750, 570 and 160 bp in length (Fig. 2 lanes 2, 16, and 18). One tomato line (348-III-2) displayed the homozygous Mi resistant banding pattern (Fig. 2 lane 6).
rest of tomato samples, including the hybrid lines Dacia and Lira, displayed homozygous mi susceptible banding pattern (Fig. 2).

Comparative analysis of the molecular and bioassay data demonstrates that the genotypes Mi/Mi and Mi/mi confer a resistant phenotype (the average scoring range is equal to or above 0, but below 1.0) for nematode resistance. The screening of twenty Moldavian tomato cultivars for resistance to root-knot nematode revealed the perspective of using in breeding programs of three lines: 35-IX-1 (Mi/mi), 351-IV-1 (Mi/mi), 348-III-2 (Mi/Mi) and one variety Djina (Mi/mi).

Basic PCR techniques are sensitive to changes in reaction conditions that sometimes make difficult to determine if the absence of an amplification product(s) is due to the genetic nature of the sample or due to unfavorable reaction conditions. Thus, for the specificity of analysis it is preferable to use more stable and reliable codominant markers as Rex-1. Obviously, the application of marker assisted selection is more rapid and convenient than traditional selection for the trait and can greatly improve the deficiency of tomato breeding for resistance to root-knot nematodes.

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