Detection of Root Knot Nematode *Meloidogyne incognita* by PCR

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Abstract

It is indispensable to have accurate and speedy method of nematodes detection considering their great deal of malignancy nematodes. For identification of *Meloidogyne incognita* genetic primers were designed and the procedure was attested by Polymerase Chain Reaction.

Keywords: genus *Meloidogyne*; PCR; primer; extraction

INTRODUCTION

Root – knot nematodes (RKN) from the genus *Meloidogyne* are widespread almost all over the world and their importance increased during last ten years. Losses of yield caused by these nematodes mainly in tropical and subtropical areas are very important. Parasitic style of life on plant roots together with root hyperplasia and root – knoting cause debilitating of root system (TAYLOR 1971).

Highly infested plants delate their development and during warm days they can wilt. Highly infested young plants can die without root – knot forming. If infestation is not so high symptoms on upper parts of plants are not well visible and such plants can be overlooked in field. That is why symptomless plants should be checked too (WILLIAMSON *et al*. 1997). Host range of *Meloidogyne* species is very vide, about 350 plant species from various families. Weeds can serve very well as reservoir plants (LIŠKOVÁ & STURHAN 1997).

Short generation cycle enables higher harmfulness of RKN. Development time depends on temperature and may be between 26–56 days. Under these conditions there may be 1–5 generations per year (DECKER 1969). Surviving as eggs even in dry substrate makes from RKN very dangerous parasites of plants.

Prevence together with fast, exact and cheap diagnostics are bases for effective plant protection against RKN. Methods using morphometric characteristics currently used for RKN detection are personnel demanding. Diagnostics on the base of DNA analysis using molecular markers may be an alternative. Its speed, accuracy and sensitivy are good prerequisites for reliable diagnosis. Population of *M. incognita* from glasshauses of Czech University of Agriculture in Prague was maintained on tomato plants variety Stupické in climatized room (18–20°C, 85% air humidity, 16 h day).

MATERIALS AND METHODS

DNA extraction

The aim was optimalization of DNA extraction from various materials and the protocols should be usable at the most variable conditions. DNA was extracted from females preparated from roots, invasion larvae, root – knots and soil. DNA from invasion larvae and females was isolated using proteinase K, DNA from root – knots was isolated by classic fenolic extraction. DNA from soil was extracted using kit from Q-biogene. DNA from *M. arenaria, M. fallax, M. chitwoodi, M. javanica* and *M. hapla* extracted by the same way was used as a control.

PCR

Primers were designed from published sequence of *Meloidogyne incognita* DNA coding for esophaegal gland protein SEC – 1. Predicted lenght of product

is 502 bp. Amplification should be specific for *Meloidogyne incognita* DNA only.

25 μl reaction mixture consisted from:

1. 2.5 μl of buffer for *Taq* DNA polymerase
2. 1.5 mM of $MgCl_2$
3. 0.25 μl of dNTP (final concentration 0.2 mM each d NTP)
4. 0.4 μl + 0.4 μl of each primer
5. 2 units of *Taq* DNA polymerase
6. dd H_2O (water to 24 μl)
7. 1 μl of extracted DNA
8. 1–2 drops of mineral oil.

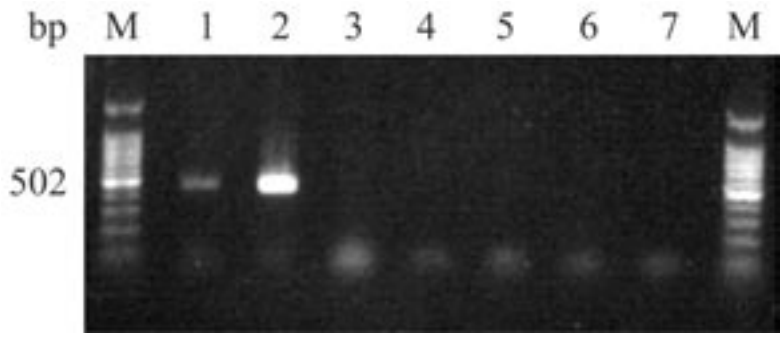
PCR was done in a PTC 200 thermocycler (MJ Research) according to the program:

1. 94°C for 1 minute
2. 60°C for 1 minute
3. 72°C for 1 minute 30 seconds
4. from step 2. 40×
5. 72°C for 5 minutes
6. 4°C end.

For visualization of productes electrophoresis in 1% agarose gel using ethidium bromide and UV transillluminator was used.

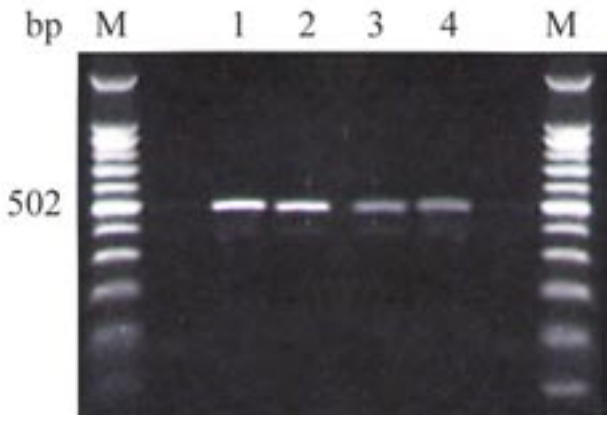
RESULTS AND DISCUSSION

Designed primers gave very specific results with DNA from *M. incognita*. DNA from other species was never amplified (Figure 1). DNA of *M. incognita* from all sources (females, invasion larvae, root – knots and soil) was easily amplified (Figure 2). Optimal annealing temperature for the primers was 60°C. The number of cycles was adjusted to the DNA from various material (40 for females, root – knots and larvae, 50 for soil). PCR proved to be a powerful tool for nematode determination. It is very specific, relatively fast and sensitive (one female or larva gives sufficient amount of DNA for analysis). On the contrary, for determination using morphometric characteristics more individulas are ussualy need. Our method is quite usable for routine determinations of nematodes. So, it completes the group of molecular methods of nematode diagnostics (WILLIAMSON *et al*. 1997; ZIJLSTRA 1997; ZIJLSTRA *et al*. 1995, 1997). However, this is probably the first case when the gene outside of ITS regions was used for primer designation.



Line 1: DNA extracted from *M. incognita*
Line 2: DNA extracted from *M. incognita*
Line 3: DNA extracted from *M. arenaria*
Line 4: DNA extracted from *M. hapla*
Line 5: DNA extracted from *M. chitwoodi*
Line 6: DNA extracted from *M. fallax*
Line 7: DNA extracted from *M. javanica*

Fig. 1. Results of PCR with using different species of *Meloidogyne*



Line 1: DNA extracted from females
Line 2: DNA extracted from root – knot
Line 3: DNA extracted from invasion larvae
Line 4: DNA extracted from soil

Fig. 2. Results of PCR

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