

Защита растений Plant protection

DOI 10.22363/2312-797X-2020-15-4-353–362
UDC 633.491:632.651:577.21

Research article / Научная статья

Diagnosis of potato rot nematode *Ditylenchus destructor* using PCR-RFLP

Niloufar Mahmoudi^{1*}, Elena N. Pakina¹,
Liudmila A. Limantceva², Anton V. Ivanov²

¹Peoples' Friendship University of Russia (RUDN University), Moscow, Russian Federation

²All-Russian Plant Quarantine Center, Moscow region, Russian Federation

*Corresponding author: niloofarmahmoodi@ymail.com

Abstract. During an investigation of nematodes in the Moscow region of Russia in 2019, a known species *Ditylenchus destructor* was recovered from tubers of potato plants. The genus *Destructor* is one of the most problematic genera of plant-parasitic nematodes. The numerous species reported for this genus have been cited from various sources. Due to the morphological similarity of many species and the lack of separation characteristics, the identification of *D. destructor* is difficult. Molecular taxonomy and phylogeny were used to confirm the identification. In the current study, PCR-RFLP illustrative models for the amplification of the ITS-rRNA gene were provided with two enzymes that could recognize *D. destructor* in potato tubers. Analysis of the rDNA sequences spanning both ITS1-ITS2 regions was carried out on the collected populations. The digestion of the PCR product of the ITS1-5.8S-ITS2 region with the enzyme TaqI produced three fragments; 100, 190, 550, and with Tru1I, two fragments were produced; 300 and 480 bp. The obtained DNA sequences were compared with those DNA sequences deposited in GenBank of populations isolated in other countries. The results showed no distinction between populations isolated from different host plant species, including populations found in the Russian Federation. New sequences from ITS-rRNA were deposited in the GenBank under accession number MN122076, MN658597, MN658599, MN658637, MN658638.

Keywords: ITS-rRNA, molecular diagnostics, PCR-RFLP, *Ditylenchus destructor*, potato rot nematode, plant pest

Conflicts of interest. The authors declared no conflicts of interest.

Acknowledgments. The research has been conducted with the support of the RUDN University Program «5—100».

Article history:

Received: 13 September 2020. Accepted: 16 October 2020

© Mahmoudi N., Pakina E.N., Limantceva L.A., Ivanov A.V., 2020



This work is licensed under a Creative Commons Attribution 4.0 International License
<https://creativecommons.org/licenses/by/4.0/1>

For citation:

Mahmoudi N, Pakina EN, Limantceva LA, Ivanov AV. Diagnosis of potato rot nematode *Ditylenchus destructor* using PCR-RFLP. *RUDN Journal of Agronomy and Animal Industries*. 2020; 15(4):353–362. doi: 10.22363/2312-797X-2020-15-4-353-362

Introduction

Nematodes are an important group of organisms having evident biological progress, characterized by the high amount of density growth, the broad spectrum of spreading, and having numerous intraspecific variable symptoms. They are listed among the quarantine pests in some European countries [1–3] and they were also evaluated as a serious pest in the globe [4]. *Ditylenchus destructor* could be a significant parasite in different host plants. Polyphagous nematode infects large diversity of plants in cropping systems, when there are no host plants, it is capable of feeding and reproducing on mycelia of many fungi. All nematode parasites undergo a process of host invasion, in which they reproduce, disperse, and pursue new hosts [5–8]. Recently, morphological identification has been investigated to recognize *D. destructor*, but morphology cannot be used when eggs, juveniles, and males are the only resources available. Moreover, the genus *Ditylenchus* includes several species with small interspecific morphological differences, and *D. destructor* usually occurs simultaneously with other nematode species. Without taxonomic expertise, the distinguishing of *D. destructor* from other species is difficult. There are some methods using PCR with specific primers designed based on nucleotides combination in the ITS rRNA gene which were also developed for the detection of *D. destructor* [2, 5, 9–11]. Molecular identification of *D. destructor* is based on the specific sequences of rDNA region by the conventional PCR methods [8, 12] and the most customarily used regions are the large subunits [13], small subunits and the internal transcribed spacer (ITS) of ribosomal RNA [14, 15]. Recently, several molecular practices have been evaluated and suggested by the European and Mediterranean Plant Protection Organization (EPPO) for identifying *D. destructor*, i.e. a PCR-RFLP-based assay of ITS fragment [16]. This research attempts to use EPPO protocol for the characterization and identification of *D. destructor* nematode. The objective of this study was to identify *D. destructor* that was recently found in potato tubers in the Russian Federation, by the means of the PCR-RFLP technique.

Materials and methods

Nematode population. Potato rot nematodes (*D. destructor*) were extracted from potato tubers (*Solanum tuberosum* L.) in the Russian Federation. Species identification was confirmed through morphological and molecular methods.

For the preparation of nematodes, a tipped pipette was used for picking individual nematodes in a suspension, and a sucking tipped pipette was prepared by burning the tips of two Pasteur pipettes pressed against each other. The pipettes were then pulled apart after melting began, resulting in a tiny syringe-like opening, which served the purpose of sucking nematodes from the suspension with the capillary action.

For morphometric analysis, nematodes (males and females) were picked for each population by sucking with the tipped pipette and placing them onto a glass slide (Menzel GmbH, Braunschweig, Germany), thus creating temporary slide mounts. Two drops of distilled water were added into each glass slide. The slides were then heated at 55 °C for 3...5 seconds on a hot plate. Thereafter, covered slides were placed on the water droplets, and samples were mounted on a ZEISS Axioskop50 microscope equipped with a camera. *D. destructor* remained straight when killed by heat (approximately 60 °C), a typical feature of *Ditylenchus* spp. Morphometric data and light microscopic images were obtained from digital images on a computer screen with the aid of AxioVision software version 4.8.2 (Carl Zeiss MicroImaging GmbH, Jena, Germany) (Fig. 1).

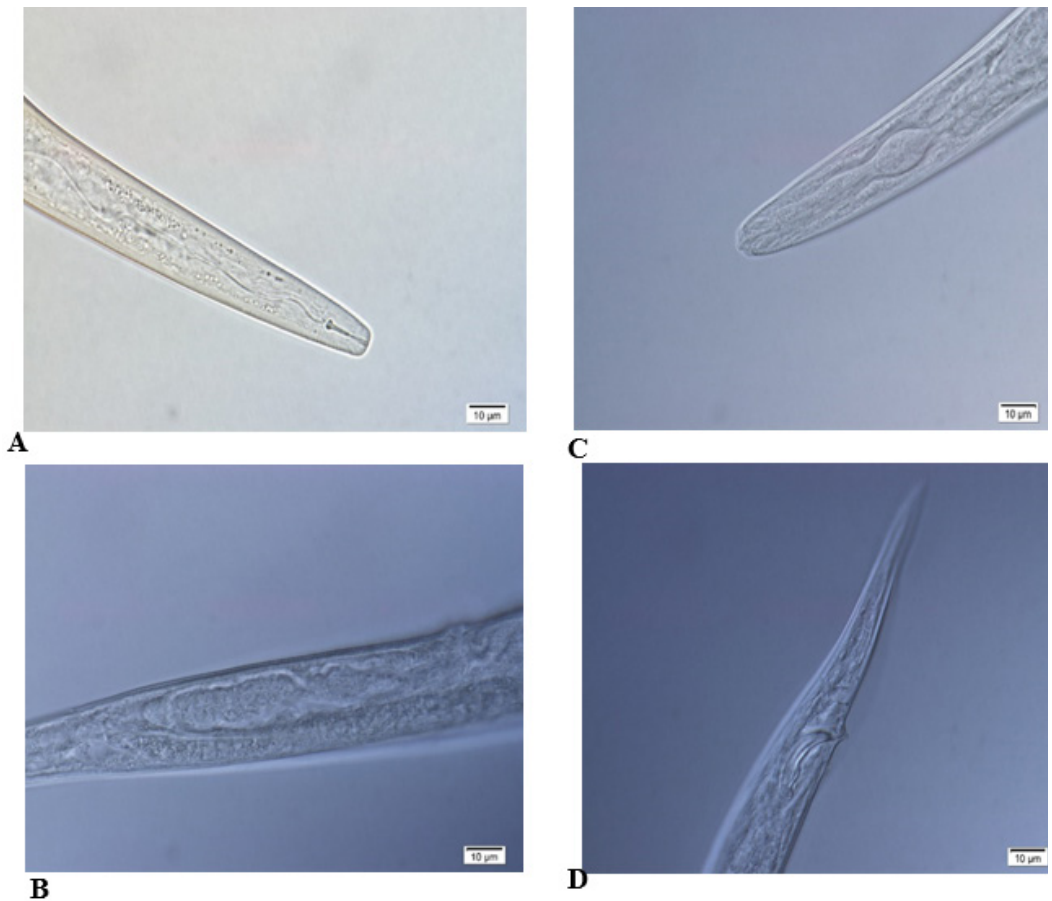


Fig. 1. Micrographs of diagnosis characteristics for *D. destructor* found in potato tubers: A – Female head and stylet; B – Female vulva, anus distance; C – Male head and stylet; D – Male bursa and tail

DNA extraction was carried out by treating the specimens with Proteinase K, which was performed by removing proteins without using organic solvents in the extraction process. A DNA-Ekstran-2 set No EX-511-100 (Synthol, Moscow) was applied for this purpose. Nematodes were extracted from the infected potato tubers. About 6...10 nematodes were collected from the potatoes and put in 20 µl lysis buffer and crashed,

then 280 µl of lysis solution and 1 µl mercaptoethanol were added. Thereafter, 10 µl of Proteinase K was added to the tubers.

The mixture was then vortexed and incubated at 55 °C for 24 hours. By the next day, 100 µl of the precipitated solution was put into tubes; vortexed for 20 seconds, and centrifuged at 13000 rpm for 5 minutes. Subsequently, 2 µl of glycogen was put into new tubes and the supernatant derived from the last stage was also added to them. The tubes were manually (by hand) shaken 10 to 14 times, then 300 µl of precipitating solution was added and shaken manually for 10 to 14 times before being centrifuged at 13000 rpm for 5 minutes. At this point, DNA became visible at the bottom of the tubes. In the next step, 400 µl of wash solution was added and the contents were shaken, and then centrifuged at 13000 rpm for 2 minutes. One more time, the extra solution was discarded. Afterward, the tubes only had the DNA in them and were opened, then put in the oven at 37 °C for 12...15 minutes to evaporate the alcohol. Finally, 50 µl DNA dissolution was added, then shaken, and also put in the oven at 60 °C for 5 minutes.

PCR. The PCR reaction was performed with samples (final volume 25 µl), containing 5 µl of 10X reaction buffer, 1 µl of each primer; universal primers 18S (5'-TTG ATT ACG TCC CTG CCC TTT-3') and 26S (5'-TTT CAC TCG CCG TTA CTA AGG-3') (Table 1), 1 µl DNA and 17 µl H₂O. Amplification conditions for the reaction were as follows: denaturation at 95 °C for 1 minute and 30 seconds followed by 40 cycles, of 45 seconds at 95 °C; primer annealing for 40 seconds at 50 °C; elongation for 1 minute at 70 °C; final elongation at 72 °C for 10 minutes.

Table 1

The primers used in the study

Primer name	Sequence (5'-3')	Amplified region	Source
18S	(5'-TTG ATT ACG TCC CTG CCC TTT-3')	ITS1-5.8s-ITS2 rRNA	Wendt et al., 1993
26S	(5'-TTT CAC TCG CCG TTA CTA AGG-3')	ITS1-5.8s-ITS2 rRNA	Wendt et al., 1993

PCR-RFLP. In carrying out RFLP analysis, 30 µl of the amplification product was digested with one of the restriction enzymes (Tru1I and TaqI) in a buffer stipulated by the supplier. Each digestion reaction involved of 2 µl of 10X reaction buffer, 10 µl of the direct PCR product, and 1 µl of restriction enzyme (10 µg⁻¹ µl) thus increasing to a total volume of 17 µl with double distilled water. The digestion mixture was incubated for 5 minutes at 65 °C. The digested DNA fragments were separated on buffered (0.5 % TAE) 1 % agarose gel which contained 15 µl of 10,000X GelRed.

Sequence analysis was performed by the commonly accepted protocol of utilizing the Genetic Analyser AB-3500. Primitive comparison of sequencing results with the GeneBank genetic sequence database was done by the NCBI BLAST website (<http://www.ncbi.nlm.nih.gov/BLAST>) (Table 2). BioEdit v.7.0.5.3, sequence alignment editor was used for checking the sequence, editing, and alignment. The tree diagrams were created by using the maximum likelihood technique (ML method) available in Mega 10 software. Bootstrap Test via building 1000 alternative trees was used to analyze and confirm the validity of the tree diagrams. The results are presented in percentage values, the DNA sequences available in the GeneBank homologous to those examined were analyzed along with the newly sequenced. These sequences were deposited in the NCBI database with access numbers: MN122076, MN658597, MN658599, MN658637, MN658638.

Table 2

Reference sequences used in the phylogenetic analysis (www.ncbi.nlm.nih.gov)

Species	Accession Number	Country	Host Plant
<i>D. destructor</i>	MH992393	China	Potato
<i>D. destructor</i>	MN016954	Poland	Potato
<i>D. destructor</i>	MN016967	Poland	Potato
<i>D. destructor</i>	EU400636	China	Sweet potato
<i>D. destructor</i>	EU400627	South Korea	Sweet potato
<i>D. destructor</i>	EF208213	China	Potato
<i>D. destructor</i>	JZ133325	China	Sweet potato
<i>D. destructor</i>	JZ133407	China	Sweet potato
<i>D. destructor</i>	JZ128929	China	Sweet potato
<i>D. destructor</i>	JZ128830	China	Sweet potato
<i>D. destructor</i>	FJ707365	Czech Republic	Potato
<i>D. destructor</i>	MG673926	China	Carrot
<i>D. destructor</i>	MN122076	Russian Federation	Potato
<i>D. destructor</i>	MN658597	Russian Federation	Potato
<i>D. destructor</i>	MN658599	Russian Federation	Potato
<i>D. destructor</i>	MN658637	Russian Federation	Potato
<i>D. destructor</i>	MN658638	Russian Federation	Potato
<i>D. destructor</i>	JZ133328	China	Sweet potato
<i>D. dipsaci</i>	MG676655	Japan	Phlox subulata
<i>D. dipsaci</i>	MG676656	Japan	Phlox subulata
<i>D. dipsaci</i>	MG676657	Japan	Phlox subulata
<i>D. dipsaci</i>	GQ469497	Czech Republic	Allium sativum
<i>Meloidogyne javanica</i>	AY545998	USA	–
<i>Meloidogyne javanica</i>	AY545997	USA	–
<i>D. dipsaci</i>	KY348765	Mexico	Alfalfa1
<i>D. dipsaci</i>	KT806479	China	Medicago sativa

Results and discussion

Using the RFLP-PCR and nematode DNA extracted from potato tubers, PCR fragments were successfully amplified at the expected product size for primer set in *D. destructor* samples. PCR-RFLP showed the distinction of *D. destructor* with similar species such as *D. dipsaci* and *D. myceliophagus* [17]. In some populations of sweet potato, the RFLP method indicated different band lengths [18]. The amplification of ITS1-5.8S-ITS2 of the ribosomal DNA produced a fragment of almost 1000 bp. Nowadays, the molecular diagnostic of *D. destructor* is primarily based on the specific sequences of some conserved regions by the conventional PCR, and the most commonly used regions are small subunits and internal transcribed spacer (ITS) of ribosomal RNA [2].

The digestion of the PCR product of the ITS1—5.8S-ITS2 region with the enzyme TaqI produced three fragments; 100, 190, 550, and with TruII, two fragments were produced; 300 and 480 bp (Fig. 2). ITS1 differed in length from 315 to 473 bp, 5.8S measured *ca* 154bp and ITS2 was 207 bp. The identified distinction in length in the ITS1 was affected by the insertion of 57 to 188 bp in the entire length in some *D. destructor* [2]. A mentioned primer set can be used for specific identification of *D. destructor*.

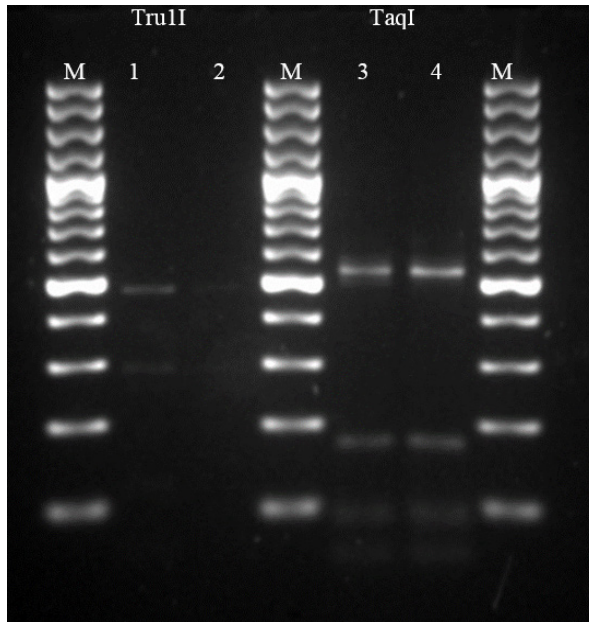


Fig. 2. Restriction fragment length polymorphisms of the ITS region amplified using 18S and 26S primers for a, *D. destructor* using restriction enzymes TruII (LANE1, 2) and TaqII (Lane 3, 4). Lane M is the (100 bp) molecular marker

All populations from potatoes were identified as *D. destructor* by DNA sequencing. DNA fragments produced bands with the primer set tested.

Diagnostic of *D. destructor* is difficult by the morphological identification only. Hence, molecular techniques help scientists to identify this nematode accurately. It allows to the suggestion that *D. destructor* may present a species or subspecies complexity]18[.

Recently, methods and knowledge of molecular biology have been investigated as a powerful practice to identify various nematode species [13, 17]. It was also revealed that rDNA ITS regions can be desirably used for phylogenetic analyzes [5, 16].

Similar research by BLAST of the sequences of *D. destructor* was found in potato tubers with sequences of the GenBank from both markers of the rDNA previously deposited in the GenBank displayed three clades of *D. destructor*, *D. dipsaci*, and *Meloidogyne javanica* as an outgroup species. Our deposited sequences formed a highly supported (PP = 100 %) with whole populations that were involved from China, Czech Republic, and South Korea.

Phylogenetic relationships within potato rot nematode as inferred from the ITS1-5.8S-ITS2 rRNA gene sequences using Maximum Likelihood Method inference is given in Fig. 3. In the GenBank database, there are many sequences of *D. destructor* retrieved from various plants and geographical regions [5, 7].

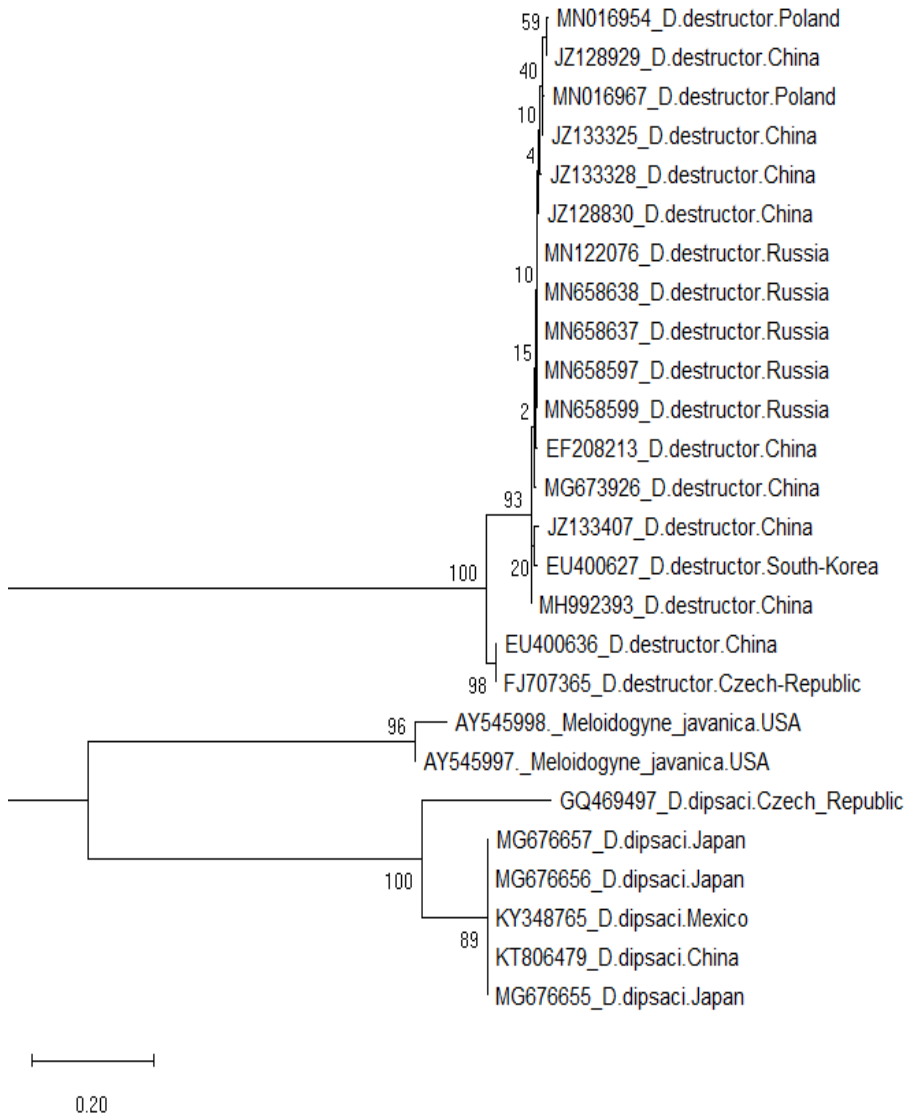


Fig. 3. Maximum Likelihood Method of the alignments of internal transcribed spacer 1 (ITS1)-5.8S-ITS2 of the *D. destructor*

Conclusions

The accurate identification of nematode is a significant step for effective control of the host plant. It must be recognized that species determinations have an essential practical application in nematology. Regulatory decisions depend on quick and also accurate identification. Overall, economic management options require a greater resolution in nematode identification. Based on the PCR-RFLP restriction pattern obtained with the Tru1I, TaqI, it is possible to indicate that the rot nematode *D. destructor* was found in potato tubers in the Russian Federation territory. It is necessary to carry out studies of differential hosts in the populations and use new sequencing to obtain useful molecular markers for the differentiation of the *D. destructor* in the Russian Federation.

References

1. Bae CH, Szalanski AL, Robbins RT. Genetic variation of *Hoplolaimus columbus* populations in the United States using PCR-RFLP analysis of nuclear rDNA ITS regions. *Journal of nematology*. 2009; 41(3):187–193.
2. Ding SW, Yang SH, Wu WJ, Xie H, Xu CL. Rapid diagnosis of *Ditylenchus destructor* by loop-mediated isothermal amplification assay based on 28S rRNA sequences. *European Journal of Plant Pathology*. 2019; 153(4):1165–1175. doi: 10.1007/s10658-018-01633-7
3. Jeszke A, Dobosz R, Obrępańska-Stęplowska A. A fast and sensitive method for the simultaneous identification of three important nematode species of the genus *Ditylenchus*. *Pest management science*. 2015; 71(2):243–249. doi: 10.1002/ps.3788
4. Ji L, Wang JC, Yang XL, Huang GM, Lin MS. PCR-RFLP patterns for differentiation of three *Ditylenchus* species. *Journal of Nanjing Agricultural University*. 2006; 29(3):39–43. doi: 10.7685/j.issn.1000-2030.2006.03.008
5. Mahmoudi N, Naserzadeh Y, Pakina EN, Limantceva LA, Nejad DK. Molecular identification of *Ditylenchus destructor* nematode with PCR Species-Specific primers in the Moscow region. *RUDN Journal of Agronomy and Animal Industries*. 2019; 14(4):430–436. doi: 10.22363/2312-797X-2019-14-4-430-436
6. Liu B, Mei YY, Zheng JW. Species-specific detection of inter-populations of *Ditylenchus destructor*. *Journal of Zhejiang University (Agriculture & Life Sciences)*. 2007; 33:490–496
7. Mahmoudi N, Pridannikov M, Zargar M, Naserzadeh Y, Limantceva L, Pakina E. Molecular diagnostics of *Ditylenchus destructor* based on the ITSrDNA from Iran and Russia Federation. *Research on Crops*. 2020; 21(1):151–155. doi: 10.31830/2348-7542.2020.025
8. Marek M, Zouhar M, Douda O, Mazakova J, Rysanek P. Bioinformatics-assisted characterization of the ITS1-5' 8S-ITS2 segments of nuclear rRNA gene clusters, and its exploitation in molecular diagnostics of European crop-parasitic nematodes of the genus *Ditylenchus*. *Plant Pathology*. 2010; 59(5):931–943. doi: 10.1111/j.1365-3059.2010.02322.x
9. Oliveira CM, Monteiro AR, Blok VC. Morphological and molecular diagnostics for plant-parasitic nematodes: working together to get the identification done. *Tropical Plant Pathology*. 2011; 36(2): 65–73. doi: 10.1590/S1982-56762011000200001
10. Mizen KA, Caubel G, Plowright RA. *Ditylenchus* species. In: Cook R, Starr JL, Bridge J. (eds.) *Plant resistance to parasitic nematodes*. CABI Publishing; 2002. p.107–139.
11. Powers TO, Szalanski AL, Mullin PG, Harris TS, Bertozzi T, Griesbach JA. Identification of seed gall nematodes of agronomic and regulatory concern with PCR-RFLP of ITS1. *Journal of Nematology*. 2001; 33(4):191–194.
12. Smith IM, Mc Namara DG, Scott PR, Harris KM. *Quarantine pests for Europe*. Cambridge: University Press; 1992.
13. Subbotin SA, Madani M, Krall E, Sturhan D, Moens M. Molecular diagnostics, taxonomy, and phylogeny of the stem nematode *Ditylenchus dipsaci* species complex based on the sequences of the internal transcribed spacer-rDNA. *Phytopathology*. 2005; 95(11):1308–1315. doi: 10.1094/PHYTO-95-1308.
14. Deimi AM, Zheng J, Chizhov VN, Subbotin SA. Length variation and repetitive sequences of internal transcribed spacer of ribosomal RNA gene, diagnostics and relationships of populations of potato rot nematode, *Ditylenchus destructor* Thorne, 1945 (Tylenchida: Anguinidae). *Nematology*. 2011; 13(7):773–785. doi: 10.1163/138855410X551923
15. Thorne G. *Ditylenchus destructor* n. sp. the potato rot nematode, and *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936, the teasel nematode (Nematoda: Tylenchidae). *Proceedings of the helminthological Society of Washington*. 1945; 12(2):27–34.
16. Wan F, Peng DL, Yang YW, He YQ. Species specific molecular diagnosis of *Ditylenchus destructor* populations occurring in China. *Acta Phytopathologica Sinica*. 2008; 38(3):263–270.
17. Wendt KR, Swart A, Vrain TC, Webster JM. *Ditylenchus africanus* sp. N. from South Africa; a morphological and molecular characterization. *Fundamental and Applied Nematology*. 1995; 18(3):241–250.
18. Willett DS, Martini X, Stelinski LL. Chemoecology and Behavior of Parasitic Nematode — Host Interactions: Implications for Management. In: Tabata J. (ed.) *Chemical Ecology of Insects*. Boca Raton: CRC Press; 2018. p.91–113. doi: 10.1201/9781351228398.

About authors:

Mahmoudi Niloufar — PhD candidate, Department of Agrobiotechnology, Agrarian and Technological Institute, Peoples' Friendship University of Russia, 8/2, Miklukho-Maklaya st., Moscow, 117198, Russian Federation; e-mail: niloofarmahmoodi@yandex.com
ORCID iD: 0000–0002–8229–4852

Pakina Elena Nikolaevna — Associate Professor, Candidate of Biological Sciences, Agro-Biotechnological Department, Agrarian and Technological Institute, Peoples' Friendship University of Russia, 8/2, Miklukho-Maklaya st., Moscow, 117198, Russian Federation; e-mail: e-pakina@yandex.ru

Limantseva Liudmila Alekseevna — Senior Scientist, All-Russian Plant Quarantine Center, 32, Pogranichnaya st., Bykovo vill., 140150, Ramenskoye, Moscow region, Russian Federation; e-mail: Limantseva.liudmila@vniikr.ru

Ivanov Anton Vladislavovich — Junior Researcher, All-Russian Plant Quarantine Center, 32, Pogranichnaya st., Bykovo vill., 140150, Ramenskoye, Moscow region, Russian Federation; e-mail: Ivanov.anton@vniikr.ru

Диагностика стеблевой нематоды картофеля *Ditylenchus destructor* с использованием PCR-RFLP

Н. Махмуди^{1*} Е.Н. Пакина¹, Л.А. Лиманцева², А.В. Иванов²

¹Российский университет дружбы народов, г. Москва, Российская Федерация

²Всероссийский центр карантина растений, Московская обл., Российская Федерация

*niloofarmahmoodi@yandex.com

Аннотация. При исследовании нематод в Московской области в 2019 г. был выделен известный вид *Ditylenchus destructor* из клубней картофеля. Род деструктор — один из наиболее проблемных родов растительных паразитарных нематод. Многочисленные виды, описанные для этого рода, были приведены в различных источниках. Из-за морфологического сходства многих видов и отсутствия раздельных признаков идентификация *D. destructor* затруднена. Для подтверждения идентификации использовалась молекулярная систематика и филогения. В настоящем исследовании иллюстративные модели PCR-RFLP для амплификации гена ITS-рДНК были снабжены двумя ферментами, которые могли распознавать *D. destructor* в клубнях картофеля. На собранных популяциях был проведен анализ последовательностей рДНК, охватывающих оба региона ITS1-ITS2. В результате амплификации участка ITS1-5.8 с-ITS2 с ферментом TaqI были получены фрагменты длиной 100, 190 и 550 п.н., а с ферментом Tru1I были получены два фрагмента — 300 и 480 п.н. Полученные последовательности ДНК сравнивали с последовательностями ДНК, депонированными в Генбанке популяций, изолированных в других странах. Полученные результаты не выявили различий между популяциями, выделенными из различных видов растений-хозяев, включая популяции, обнаруженные в Российской Федерации. Новые последовательности из ITS-рДНК были депонированы в Генбанк под регистрационными номерами MN122076, MN658597, MN658599, MN658637, MN658638.

Ключевые слова: ITS-рДНК, молекулярная диагностика, PCR-RFLP, *Ditylenchus destructor*, стеблевая картофельная нематода, вредитель растений

Заявление о конфликте интересов: Авторы заявляют об отсутствии конфликта интересов.

Финансирование. Благодарности. Исследование проведено при финансовой поддержке Программы РУДН «5—100».

История статьи:

Поступила в редакцию: 13 сентября 2020 г. Принята к публикации: 16 октября 2020 г.

Для цитирования:

Mahmoudi N., Pakina E.N., Limantseva L.A., Ivanov A.V. Diagnosis of potato rot nematode *Ditylenchus destructor* using PCR-RFLP // Вестник Российского университета дружбы народов. Серия: Агрономия и животноводство. 2020. Т. 15. № 4. С. 353–362. doi: 10.22363/2312-797X-2020-15-4-353-362

Об авторах:

Махмуди Нилуфар — аспирант, Агробиотехнологический департамент, Аграрно-технологический институт, Российский университет дружбы народов, Российская Федерация, 117198, г. Москва, ул. Миклухо-Маклая, д. 8/2; e-mail: niloofarmahmoodi@yandex.com

ORCID iD: 0000-0002-8229-4852

Пакина Елена Николаевна — доцент, кандидат биологических наук, Агробиотехнологический департамент, Аграрно-технологический институт, Российский университет дружбы народов, Российская Федерация, 117198, г. Москва, ул. Миклухо-Маклая, д. 8/2; e-mail: e-pakina@yandex.ru

Лиманцева Людмила Алексеевна — старший научный сотрудник, Всероссийский центр карантина растений, Российская Федерация, 140150, г. Раменское, п. Быково, ул. Пограничная, д. 32; e-mail: Limantseva.ludmila@vniikr.ru

Иванов Антон Владиславович — младший научный сотрудник, Всероссийский центр карантина растений, Российская Федерация, Московская область, 140150, г. Раменское, п. Быково, ул. Пограничная, д. 32; e-mail: Ivanov.anton@vniikr.ru