Using DNA markers for characterisation of tomato resistance against root nematode *Meloidogyne incognita*

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ABSTRACT

CAPS (Cleaved Amplified Polymorphic Sequence) method and standard infection tests were used for the study of tomatoes (*Lycopersicon esculentum* L.) resistance against the root-knot nematode (*Meloidogyne incognita* Kofoit and White). The CAPS method was used to determine genotypic constitution of M_i gene in ten Czech and four foreign varieties of tomatoes. Similarly were verified one hundred and twenty individuals of F_2 segregated progeny of Nema variety and the same number of F_2 progeny of Petopride variety. A comparison of results of infection test with results of molecular-genetic analyse of DNA showed 100% congruence in detection resistant (susceptible respectively) genotypes. Result of χ^2 test confirmed in the level of mathematical expectation $P \in (0.5, 0.7)$ congruence between actually obtained and theoretical segregation ratios. The CAPS method can be recommended for fast and precise determination of resistance or susceptibility of tomato plants against the root-knot nematodes. The goal of the method is the possibility of detection of genotypic constitution of M_i gene that can significantly accelerate the process of creation of new resistant varieties.

Keywords: tomato; *Lycopersicon esculentum* L.; *Meloidogyne incognita* Kofoit and White; resistance; CAPS; DNA; marker; PCR

Genus *Meloidogyne*, especially *Meloidogyne incognita* Kofoit and White, is in an economical view an important pathogen, and is considered as the most dangerous pest of in greenhouse-grown plants. Females of *M. incognita* make on the roots of host plants 4–5 cm galls that form on the base of fusion of few smaller galls. Life cycle and diffusion of the nematode studied also Fassuliotis and Connick (1989), Castagnone-Sereno and Kermarrec (1991) and Beek (1997).

M. incognita is spread out in all five live continents and makes large economical losses in many countries inclusive of Czech Republic. Quantity of over- and underground part of tomato plants is smaller in consequence of attack by the pest (Fortnum et al. 1991).

Resistant breeding is one of the most effective methods of tomato protection. The biochemical mechanism of tomato resistance against nematodes studied for example Bajaj (1983), Huang (1983) and Cook (1991).

Species *Lycopersicon peruvianum* L. is considered as the donor of M_i resistance gene against *Meloidogyne incognita*. Except others, Veremis and Roberts (1996) studied the complexity of resistance genes that come from *L. peruvianum*. Later other M_i genes were identified (M_i1, M_i2, M_i3). These genes are localised in short arm and in the pericentromeric region of chromosome VI of *L. esculentum* (Wordragen et al. 1994).

Genetic and segregation analyses confirmed that the M_i gene is closely linked to the gene of resistance against aphids (Kaloshian et al. 1995, Vos et al. 1998). Selection based on *Mi* gene allows simultaneously chosen genotypes resistant against aphids.

All techniques of molecular-genetic markering are always connected to field and greenhouse tests of resistance. By means of field tests primary material with presumptive resistance is selected and then analysed by DNA makers. It is known that resistance prove only the dominant homozygotes and heterozygotes. The detection of the genotypic constitution of *Mi* gene is based technically on the CAPS method (Williamson et al. 1994, Yaghoobbi et al. 1995). The detection of genes, their markering, and mapping performs a sharp acceleration of the breeding process.

Supported by the Ministry of Education, Youth and Sports of the Czech Republic, Projects MŠM 41210-0002, FRVŠ G4-1102 and ČZU 21190/1312/213110.

MATERIAL AND METHODS

We analysed fourteen varieties of tomatoes. Ten varieties of Czech provenance were susceptible and four foreign varieties were resistant against *Meloidogyne incognita*. In next step one hundred and twenty individuals of Nema's progeny (F_2 generation) and the same amount of Petopride's progeny were analysed (Table 1). The material came from breeding station Moravoseed Ltd.

The genotypes were tested in provocation conditions of the infection test for verify of declared resistance or susceptibility. An infected soil substrate was used for this test. In a container with volume of one litter, that included the sensitive variety Stupické polní rané, five eggs-pouches of *M. incognita* were added to each container. Used nematodes were identified on the base of morphological method and PCR (Tesařová et al. 2003) as *M. incognita*. After one hundred and ten days of vegetation was obtained enough amount of infected soil. Soil substrate for the test of resistance was prepared of one part of the infected substrate and nine parts of the clean substrate without galls. Experimental containers contained ten litters of the substrate and five tested plants in the stage of four leaves. Containers were placed in a greenhouse and equipped by automatic irrigation. After one hundred and ten days of vegetation the experiment was evaluated. The root system (galls presence or absence) of individual plants were analysed and photo-documented. Roots of all plants were evaluated also microscopically.

DNAs were isolated by GenElute™ Plant Genomic DNA kit (Sigma, SRN). The modified method according Willamson et al. (1994) was used for detection of genotypic constitution of M_i gene. 25 µl PCR reaction consists of: 100 ng template DNA, 1 U *Taq* polymerase, 0.5µM of each primer, 1.5mM of MgCl, 0.1mM dNTP, 1× PCR buffer. The amplification profile was as followed: 1× (94°C, 180 s), 30× (94°C, 60 s; 55°C, 120 s; 72°C, 120 s) and 1× (72°C, 480 s). Primers REX-F1 5′-TCGGAGCCTTGGTCTGAATT-3′ and REX-R2 5′-GCCAGAGATGATTCGTGAGA-3′ were used. There was obtained monomorphic A 750 bp PCR

Table 1. Analysed varieties and result of CAPS markering and infection test

Variety	Origin	Variety type	Results of CAPS markers	Result of infection test
Domino	Semo Ltd. Czech Republic	F_1 hybrid	m_im_i	susceptible
Marfa	Royal Sluis Netherlands	F_1 hybrid	M_im_i	resistant
Nema 1435	Royal Sluis Netherlands	F_1 hybrid	M_im_i	resistant
Orkado	Moravoseed Ltd. Czech Republic	F_1 hybrid	m_im_i	susceptible
Petopride II	Royal Sluis Netherlands	F_1 hybrid	M_im_i	resistant
Resyset	Royal Sluis Netherlands	F_1 hybrid	M_im_i	resistant
Sláva porýní	Moravoseed Ltd. Czech Republic	line	m_im_i	susceptible
Start S	Moravoseed Ltd. Czech Republic	F_1 hybrid	m_im_i	susceptible
Stupické skleníkové	Moravoseed Ltd. Czech Republic	line	m_im_i	susceptible
Tajfun	Moravoseed Ltd. Czech Republic	F_1 hybrid	m_im_i	susceptible
Tipo	Semo Ltd. Czech Republic	F_1 hybrid	m_im_i	susceptible
Tornádo	Semo Ltd. Czech Republic	F_1 hybrid	m_im_i	susceptible
Toro	Semo Ltd. Czech Republic	F_1 hybrid	m_im_i	susceptible
Uragan	Moravoseed Ltd. Czech Republic	F_1 hybrid	m_im_i	susceptible

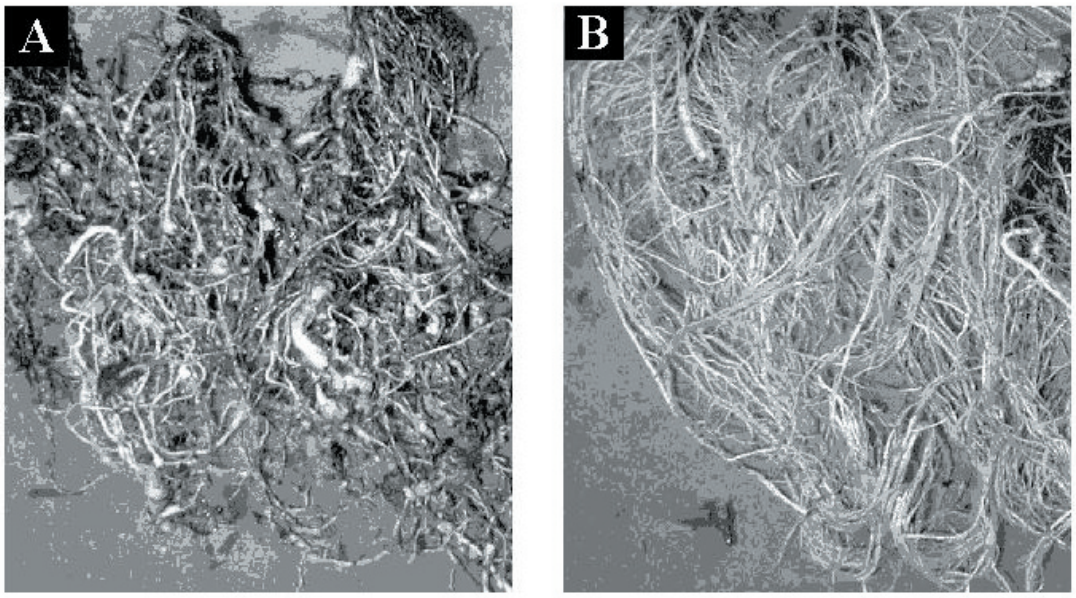


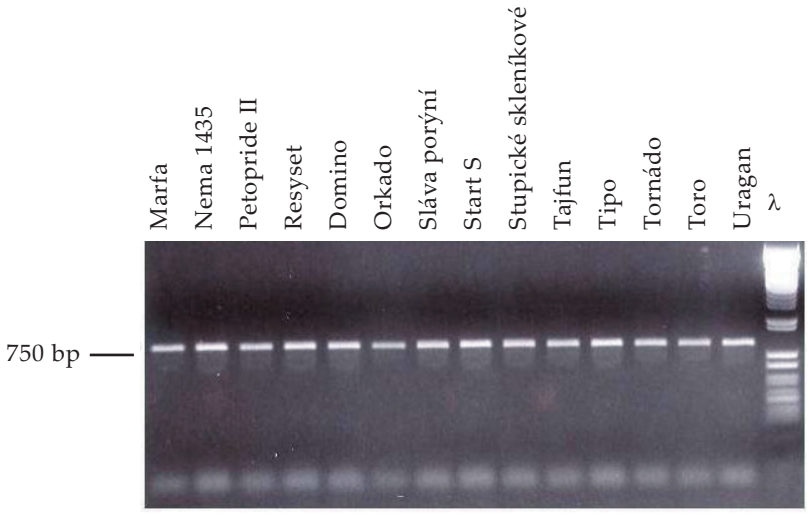
Figure 1. Roots of susceptible (A) and resistant (B) plants

product. The fragment was consequently digested by *Taq*I restriction endonuclease. PCR products were separated in 1.5% agarose gel and visualised under the UV light after ethidium bromide staining.

RESULTS AND DISCUSSION

After one hundred and ten days of cultivation, plants in infected soil had detected galls of *M. incognita* on roots of plants with declared susceptibility. All of the resistant genotypes had characteristic absence of galls. Individuals of *M. incognita* were detected only in susceptible plants. Figure 1 shows an example of root systems of resistant and susceptibility.

The 750 bp fragment (Figure 2) was obtained in all tested genotypes. This 750 bp PCR product was not in all of the recessive homozygous $m_i m_i$ digested. In the heterozygous there were three fragments (750 bp, 570 bp and 160 bp) obtained. For the dominant homozygous there were two



λ – marker λDNA/*Eco*47I(*Ava*II)

Figure 2. Electrophoreogram of monomorphic PCR product before restriction

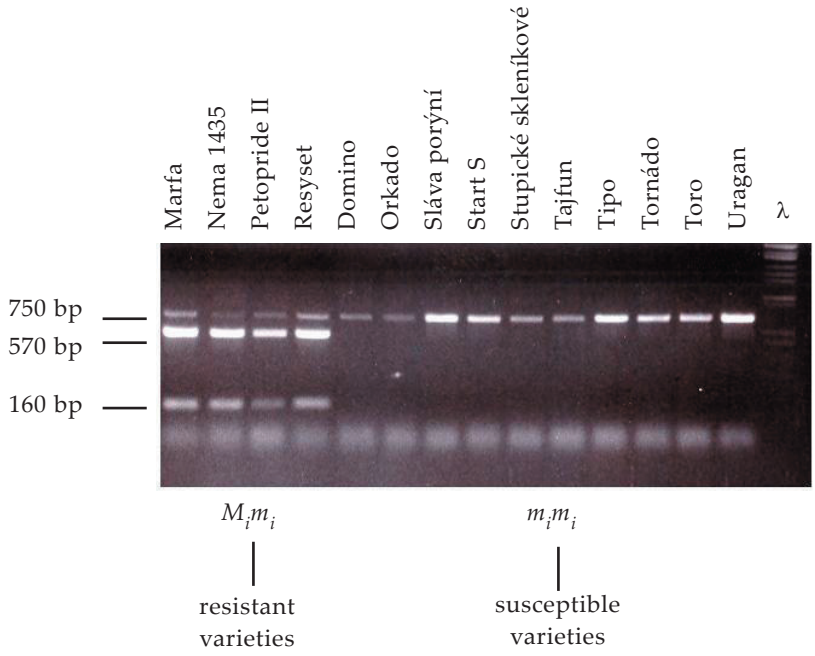


Figure 3. Electrophoreogram of separated restriction products

characteristic fragments (570 bp and 160 bp). Figure 3 presents the result of specific digestion in analysed varieties.

Digestion of the PCR product allowed determinate genotypic constitution of M_i gene described in all individuals of progenies of Nema and Petopride varieties. Detected genotypes by means of CAPS marker are summarised in Table 1. Figure 4 represents prototypal electrophoreogram of fifteen genotypes of segregated F_2 progeny.

There is a possibility to determine the dominant homozygous, heterozygous and recessive homozygous in individual genotypes in M_i gene. The result of bulked analyse of F_2 progenies of Nema and Petopride varieties is shown in Table 2. For verification of the segregation ratio of all tested genotypes we used the χ^2 test. Its result is summarised in Table 3.

The real obtained segregation ratio of Nema progeny corresponds to theoretical ratio of monohybrid $1M_iM_i$:$2M_im_i$:$1m_im_i$. The mathematical expectation is in interval $P \in (0.5, 0.7)$. Phenotypic segregation is identical to theoretical ratio 3:1 [resistant: susceptible with mathematical expectation $P \in (0.3; 0.5)$].

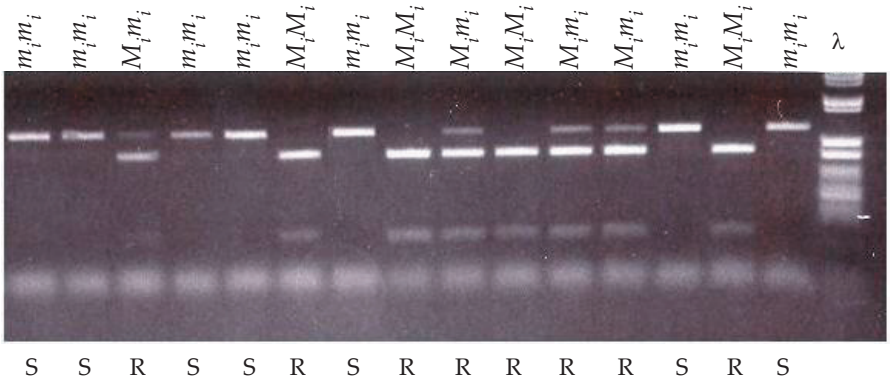


Figure 4. Electrophoreogram of bulked analysis in F_2 generation of variety Petopride II

Table 2. CAPS markers segregation in F_2 generation

Genotype	Genotypic segregation			Phenotypic segregation	
	M_iM_i	M_im_i	m_im_i	resistant	susceptible
Nema 1435 F_2	32	62	26	94	26
Petopride II F_2	34	57	29	91	29

Table 3. Evaluation of segregation by means of χ^2 test

Genotype	Genotypic segregation		Phenotypic segregation	
	χ^2	expectation P	χ^2	expectation P
Nema 1435 F_2	0.733	(0.5; 0.7)	0.711	(0.3; 0.5)
Petopride II F_2	0.716	(0.5; 0.7)	0.078	(0.7; 0.8)

Similar results were obtained also in the cause of evaluation of Petopride progeny segregation. The expectation of phenotypic segregation congruence was in the interval $P \in (0.7; 0.8)$. Resultant values of mathematical expectations unequivocally confirm monogenic ground of this resistance and correspond to the works of Vos et al. (1998).

Regression and correlation comparison of results of infection tests and molecular-genetic analyses of tested genotypes was accomplished. The value of correlation coefficient was 1.00. Resistance and susceptibility of all genotypes was unequivocal by means of both methods determined. But only molecular-genetic analysis allowed detection of genotypic constitution of M_i gene in tested plants. Only dominantly homozygous constitution of M_i gene allows production of resistant lines and following F_1 hybrids varieties of tomato with declared resistance. Using the CAPS marker according to Williamson et al. (1994) enabled unequivocal determination of genotypic constitution of M_i gene. It is possible to use this method in very early ontogeny stage of studied plants and it is not necessary to pursue real and time-consuming infection tests. Simultaneously this method allows the selection of dominant homozygotes and there is no use for the testing of resistant plants in the next generations.

Said facts indicate that the possibility may exist to speed up the creation of new resistant varieties by means of the CAPS method. The DNA marker according to Williamson et al. (1994) is credibly usable in the breeding of new varieties also in Czech Republic. Similarly tight linkage between genes M_i and M_{eu} (gene for resistance against aphid *Macrosiphum euphorbiae* Thomas) (Fazal et al. 1994, Kaloshian et al. 1995 and Vos et al. 1998) allows the parallel creation of varieties with another type of resistance.

Possible application of studied markers in practical breeding will be further investigated.

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Received on February 20, 2003

ABSTRAKT

Využití DNA markerů pro charakterizaci rezistence rajčete vůči kořenovému háďátku *Meloidogyne incognita*

Pro studium rezistence rajčete (*Lycopersicon esculentum* L.) vůči háďátku *Meloidogyne incognita* Kofoit a White byly využity standardní infekční testy a zároveň metoda CAPS (Cleaved Amplified Polymorphic Sequence). Metodou CAPS byla jednoznačně určena alelická sestava genu M_i u deseti českých a čtyř zahraničních odrůd rajčat. Rovněž bylo prověřeno 120 jedinců segregující F_2 generace odrůdy Nema a 120 jedinců segregující F_2 generace odrůdy Petopride. Srovnání výsledků infekčních testů s molekulárně genetickou analýzou DNA vykazovalo 100% shodu v detekci rezistentních, resp. senzitivních genotypů. Výsledek χ^2 testu potvrdil s pravděpodobností $P \in (0{,}5; 0{,}7)$ shodu mezi skutečně získanými a teoreticky očekávanými štěpnými poměry. Metodu CAPS lze jednoznačně doporučit pro rychlé a přesné určení rezistence, resp. senzitivity rostlin rajčete vůči háďátkům rodu *Meloidogyne*. Metoda navíc umožňuje jednoznačné určení alelické sestavy genu M_i, což může velmi urychlit proces tvorby nových rezistentních odrůd.

Klíčová slova: rajče; *Lycopersicon esculentum* L.; *Meloidogyne incognita* Kofoit a White; rezistence; CAPS; DNA; marker; PCR

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