Study of European and Czech populations of potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) by RAPD method

P. Sedlák[1], M. Melounová[1], S. Skupinová[1], P. Vejl[1], J. Domkářová[2]

[1]*Czech University of Agriculture in Prague, Czech Republic*
[2]*Potato Research Institute, Ltd., Havlíčkův Brod, Czech Republic*

ABSTRACT

Potato cyst nematodes (PCN) are the big problem in worldwide planting of potatoes and another *Solanaceous* plants. Identification of individual pathotypes according to international scheme is very demanding but a very important part of the phytosanitary process to control these pests. Molecular genetic identification of different plant and animal species or individuals is a very interesting way at the present time and let's hope that it will be important in future. This report presents results of the RAPD study of nine different real PCN populations. There were five *Globodera rostochiensis* populations and four *G. pallida* populations. Pathotypes Ro2, Ro2/3, Ro4, Ro5, Pa2 and Pa3 were from European populations; population Ro1 and X were of Czech provenance. Genetics variable of these populations was described by a set of six decameric primers (OPA 07, OPG 03, OPG 05, OPG 08, OPG 10 and OPG 13). Genetic dissimilarity was by Gel Manager for Windows evaluated. Detectable differences behind all populations were found and the dendrogram was compiled. The unknown population *X* was sorted into group of *Globodera pallida* species subgroup of Pa2 consequently.

Keywords: potato cyst nematodes; *Globodera rostochiensis*; *G. pallida*; pathotypes; RAPD

Potato cyst nematodes (PCN) *Globodera rostochiensis* (Wollenweber, 1923) Behrens, 1975 (yellow potato nematode) and *Globodera pallida* (Stone, 1973) Behrens, 1975 (white potato nematode) are recently sorted into phylum *Nematoda*, order *Tylenchida*, family *Heteroderidae*. For these plant parasitic species there is a typically sharp sex dimorphism. Opposite of males the females are spherical and sessile, and live in the root system of *Solanaceous* plants. Lifeless females (cysts) stay in the soil for a few years and are a source of new infections (Smith et al. 1992). European PCN populations are divided into eight groups (pathotypes Ro1, Ro2, Ro3, Ro4, Ro5, Pa1, Pa2 and Pa3) base their different pathogenic characters (Kort et al. 1977). With exception to these pathotypes there exist other populations in the Andean region that have never been introduced into a different part of the world. Control of these pests is very difficult. For these reasons PCN belong to the quarantine list A2 of EPPO (Europe Plant Protection Organization). Apart from the biological method of cultivation on differential clones were for distinguishing of individual pathotypes in the past used some progressive techniques of molecular biology such as isoelectric focusing analyse (Fox and Atkinson 1984), SDS-PAGE (Bakker and Bouwman-Smits 1988), immunological studies by ELISA (Enzyme-Linked Immunosorbent Assay) (Schots et al. 1989) or first methods of detection nucleic acids variable like RFLP (Burrows and Boffeye 1986 and Schnick et al. 1990). And at the present time are the most expanded methods based on the polymerase chain reaction (PCR). The most usable PCR method for taxonomic studies is often presented RAPD (Random Amplified Polymorphic DNA) (Williams et al. 1990, Roosien et al. 1993, Folkertsma et al. 1994, Blok et al. 1997, Bendezu et al. 1998, Thiery et al. 1997 and Fullaondo et al. 1999). Other methods based on PCR like STS e.g. are used as well (Zouhar et al. 2000 and Skupinová et al. 2002).

MATERIAL AND METHODS

Biological material. Cysts of different European populations inclusive of Czech were obtained in cooperation with the Department of Plant Protection

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Table 1. Collections of PCN evaluated by RAPD

Collection	Provenance	Number of cysts
Ro1	Šluknov – Czech Republic (DPP)	100
Ro2	Obersteinbach – Germany (SPA)	98
Ro2/3	Obersteinbach – Germany (DPP)	50
Ro4	Harmerz – Germany (DPP)	100
Ro5	Harmerz – Germany (SPA)	95
Pa2	Kalle – France (SPA)	92
Pa3	Chavornay – France (SPA)	70
Pa3	Delmsen – Germany (SPA)	83
X	Unknown – Czech Republic (DPP)	4

Table 2. Used oligonucleotides

Primer	Sequence
OPG 03	5′-GAGCCCTCCA-3′
OPG 05	5′-CTGAGACGGA-3′
OPG 08	5′-TCACGTCCAC-3′
OPG 10	5′-AGGGCCGTCT-3′
OPG 13	5′-CTCTCCGCCA-3′
OPA 07	5′-GTGGGCAAAG-3′

at the Czech University of Agriculture in Prague (DPP) and the State Phytosanitary Administration of the Czech Republic in Prague-Ruzyně (SPA). Collections provenance and amount of evaluated cysts are shown in Table 1.

DNA isolation. DNA was isolated from individual cysts by simple and not time-consuming under mentioned method of extraction in TE (10mM Tris-HCl pH 8.1, 10mM EDTA) buffer. Individual cyst in sterile polypropylene tube (Eppendorf) with 5 µl 1× TE buffer was disrupted and moved into liquid nitrogen for fifteen minutes. At the next step the cyst was perfectly homogenised and the solution was diluted with new 5 µl portion of 1× TE buffer. Dilution was furthermore incubated in water bath (60°C for two hours). Finally the homogenate was centrifuged for 20 min (5000 rpm) and water part (supernatant) was carefully moved into a new sterile tube and stored in an icebox (–20°C).

RAPD analyse. All isolates were analysed by set primers described in Table 2.

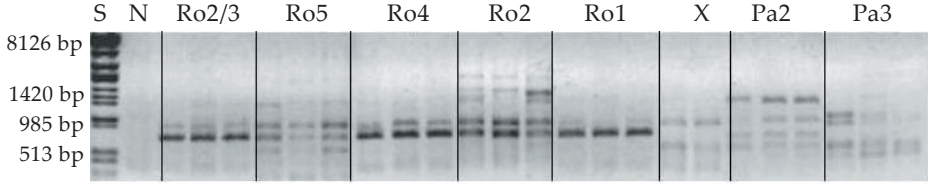
RAPD conditions were optimised. Each 25 µl reaction contained 10 ng of DNA, 0.7 unit of *Taq* DNA polymerase, 2.5mM of $MgCl_2$, 0.2mM of dNTP (MBI Fermentas, Litva) and 25 ng of primer. RAPD was realised in the next steps: 1× (94°C for 240 s), 40× (94°C for 30 s, 36.5°C for 45 s, 72°C for 90 s) and 1× final extension (72°C for 300 s).

Amplified fragments of DNA were separated in 1.2% agarose gel (120V for 90 min), stained by ethidium bromide and visualised by UV-light. UPGMA was processed by software Gel Manager for Windows (Biosystematica, GB).

RESULTS

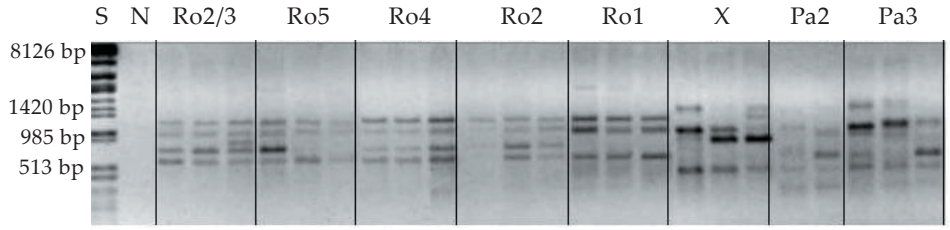
Finally were chosen six primers from the total amount of one hundred tested oligonucleotides (Table 2). These primers offered a sufficient amount of polymorphic bands that were characteristic for single populations. It was very important to obtain these bands in individual repetition of experiments. Typical RAPD profiles of individual populations obtained by primers OPG 08 and OPG 13 are shown at Figures 1 and 2. All reproducible polymorphic bands with intra-population occurrence higher than 50% were evaluated by UPGMA.

Figure 3 presents results of UPGMA analyse of population's variable. Finally were evaluated 63 se-



S – λ DNA/*Eco*47I(*Ava*II)
N – negative control
X – population X (unknown pathotype)

Figure 1. Characteristic RAPD profiles of individual populations (used primer OPG 08)



S – λ DNA/*Eco*47I(*Ava*II)
N – negative control
X – population X (unknown pathotype)

Figure 2. Characteristic RAPD profiles of individual populations (used primer OPG 13)

lected polymorphic bands. All populations were divided into two main groups. These groups corresponded to single species, *Globodera rostochiensis* and *G. pallida.* From the graph there is an obvious structure of single species populations. The main point of this analyse is identification of the unknown cysts in the Czech provenance signposted as X. Finally these cyst were identified as *Globodera pallida* pathotype Pa2. This result supports the results of Skupinová et al. (2002).

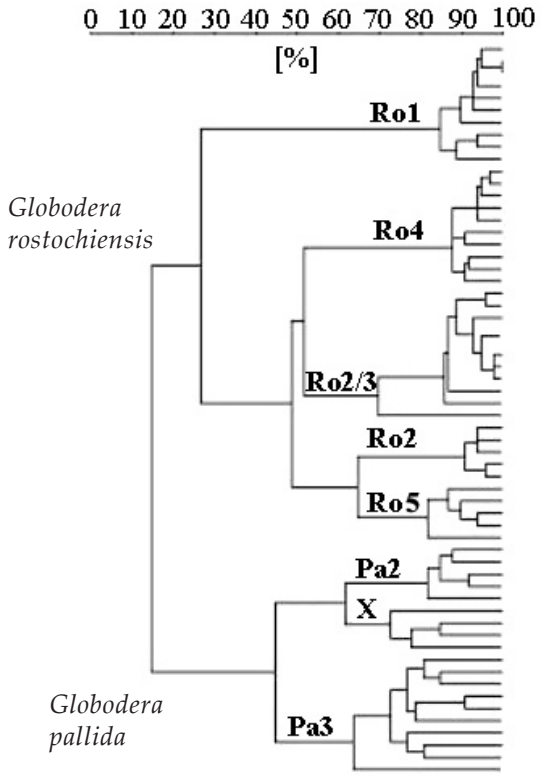


Figure 3. UPGMA scheme of studied populations by means of used RAPD markers

DISCUSSION

The used method of DNA isolation was able to obtain a sufficient amount of nucleic acid that was capable of non-problematic amplification. Different authors present other methods of monocystic isolation with additional phenol purification (Roosien et al. 1993). One cyst represents a mixture of genetically similar objects of common origin. For this reason it is possible to consider this subpopulation as one original object. Some authors analysed DNA from larger amounts of cysts en masse isolated (Folkertsma et al. 1994).

Used decameric primers are standard RAPD sequences for taxonomic studies. Roosien et al. (1993), Folkertsma et al. (1994), Thiery et al. (1997), Phillips et al. (1998), Blok et al. (1998) used these sequences also and Bendezu et al. (1998) used the primer OPA 7. All methods (DNA isolation, RAPD profiles and electrophoresis) were designed and optimised. For this reason some small differences occurred mutually published and our results also. Particularly the main differences were in the number of detected polymorphic or non-specific bands. This problem is the largest handicap of RAPD. The causes of this problem are evidently significant differences among technical equipment of individual laboratories and specific skills of scientific personal (Innis et al. 1990). Studied *Globodera's* collections were taken from different workplaces and presented important populations from some European localities. Said authors studied two populations of our assortment (Ro5 Harmerz – Germany and Pa2 Kalle – Germany). Total variable was evaluated by UPGMA on the base of the Dice's coefficient of similarity. An analogous way was also used for evaluation of wheat (*Triticum aestivum* L.) and potato (*Solanum tuberosum* ssp. *tuberosum* L.) variable (Vejl 1995, Čurn et al. 1995) and in nematologist studies as well (Folkertsma et al. 1994, Bendezu et al. 1998).

CONCLUSION

All populations were distinguished by described methods and used primers. Unknown population X (Czech Republic) was identified as *Globodera pallida* pathotype Pa2. This result was not supported by an infection test. Described method and primers are fully usable in similar population studies. It was not possible to design some generally usable electrophoretic etalon of individual pathotypes. There is recommended to optimise conditions of method to the conditions of each laboratory before it is used.

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ABSTRAKT

Studium evropských a českých populací karanténních háďátek bramboru (*Globodera rostochiensis* a *G. pallida*) RAPD metodou

Cystotvorná karanténní háďátka bramboru jsou velkým problémem celosvětového pěstování bramboru a jiných rostlin z čeledi lilkovitých. Identifikace jednotlivých patotypů podle mezinárodního patotypového schématu je velmi obtížnou, ale také velmi důležitou součástí fytosanitárního procesu ochrany bramboru vůči těmto patogenům. V současnosti je velmi zajímavou cestou identifikace různých rostlinných i živočišných druhů pomocí molekulárně genetických markerů. Práce shrnuje výsledky RAPD studie devíti různých populací háďátka bramborového; evropské populace zastoupené patotypy Ro2, Ro2/3, Ro4, Ro5, Pa2 a Pa3 a populace pocházející z ČR (patotyp Ro1 a neznámá populace X). Genetická variabilita byla charakterizována pomocí sady šesti dekamerických primerů (OPA 07, OPG 03, OPG 05, OPG 08, OPG 10 a OPG 13). Podobnost populací byla vyhodnocena programem Gel Manager for Windows. Byly odhaleny významné rozdíly mezi jednotlivými populacemi a na jejich základě sestaven dendrogram. Neznámá populace *X* byla identifikována jako *Globodera pallida*, patotyp Pa2.

Klíčová slova: háďátko bramborové; *Globodera rostochiensis*; *G. pallida*; patotypy; RAPD

Corresponding author:

Ing. Petr Sedlák, Česká zemědělská univerzita v Praze, 165 21 Praha 6-Suchdol, Česká republika
phone: + 420 224 382 562, fax: + 420 234 381 837, e-mail: sedlak@af.czu.cz