

MORPHOMETRIC AND MOLECULAR ANALYSIS OF POTATO CYST NEMATODES FROM SERBIA

Jasmina BA I¹, Barbara GERI STARE², Saša ŠIRCA³, Gregor UREK⁴

¹Agricultural Extension Service Institute Tamiš, Serbia

^{2,3,4}Agricultural Institute of Slovenia, Ljubljana

ABSTRACT

Potato cyst nematodes (PCN) *Globodera rostochiensis* and *G. pallida* belong to the major potato parasites in temperate regions. These quarantine pests are regulated by EU directive 2007/33/EC. The morphological characters of these sibling species may overlap and molecular method of identification is needed in such cases. A precise identification is crucial for the phytosanitary system of every country including Serbia. *G. rostochiensis* and *G. pallida* are present in Serbia since 1999 and 2005 respectively. Both species were detected in soil samples originated from seed potato fields in three districts (Zlatibor, Ma va and Moravica) during official surveys. In accordance with phytosanitary measures for prevention of their further spreading, an official program for control of ware potato fields has been carried out for the presence of PCN in these quarantine districts since 2009. During 2009-2011, the PCN originated from ware potato fields from Serbia were analyzed by morphometric method and their identity was confirmed using real-time PCR method. The morphometrical characters of five PCN populations from locations of Gojna Gora (Moravica District), Tabanovi i and Ponikve (Zlatibor District) were studied. Morphometric analyses of ten cysts and ten second-stage juveniles originated from these locations established the presence of *G. rostochiensis*. All morphological values from these different populations are very close. The characters that vary most are J2 body length, J2 tail length (including hyaline part of tail) and cyst distance-vulval basin to anus. Molecular analysis with real-time PCR identified all tested samples as *G. rostochiensis* and confirmed morphometrical identification.

Key words: *Globodera rostochiensis*, morphology, potato cyst nematodes, real-time PCR

1 INTRODUCTION

The potato cyst nematodes (PCN) *Globodera rostochiensis* (Wollenweber) Behrens and *G. pallida* (Stone) Behrens are among the most dangerous potato pests worldwide. These species are listed as A2 quarantine organisms in Europe and regulated by EU directive 2007/33/EC on the control of potato cyst nematodes (EUR-lex, 2007). *G. rostochiensis* and *G. pallida* are morphologically and morphometrically closed related (Stone, 1973a, b). The morphological characters of these sibling species may overlap and molecular method of identification is needed in such cases. A precise identification is essential for the phytosanitary system of every country including Serbia. *G. rostochiensis* and *G. pallida* are present in Western Serbia since

¹ dr., Novoseljanski put 33, 26000 Pan evo, Serbia

² dr., Hacquetova 17, SI-1000 Ljubljana

³ dr., prav tam

⁴ doc. dr., prav tam

1999 and 2005 respectively (Krnjai *et al.*, 2002; Krnjai *et al.*, 2006; Radivojevi *et al.*, 2006). Both species were detected in soil samples originated from seed potato fields during official surveys. *G. rostochiensis* was found in three districts (Zlatibor, Ma va and Moravica) while *G. pallida* was reported only on location of Javor mountain in Zlatibor District. In accordance with phytosanitary measures for prevention of their further spreading, an official program for control of ware potato fields has been carried out for the presence of PCN in these quarantine districts since 2009. According to the official data of the Statistical Office of the Republic of Serbia, potatoes are grown on 75.449 ha in 2012 (Statistical Office of the Republic of Serbia, 2012). The PCN collected from ware potato fields in Serbia during 2009 - 2011 were analyzed by morphometric and molecular methods. The aim of this study was to identify these PCN populations unambiguously based on morphological characters and using real-time PCR method.

2 MATERIALS AND METHODS

Five PCN populations from Western Serbia from locations of two districts (Moravica and Zlatibor) were studied: Galjen-Gojna Gora, Ravni Gaj-Gojna Gora, Tabanovi i-Bakionica Loret, Ponikve-Stapari and Ponikve-Mala Njiva. Morphological and morphometrical characters of ten cysts and ten second-stage juveniles (J2s) were analysed. The cysts were extracted by flotation method using Fenwick can. The second-stage juveniles were obtained from cysts pre-soaked in water by cutting the cysts and fixed in TAF (triethanolamine-formalin). Cyst vulval cones and J2s were mounted on glycerol and prepared as semi-permanent microscopic slides. The specimens were examined using a light microscopy Leica DM 1000 LED connected to a DFC 295 digital camera. Photomicrographs of the specimens were analysed with Leica Application Suite by interactive measurements and diagnostic characters were measured. The morphological identification of PCN was based on combination of J2 and cyst characters: body length, stylet length, tail and hyaline part of tail length, Granek's ratio (the distance from anus to the nearest edge of vulval basin divided by vulval basin diameter) and number of cuticular ridges between vulva and anus. Specimens were morphometrically identified on the basis of taxonomic keys (Fleming and Powers, 1998). DNA was extracted from 5-28 cysts of each of the studied populations collected in three consecutive years (2009-2011). The Promega Genomic DNA Wizard purification kit was used after a slight modification of the manufacturer's instructions. Molecular identification of PCN was performed with real-time PCR method and sybr green chemistry as described previously (Ba i *et al.*, 2008).

3 RESULTS AND DISCUSSION

Measurements of J2s and cysts of potato cyst nematodes from 5 locations from Serbia are listed in Table 1. Morphometrical analyses of ten cysts and ten J2 per population established the presence of *G. rostochiensis* in all soil samples. Morphometrics fitted within the ranges of previous *G. rostochiensis* reports and coincided with mean values of characteristics indicated in the literature (OEPP/EPPO, 2009). The mean vulval basin diameter value from Ponikve-Mala Njiva population was slightly greater (19.03 μm) than the mean diameter value (< 19 μm) given in the taxonomic keys (Fleming and Powers, 1998). However, essential morphological and morphometrical characters, such as the juvenile stylet length, the stylet knobs anteriorly flattened to rounded, without forward projections, the number of the cuticular ridges and Granek's ratio indicated that the specimens were *G. rostochiensis*.

Table 1: Morphometrical characters of J2s and cysts of *Globodera rostochiensis* from 5 locations from Serbia (n=10). All measurements are in μm , presented as mean \pm standard deviation with the range in parentheses.

	Galjen-Gojna Gora (Moravica District)	Ravni Gaj-Gojna Gora (Moravica District)	Tabanovići-Bakionica Loret (Zlatibor District)	Ponikve-Stapari (Zlatibor District)	Ponikve-Mala Njiva (Zlatibor District)
J2 body length	438,08 \pm 35,44 (410,40-469,15)	444,87 \pm 26,71 (404,36-480,63)	465,62 \pm 14,32 (444,27-492,68)	456,85 \pm 20,49 (413,22-478,14)	441,93 \pm 24,31 (411,93-478,68)
J2 stylet length	20,81 \pm 1,14 (19,14-22,18)	21,60 \pm 1,12 (19,23-22,47)	21,92 \pm 0,80 (20,05-22,74)	21,09 \pm 0,84 (19,80-22,66)	21,88 \pm 0,94 (20,04-22,87)
J2 tail length	45,25 \pm 4,71 (37,05-51,15)	42,81 \pm 2,92 (37,79-46,71)	39,99 \pm 2,87 (37,69-47,18)	47,13 \pm 5,50 (38,60-54,43)	45,35 \pm 4,41 (37,69-51,40)
J2 hyaline part of tail	25,59 \pm 2,97 (20,60-29,85)	26,24 \pm 2,41 (21,11-29,80)	26,46 \pm 2,66 (21,50-31,18)	25,73 \pm 3,30 (20,60-30,00)	26,70 \pm 3,15 (21,57-32,00)
Vulval basin diameter	18,59 \pm 2,05 (14,52-22,10)	18,06 \pm 1,07 (16,36-19,59)	17,74 \pm 1,37 (15,09-19,52)	18,20 \pm 2,38 (15,60-22,02)	19,03 \pm 2,34 (15,02-22,14)
Distance-vulval basin to anus	63,10 \pm 7,74 (50,50-75,15)	64,02 \pm 8,62 (50,16-78,03)	72,00 \pm 13,95 (48,95-86,19)	65,07 \pm 11,11 (49,82-87,20)	70,71 \pm 11,89 (49,13-85,72)
Granek's ratio	3,45 \pm 0,71 (2,52-4,84)	3,55 \pm 0,59 (2,72-4,76)	4,03 \pm 0,64 (2,86-4,78)	3,63 \pm 0,87 (2,59-5,58)	3,78 \pm 0,89 (2,41-5,34)
No. of cuticular ridges	20,80 \pm 3,29 (16-27)	22,70 \pm 3,02 (18-27)	23,10 \pm 0,64 (19-28)	20,70 \pm 2,11 (17-24)	24,20 \pm 3,25 (18-28)

All morphological values from the analysed populations were very close. The characters that vary most are J2 body length, J2 tail length (including hyaline part of tail) and cyst distance-vulval basin to anus.

On average, population from Galjen-Gojna Gora have the shortest J2s (438,08 µm) with the lowest stylet (20,81 µm). The longest J2s are from Tabanovi i-Bakionica Loret (465,62 µm). The specimens from this population have the highest stylet (21,92 µm) and the shortest tail (39,99 µm). On average, the J2s from Ponikve-Stapari have the longest tail (47,13 µm). As for the morphometrics of cysts, the specimens from Galjen-Gojna Gora population have the lowest mean value of Granek's ratio (3,45) while the specimens from Tabanovi i-Bakionica Loret have the highest value (4,03). Finally, the specimens from Ponikve-Stapari have the lowest mean number of cuticular ridges between vulva-anus (20,70) and the specimens from Ponikve-Mala Njiva have the highest mean value (24,20).

Molecular identification with real-time PCR determined all samples from five locations as *G. rostochiensis*. The peak of dissociation curve in the range of 86.7 to 87.1°C indicated the presence of *G. rostochiensis*. All samples tested positive for *G. rostochiensis* and mixed PCN populations were not determined.

4 CONCLUSIONS

The correct and rapid identification of PCN is essential for its control. This can be achieved with combining morphological and molecular based methods of identification. With this approach all five populations from Western Serbia were identified as *G. rostochiensis*.

5 REFERENCES

- Ba i , J., Geri Stare B., Širca S., Urek, G. 2008. Analyses of *Globodera rostochiensis* and *G. pallida* from Serbia by morphometrics and real-time PCR. Russian Journal of Nematology, 16, 1: 63-65.
- EUR-lex. 2007. EUR-lex Access to European Union Law-Council Directive 2007/33/EC of 11 June 2007 on the control of potato cyst nematodes and repealing Directive 69/465/EEC. <http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32007L0033:en:NOT>.
- Fleming, C.C., Powers, T.O. 1998. Potato cyst nematode diagnostics: morphology, differential hosts and biochemical techniques. In: Marks R.J. and Brodie B.B. (eds.) Potato Cyst Nematodes-Biology, Distribution, Control. CAB International, Wallingford (GB): 95-99.
- Krnjai , ., Lamberti, F., Krnjai , S., Ba i , J., ali , R. 2002. First record of the potato cyst nematode (*Globodera rostochiensis*) in Yugoslavia. Nematologica Meditteranea, 30: 11-12.
- Krnjai , ., Oro, V., Gladovi , S., Trkulja, N., Š eki , D., Kecovi , V. 2006. New records of the potato cyst nematodes in Serbia. Zaštita Bilja / Plant Protection, 53 (4), 243: 147-156.
- OEPP/EPPO 2009. *Globodera rostochiensis* and *Globodera pallida* PM 7/40 (2). Bulletin OEPP/EPPO Bulletin, 39: 354-368.
- Radivojevi , M., Krnjai , ., Gruji , N., Oro, V., Gladovi , S., Madani, M. 2006. The first record of potato cyst nematode *Globodera pallida* (Stone, 1973) from Serbia. In: Programme and Abstracts of 58th International Symposium on Crop protection, 23rd May 2006, Ghent, Belgium, 2006: 203.
- Statistical Office of the Republic of Serbia. 2012. Areas: agriculture and fishery: production of late crops, fruits and grapes: potatoes: harvested area, ha, <http://webrzs.stat.gov.rs/WebSite/Public/ReportResultView.aspx?rptKey=indld%3d1301IND01%2635%3d6%2c7%2c8%262%3d%23Last%231%2639%3d03000%2c07000%2c08000%2c11000%2c12000%2c13000%2c19000%2c29000%2c31000%2c33000%266%3d1%2c2%2c3%26sAreald%3d1301%26dType%3dName%26IType%3dEnglish>
- Stone, A.R. 1973a. CIH Description of Plant-Parasitic Nematodes No.16 *Globodera rostochiensis*. CAB International, Wallingford (GB), cited in: OEPP/EPPO 2009. *Globodera rostochiensis* and *Globodera pallida* PM 7/40 (2). Bulletin OEPP/EPPO Bulletin, 39: 356 pp.
- Stone, A.R. 1973b. CIH Description of Plant-Parasitic Nematodes No. 15 *Globodera pallida*. CAB International, Wallingford (GB), cited in: OEPP/EPPO 2009. *Globodera rostochiensis* and *Globodera pallida* PM 7/40 (2). Bulletin OEPP/EPPO Bulletin, 39: 356 pp.