

DOI: 10.37317/pbss-2019-0008

Anna Podlewska-Przetakiewicz ^{*1}; Dorota Milczarek ²

¹ Plant Breeding and Acclimatization Institute-National Breeding Institute, Research Centre Radzikow, Department of Plant Pathology, Radzikow, 05-870 Blonie, Poland;

² Plant Breeding and Acclimatization Institute – National Research Institute, Research Centre Młochów, Platanowa 19, 05-831 Młochów, Poland

* Corresponding author e-mail: a.przetakiewicz@ihar.edu.pl

MOLECULAR METHODS OF CHARACTERIZATION AND IDENTIFICATION OF *GLOBODERA ROSTOCHIENSIS* AND *GLOBODERA PALLIDA* POPULATIONS

ABSTRACT

The cyst nematodes belonging to the genus *Globodera* are big worldwide problem in countries where *Solanaceae* plants growing. Knowledge of species-composition in populations of *Globodera rostochiensis* and *Globodera pallida* is very important for selection of appropriate measure of nematode regulations occurrence. Inter- and intraspecific variability among species of *Globodera rostochiensis* and *Globodera pallida* were studied intensively with the use of molecular analyses of DNA methods. This review summarizes and compares of methods chosen to distinguish between *Globodera*, both pathotypes and species.

Key words: *Globodera*; inter-and intraspecies identification; molecular methods; potato cyst nematode

INTRODUCTION

Globodera rostochiensis (Wollenweber) Behrens and *G. pallida* (Stone) Behrens belongs to the most economically important pests of potato causing up to 50% of yield damages (Nicol *et al.*, 2011). They were introduced into Europe from South America in the 1600s (Evans *et al.*, 1975; Baldwin and Mundo-Ocampo, 1991) and knowledge of the genetic characteristics of each separate introduction might form the basis for classifying the various populations and virulence groups. Pathotype scheme proposed by Kort in 1977 differentiates some pathotypes on the basis of qualitative differences in relation to major resistance genes and others on the basis of quantitative differences in reproduction of populations on differential hosts with polygenic resistance (Kort *et al.*, 1977).

Communicated by Grzegorz Żurek

Nowadays, many molecular techniques based on DNA analysis is used to searching for variations among nematode populations comes from different geographical regions. Molecular data could be a powerful source of information for the systematics of nematodes and conduct to the determine the range of genetic diversity in relation to virulence of potato cyst nematode (PCN) populations (Hyman and Powers, 1991; Ferris *et al.*, 1991; Baldwin, 1992; Ferris and Ferris, 1992; Bossis and Mugniery, 1993; Powers and Adams, 1993; Blok *et al.*, 1998). Reliable identification and characterization of nematode populations is a prerequisite to studying their genetic variation, interaction with host plants, virulence and to many aspects of PCN control and management. The choice of molecular technique used to the estimation of genetic diversity of populations depends on research question.

MOLECULAR TECHNIQUES OF PCN CHARACTERIZATION

rDNA

Region of rDNA in eukaryotic species consists three ribosomal RNA genes - 18S, 28S, 5.8, internal transcribed regions – ITS 1 and ITS2 and an external non-transcribed spacer region. In nematodes three ribosomal genes are the best characterized gene regions (Powers, 2004). They are highly conserve but the comparative analysis of differences in coding and non-coding regions of ribosomal DNA (rDNA) can be a popular method for inter- and intraspecies identification of many organisms. DNA sequencing of ITS regions within rDNA allows revealing single base-pair substitution and analysis of this regions has been used to examine phylogenetic relationships between population of the same species. Phylogenetic analysis of 41 ITS region sequences of *Globodera* parasitizing solanaceous plants obtained by Subbotin and co-authors (2000) suggest that the rDNA in genome of *G. rostochiensis* and *G. pallida* populations is present as a mixture of haplotypes with different sequences. Ferris *et al.*, (1993) compared sequence data from internal transcribed spacer (ITS) of the *Globodera* spp. and found that they have characteristic inter- and intraspecific variations and that the choice of region in the rDNA can influence the amount of variability detected between isolates.

In 1998 Blok studied intraspecific variation of 18 population of *G. pallida* from Europe and South America in relation to their original introduction into Europe amplifying the repeated region of ITS1/5,8/ITS2 together with 3' end of 18S and 5' end of 28S gene. They found one population of *G. pallida* from South America more distinct then the others, in different molecular method: RFLP and sequence analysis (Blok *et al.*, 1998) and earlier in SSR and RAPD method (Blok *et al.*, 1997) in comparison of ITS2 region of all tested populations. Identical approaches carry out on 16 Ukrainian population of *G. pallida* and *G. rostochiensis* by Pypylenko *et al.* (2008) shows similarity of *Globodera pallida* population in 97,8% based on sequences 938bp in comparison to published sequences of European population of PCN described by Blok *et al.* (1998) and Subbotin *et al.* (2000). Polymorphism of sequence of the first inter-

nal transcribed spacer (ITS1) has been widely used to identifying and assessing genetic variability of three Russian population of *Globodera rostochiensis* comes from different geographical localities (Chrisanfova *et al.*, 2008). Low variation of the ITS-1 sequences and high conservativeness of this genome region failed to distinguish the isolates groups within the species with the used of this particular method. Comparative analysis of rDNA (fragments ITS1/5.8S/ITS2) sequences of 16 polish *Globodera* populations show 97-98% identity in *G. rostochiensis* and 94-95% identity in *G. pallida* isolates and allow for splitting populations into three phylogenetic groups originate from Central and Eastern part of the country (Nowaczyk *et al.*, 2011). Molecular characterization of Serbian *Globodera* isolates reported by Oro *et al.* (2012) based on comparison of variation occurred in ITS-1 region assumed that possible ancestors of PCN population originated from Peru but presence of *G. pallida* is not the result of direct import of infected potatoes from Latin America but from England where the population from York has the same sequence like Serbian ones.

mtDNA

Animal mitochondrial genomes (mtDNA) are often used in phylogenetic and population studies. Due to maternal mode of inheritance and lack of recombination mtDNA seems to be the best marker for searching of evolutionary connection between populations (Avice, 1994; Harrison, 1989; Birky, 2008). At present there are available a complete nucleotide sequences for 76 species which revealed differences in size, structure and gene content (Armstrong *et al.*, 2000). The mtDNA encodes the 22 transfer RNAs, two ribosomal RNAs and 12 or 13 proteins involved in transport of electrons and oxidative phosphorylation (Pont-Kingdon *et al.*, 1995). In metazoan mtDNA can occur as a single circular molecules, a linear chromosome or two 8-kb linear molecules (Bridge *et al.*, 1992).

In 2000 Armstrong provided evidence about multipartite structure of mtDNA of *Globodera pallida*. Molecular approaches carry out on British population of *G. pallida* reveal at least 6 small, circular mitochondrial DNAs (scmtDNAs I - VI) differ in size. These scmtDNA contained mitochondrial gene coding sequences and about 2kb non-coding region common to all small circular mitochondrial DNAs (Armstrong *et al.*, 2000). The authors suggested that scmtDNAs are present in all *G. pallida* but their frequencies vary between different populations (Armstrong *et al.*, 2007). For example, while scmtDNAs I and III were not detected in several UK populations, scmtDNA IV was present in all population analyzed. Last study shows that scmtDNA IV seems to be the most evolutionary stable than other subgenomic circles and may be used for studying genetic interactions between populations. Moreover, only scmtDNA IV contains rRNA genes which are necessary for the translation of mitochondrial protein. The scmtDNA IV region can decide populations as monophyletic what indicates this DNA region may be a useful marker for *G. pallida*. Analysis of scmtDNA sequences variation can be a powerful tool to research relationship between European *G. pallida* populations and their South American ancestors (Armstrong *et al.*, 2007).

On base of maternal mode of mtDNA inheritance and theory that recombinant products are indistinguishable from their progenitor molecules, Hoolahan in 2012 investigated past and contemporary recombination of British population of *G. pallida*. Past recombination was detected and confirmed between a South American and several UK population of white potato cyst nematode. In their study, progeny from experimental crosses of tested population of *G. pallida* had no evidence of contemporary recombination between the mtDNA of the maternal and paternal populations (Hoolahan *et al.*, 2012) what support current arguments that animal mtDNA recombination events are rare (Kivisild *et al.*, 2000; Innan and Nordberg, 2002).

RFLP

Molecular analysis based on restriction digest of total DNA (Restriction Fragment Length Polymorphisms - RFLP) are being used to observe genetic variation both between and within species. Investigations of variability within population are based on a sequence information from different populations and on using various clones. Inter- and intraspecific polymorphism between reference populations of *G. rostochiensis* and *G. pallida* has been observed for the first time by Burrows and Boffey in 1986. This method was used several times to examine genetic variation between different population of *G. pallida* but the results did not correlate with a type of pathotype (De Jong *et al.*, 1989; Schnick *et al.*, 1990; Phillips *et al.*, 1992).

The studies of rDNA using PCR-RFLP method are attractive in case of small organisms like nematodes. In 1998 Blok reported variation in the multiple copies of rDNA of eighteen populations of *G. pallida* from Europe and South America. Authors conducting RFLP analysis of PCR products of ribosomal cistron out of six digestion enzymes found one that discriminated among most of the tested populations. The results support already existing studies that the majority of European population of *G. pallida* derived from one source with few exceptions (Blok *et al.*, 1998).

RFPLs analysis of ITS-PCR products were carry out by Subbotin in 2000 on group of Russian populations of *Globodera rostochiensis* and the other *Globodera* species (Subbotin *et al.*, 2000). Researchers used RFLP catalogue and sequence information from different populations or species and set of digestion enzymes to compare PCR products of nematode populations. Results of work revealed that rDNA in the genomes of *G. rostochiensis* populations is present as a mixture of haplotypes with different sequences and RFLP profiles and sequences of ITS region can be used rather for inter- than for intra-specific identification of *Globodera* species.

RAPD

Random Amplified Polymorphic DNA (RAPD) is one of the high sensitive PCR-based technique which involves the use of single 8-12 nucleotide length primers to amplification of many discrete and random DNA segments in the target genome (Welsh and McClelland, 1990). Genetic relationships between Dutch populations of *G. rostochiensis* and *G. pallida* analyzed by Folkertsma *et*

al. (1994) with the use of RAPDs has shown pathotypes designation within the first species but not with the second. They found that isolates of *G. rostochiensis* classified as one pathotype were distinguishable by a number of unique RAPD fragments. Intra-species differences in European and South American populations of *G. pallida* and two populations of *G. rostochiensis* was examined by Blok *et al.* (1997). In their study both populations of golden PCN, identified as Ro1, were similar but they reported higher dissimilarity of white PCN isolates then in a Dutch surveys. Research carried out by Bendezu *et al.* in 1998 on nine populations of *G. rostochiensis* from UK, Bolivia, the Netherlands and Germany with the use of four primers shown genomic similarity among European populations of yellow potato cyst nematode in 82% and among UK populations in 89%. Intra-species differences in virulence were investigated by Pastrik *et al.* (1995) on German *G. pallida* populations and the partially resistant potato cultivar Darwina. RAPD patterns with the use of 40 primers showed generally high similarity of selected and unselected for virulence PCN populations except of two non-homologous DNA fragments present in selected ones. Pastrik interpreted that one of this DNA fragment indicates a correlation with the particular type of virulence in individual population (Pastrik *et al.*, 1995).

RAPD technique was used by Greiner *et al.* in 2001 to reveal a possible phylogeny between populations comes from different continents and determine the origin of infestation. Authors compare Peruvian and European populations of *G. pallida* and the results showed clear intra-specific distinction between two tested groups of population. In contrary to their results Hlaoua *et al.* (2008) studied similarity of Tunisian and European populations of white potato cyst nematode and proved high similarity of both clade of populations.

Conceição *et al.* (2003) tested genetic variability of 32 populations of *Globodera rostochiensis* and three of *G. pallida* from different regions of Portugal. Populations were analyzed and compared using random amplified polymorphic DNA and sixteen primers. Results of study showed that two populations of *G. rostochiensis* appeared to be distinct from the main group of this species as well as one population of *G. pallida*. Distinct clusters were observed within both species but the clusters could not be related to the geographic proximity of the populations.

AFLP

Amplified Fragment Length Polymorphism (AFLP) is a fingerprinting technique used to detection of polymorphism in different genomic regions of DNA. This method, highly sensitive and reproducible, is based on the PCR amplification of restriction fragments from a total digest of genomic DNA (Zabeau and Vos, 1993). It based on the ligation of adapters to ends of restriction fragments and a selective PCR-based amplification with adapter-specific primers. The standard procedures of AFLP described by Vos *et al.* (1995) become widely used for the identification of genetic variation in closely related species and populations. Like RFLP and RAPD analysis, AFLPs give an information about mutation that are dispersed over the genome. The comparative study of *Globodera* species and populations using AFLP revealed greater inter- and intra-

specific variability than obtained by RAPD (Subbotin and Moens, 2006). AFLP technique were used to amplify genomic DNAs extracted from cysts of 16 Swedish and 20 other European populations of PCN, both *G. rostochiensis* and *G. pallida* from UK, Germany, Norway and the Netherlands (Manduric and Andersson, 2003). The results of analysis revealed that Swedish Ro1 populations were very similar to corresponding populations from other parts of Europe. The rest of *G. rostochiensis* populations appeared as a genetically heterogeneous group with two Swedish populations being most dissimilar. Characteristic of 9 *G. rostochiensis* populations carried out by Folkertsma *et al.* (1996) by AFLP analysis clustering them into three groups based on variants expressed by the presence or absence of polymorphic DNA fragments. These groups were distinguished by 3, 7 and 12 unique DNA fragments, respectively.

Microsatellites

Microsatellites such as Simple Sequence Repeats (SSR) or Short Tandem Repeats (STR) or Simple Sequence Length Polymorphisms (SSLP) are repetitive DNA in which short DNA motifs are repeated many times in tandem (TRs). They are unstable and can mutate at rates between 10^3 and 10^6 per cell (Gemayel *et al.*, 2012). TRs are present in coding and non-coding regions of nematode genome (Castagnone-Sereno *et al.*, 2010; Pérez-Jiménez *et al.*, 2013; Phumichai *et al.*, 2015). Particular alleles differ in number of tandem repeats and give an information about differences in nematode populations (Jarne and Lagoda, 1996). In 2013 Boucher *et al.* with the used of three sets of microsatellite markers was looking for genetic diversity and the origin of introduction of 15 populations of *G. rostochiensis* distributed worldwide. Obtained results confirm influence of genetic drift on losing of genetic diversity of tested nematode populations and indicate populations from South America less diverse than European ones.

Microsatellites markers were also used to explain of allelic richness of *G. pallida* populations introduced from South America to Europe, Africa, North America and Asia. Plantard *et al.* (2008) carried out comparative analysis of the allelic richness at seven microsatellite loci observed in the Western European populations. They found only one-third of them observed in this part of southern Peru comparable to the allelic richness observed in the northern region of Peru. The authors give an explanation that genetic variability can be a result of a single introduction of infected plant and can influence on control of quarantine nematodes on the field.

Many molecular techniques based on DNA/RNA analysis is used to searching for variations among nematode populations but for practical use there is a need to distinguish species and pathotypes. Shields *et al.* (1996) used the polymerase chain reaction to amplify a region between the 5S rRNA and spliced leader RNA genes in *G. rostochiensis* and *G. pallida*. Isolates of *G. rostochiensis* amplified a single 914 bp product and were distinguishable from *G. pallida* isolates which amplified 914 and 853 bp products or a single 853 bp product. Concordant identifications of *G. pallida* and *G. rostochiensis* isolates were obtained by using 5S-SL PCR for specific DNA probes and differential

host plant tests. Later the multiplex PCR analysis with primers GroR-GroF and PaR-PaF has been developed to distinguish the species *Globodera rostochiensis* and *G. pallida* by Fullaondo *et al.* (1999). Using a approach based on melting peak analysis of PCR products Bates *et al.* (2002) developed a semi-quantitative assay to measure the relative proportions of *Globodera pallida* and *G. rostochiensis* in a sample. The method depends on a multiplex PCR where the products of each species can be separated by their individual melting temperatures (Tm). 2% of *G. pallida* cysts in a mixture could be detected. Nakhla *et al.* (2010) developed the multiplex real-time PCR assays for the identification of the potato and tobacco cyst nematodes. They used a set of primers (PITSpf and PITs4) and a TaqMan probe (GFAMp) for the specific detection of *G. pallida*, and another set of primers (PGrtf and Prostor) for the detection of *G. rostochiensis* and the specific for *G. tabacum* primer set (PGrtf and PITSt3mr) with a TaqMan probe (GTETp).

However DNA/RNA markers could not be used to distinguish the PCN pathotypes. Hinch *et al.* (1998) developed a technique of high performance capillary electrophoresis (CE) that allows to obtain polypeptide profiles of each of the pathotypes of *G. rostochiensis* Rol-5, and *G. pallida* Pal, and mixture of Pa2 and Pa3.

CONCLUSION

Understanding of genetic diversity between populations and way of its dispersal in- and outside of origin geographic region is essential to prevent further spreading and create new methods of protection strategies. Both host range and genetic variation are important to have access to methods that enable inter- and intra-species identification of nematode populations. Classical methods of identification of PCN population based on morphological characterization and bio-tests identification are time-consuming and are more often replaced by molecular methods which are independent of environmental influence and stage of nematode. DNA-based diagnostic techniques are relatively quicker and the results seems to be more reliable in assessments of similarity of populations. For the practical purposes it is possible to distinguish the species of the evaluated nematodes on the basis of the DNA/RNA markers but up to now it is not possible to distinguish the pathotypes in a such way.

REFERENCES

- Armstrong M.R., Blok V.C., Phillips M.S. 2000. A multipartite mitochondrial genome in the potato cyst nematode *Globodera pallida*. *Genetics* 154: 181-192.
- Armstrong M.R., Husmeier D., Phillips M.S., Blok V.C. 2007. Segregation and recombination of a multipartite mitochondrial DNA in population of the potato cyst nematode *Globodera pallida*. *Journal of Molecular Evolution* 64: 689-701.
- Avise J., Nelson C., William S., Charles G. 1994. Why one-kilobase sequences from mitochondrial DNA fail to solve the hoatzin phylogenetic enigma. *Molecular and Phylogenetics Evolution* 3(2): 175-184.
- Baldwin J.G., Mundo-Ocampo M. 1991. Heteroderidae, cyst- and no-cyst forming nematodes. In: W. R. Nickle (Ed.), *Manual and Agricultural Nematology*, New York, Marcel Dekker, pp. 275-362.
- Baldwin J. G. 1992. Evolution of cyst and non-cyst forming *Heteroderidea*. *Annual Review of Phytopathology* 30: 271-290.

- Bate J. A., Taylor E. J., Gans P.T., Thomas J.E. 2000. Determination of relative proportions of *Globodera* species in mixed populations of potato cyst nematodes using PCR product melting peak analysis. *Molecular Plant Pathology* 3(3): 153-161.
- Bendezu I. F., Evans K., Burrows P.R., de Pomerai D., Canto-Saenz M. 1998. Inter and intra-specific genomic variability of the potato cyst nematodes *Globodera pallida* and *G. rostochiensis* from Europe and South America using RAPD-PCR. *Nematologica* 44: 49-61.
- Birky C.W. Jr. 2008. Uniparental inheritance of organelle genes. *Current Biology* 18: 692-695.
- Blok V.C., Phillips M.S., Harrower B.E. 1997. Comparison of British populations of potato cyst nematodes with populations from continental Europe and South America using RAPDs. *Genome* 40: 286-293.
- Blok V.C., Malloch G., Harrower M.S., Vrain T.C. 1998. Intraspecific variation in ribosomal DNA in population of the potato cyst nematode *Globodera pallida*. *Journal of Nematology* 30(2): 262-274.
- Bossis M., Mugniery D. 1993. Specific status of six *Globodera* parasites of *solanaceous* plants studied by means of two-dimensional gel electrophoresis with comparison of gel patterns by a computer system. *Fundamental and Applied Nematology* 16: 47-56.
- Boucher A.C., Mimee B., Bardou-Valette S., Belair G., Moffett P., Greiner E. 2013. Genetic diversity of the golden potato cyst nematode *Globodera rostochiensis* and determination of the origin of populations in Quebec, Canada. *Molecular and Phylogenetic Evolution* 69(1): 75-82.
- Bridge J. 1992. Nematodes. In: R. J. Hillocks (Ed.), *Cotton diseases*, Walling Ford, UK: CSB International, pp. 331-353.
- Burrows P.R., Boffey S.A. 1986. A technique for the extraction and restriction endonuclease digestion of total DNA from *Globodera rostochiensis* and *Globodera pallida* second stage juveniles. *Revue de Nématologie* 9: 199-200.
- Castagnone-Sereno, P., Danchin E.G.J., Deleury E., Guillemaud T., Malausa T., Abad P. 2010. Genome-wide survey analysis of microsatellite in nematodes, with the focus on the plant-parasitic species *Meladogyne incognita*. *BMC Genomics* 11: 598.
- Chrisanfova G.G., Charchevnikov D.A., Popov I.O., Zinovieva S.V., Semyenova S. K. 2008. Genetic variability and differentiation of three Russian populations of potato cyst nematode *Globodera rostochiensis* as revealed by nuclear markers. *Russian Journal of Genetics* 44(5): 533-538.
- Da Conceição I.L., Santos M.C.V., de Oliveira Abrantes I.M., de Almeida Santos M.S. 2003. Using RAPD markers to analyze genetic diversity in Portuguese potato cyst nematode populations. *Nematology* 5: 137-143.
- de Jong A.J., Bakker J., Roos M., Gommers F.J. 1989. Repetitive DNA and hybridization patterns demonstrate extensive variability between siblings species *Globodera rostochiensis* and *G. pallida*. *Parasitology* 99: 133-138.
- Evans K., Franco J., de Scurrah M.M. 1975. Distribution of species of potato cyst nematodes in South America. *Nematologica* 21: 365-369.
- Ferris V.R., Ferris J.M. 1992. Integration of classical and molecular approaches in nematode systematics. In: F. J. Gommers and P. W. Th. Maas (Eds.), *Nematology from Molecule to Ecosystems*, *European Society of Nematologists*, Invergowrie, Dundee, Scotland, pp. 92-100.
- Ferris V.R., Ferris J.M., Faghihi J. 1991. Biological diversity from the perspective of molecular biology. In: D. C. Dudley (Ed.), *The Unity of Evolutionary Biology*, Portland, OR, Dioscorides Press, pp. 71-76.
- Ferris V.R., Ferris J.M., Faghihi J. 1993. Variation in spacer ribosomal DNA in some cyst forming species of plant parasitic nematodes. *Fundamental and Applied Nematology* 16: 177-184.
- Folkertsma R.T., Rouppe van der Voort J.N., van Gent-Pelzer M.P.E., de Groot K.E., van den Bos W.J.R., Schots A., Bakker J., Gommers F.J. 1994. Inter- and Intraspecific variation between populations of *Globodera rostochiensis* and *G. pallida* revealed by Random Amplified Polymorphic DNA. *Genetics*, 84(8): 807-811.
- Folkertsma R.T., Rouppe van der Voort J.N., de Groot K.E., van Zandvoort P.M., Schots A., Gommers F.J., Helder J., Bakker J. 1996. Gene pool similarities of potato cyst nematode populations assessed by AFLP analysis. *Molecular Plant-Microbe Interaction* 9: 47-54.
- Fullaondo A., Barrena E., Viribay M., Barrena I., Salazar A., Ritter E. 1999. Identification of potato cyst nematode species *Globodera rostochiensis* and *G. pallida* by PCR using specific primer combinations. *Nematology* 1: 157-163.
- Gemayel R., Cho J., Boeynaems S., Verstrepen K.J. 2012. Beyond junk-variable tandem repeats as facilitators of rapid evolution of regulatory and coding sequences. *Genes* 2012(3): 461-480.
- Greiner E., Bossis M., Fouville D., Renault L., Mugniery D. 2001. Molecular approaches to the taxonomic position of Peruvian potato cyst nematodes and gene pool similarities in indigenous and imported populations of *Globodera*. *Heredity* 86: 277-290.
- Harrison R.G., 1989. Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. *Trends in Ecology and Evolution* 4: 6-11.
- Hinch J.M., Alberdi F., Smith S.C., Woodward J.R., Evans K. 1998. Discrimination of European and Australian *Globodera rostochiensis* and *G. pallida* pathotypes by high performance capillary electrophoresis. *Fundamental and Applied Nematology* 21(2): 123-128.

- Hlaoua W., Horrigue-Raouani N., Fouville D., Mugniery D. 2008. Morphological and molecular characterization of potato cyst nematode populations from Tunisia and survey of their probable geographical origin. *Biotechnology* 7(4): 651-659.
- Hoolahan A.H., Blok V.C., Gibson T., Dowton M. 2012. A comparison of three molecular markers for the identification of population of *Globodera pallida*. *Journal of Nematology* 44(1): 7-17.
- Hyman B.C., Powers T.O. 1991. Integration of molecular data with systematics of plant parasitizing nematodes. *Annual Review of Phytopathology* 28: 89-107.
- Innan H., Nordborg M. 2002. Recombination or mutational hot spots in human mtDNA?. *Molecular Biology Evolution* 19: 1122-1127.
- Jarne P., Lagoda P.J. 1996. Microsatellites, from molecules to population and back. *Trends in Ecology and Evolution* 11(10): 44-429.
- Kivisild T., Villems R., Jorde L.B., Bamshad M., Kumar S., Hedrick P., Dowling T., Stoneking M., Parsons T.J., Irwin J.A., Awandalla P., Eyre-Walker A., Smith A. 2000. Questioning evidence for recombination in human mitochondrial DNA. *Science* 288: 1931a.
- Kort J., Ross H., Rumpfenhorst H.J., Stone A.R. 1977. An international scheme for identifying and classifying pathotypes of potato cyst nematodes *Globodera rostochiensis* and *G. pallida*. *Nematologica* 23: 333-339.
- Manduric S., Andersson S. 2003. Potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) from Swedish potato cultivar – an AFLP study of their genetic diversity and relationships to other European populations. *Nematology* 5(6): 851-858.
- Nakhl, M.K., Owens K.J., Li W., Wei G., Skantar A.M., Levy L. 2010. Multiplex real-time PCR assays for the identification of the potato cyst and tobacco cyst nematodes. *Plant Disease* 94(8): 959-965.
- Nicol J.M., Turner S.J., Coyne D.L., den Nijs L., Hockland S., Maafi Z.T. 2011. Current nematode threats to world agriculture. In: J. T. Jones, G. Gheysen and C. Fenoll (Eds.), *Genomics and Molecular Genetics of Plant-Nematode Interactions*, Heidelberg, Germany, pp. 21-44.
- Nowaczyk K., Dobosz R., Budziszewska M., Kamasa J., Obrępańska-Stepłowska A. 2011. Analysis of diversity of golden potato cyst nematode (*Globodera rostochiensis*) populations from Poland using molecular approaches. *Journal of Phytopathology* 159: 759-766.
- Oro V., Oro Radovanovic V. 2012. Molecular characterization of PCN populations from Serbia. *Genetika* 44 (1): 189-200.
- Patrik K.H., Rumpfenhorst H.J., Burgermeister W. 1995. Random amplified polymorphic DNA analysis of a *Globodera pallida* population selected for virulence. *Fundamental and Applied Nematology* 18(2): 109-114.
- Perez-Jimenez M., Besnard G., Dorado G., Hernandez P. 2013. Varietal tracing of virgin olive oils based on plastid DNA variation profiling. *PLoS One* pp. 8.
- Phillips M. S., Harrower B.E., Trudgill D.L., Waugh R., Catley M.A. 1992. Genetic variation in British populations of *Globodera pallida* as revealed by isozyme and DNA analysis. *Nematologica* 38: 301-319.
- Phumichai C., Phumichai T., Wongkaew A. 2015. Novel chloroplast microsatellite (cpSSR) markers for genetic diversity assessment of cultivated and wild *Hevea* rubber. *Plant Molecular Biology Reporter* 33: 1486-1498.
- Plantard O., Pickard D., Valette S., Scurrah M., Greiner E., Mugniery D. 2008. Origin and genetic diversity of Western European populations of the potato cyst nematode (*Globodera pallida*) inferred from mitochondrial sequences and microsatellite loci. *Molecular Ecology* 17(9): 2208-2218.
- Pont-Kingdon G.A., Okada N.A., Macfarlane J.L., Beagley C.T., Wolstenholme D.R. 1995. A coral mitochondrial *mtS* gene. *Nature* 375: 109-111.
- Pypylenko L.A., Phillips M.S., Blok V.C., 2008. Characterization of two Ukrainian populations of *Globodera pallida* in terms of their virulence and mtDNA, and the biological assessment of a new resistant cultivar Vales Everest. *Nematology* 10: 585-590.
- Powers T.O., Adams B. 1993. Nucleotide sequences in nematode systematics. In: F. Lamberti, C. DeGiorgi and D. McK. Bird (Eds.), *Advances in Molecular Plant Nematology*, London, UK. Plenum, pp. 9-118.
- Powers T. 2004. Nematode molecular diagnostics: from bands to barcodes. *Annual Review of Phytopathology* 42: 367-383.
- Schnick D., Rumpfenhorst H.J., Burgermeister W. 1990. Differentiation of closely related *Globodera pallida* (Stone) populations by means of DNA restriction fragment length polymorphisms (RFLPs). *Journal of Phytopathology* 130: 127-136.
- Shield R., Fleming C.C., Stratford R. 1996. Identification of potato cyst nematode using the polymerase chain reaction. *Fundamental and Applied Nematology* 19(2): 167-174.
- Subbotin S.A., Halford P.D., Warry A., Perry R. 2000. Variation in ribosomal DNA sequences and phylogeny of *Globodera* parasitizing solanaceous plants. *Nematology* 2(6): 561-604.
- Subbotin S.A., Moens M. 2006. Molecular taxonomy and phylogeny. In: R. N. Perry and M. Moens (Eds.), *Plant Nematology*, Wallingford, UK. *CAB International*, pp. 40-721.
- Thiery M., Mugniery D. 2000. Microsatellite loci in the phytoparasitic nematode *Globodera*. *Genome* 43(1): 160-165.
- Vos P., Hogers R., Blekker M., Reijers M., van de Lee T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper M. 1995. AFLP: a new technique to DNA fingerprinting. *Nucleic Acids Research* 23(21): 4407-4414.

- Welsh J., McClelland M. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research* 18: 7213-7218.
- Zabeau M., Vos P. 1993. Selective restriction fragment amplification: A general method for DNA fingerprinting. *European Patent Application*, No.: 92402629.7. Publication number EP 0534858.