Research Note

Aphelenchus avenae (Nematoda: Aphelenchidae) under the rhizosphere of *Brassica napus*

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Summary

Soil samples under the rhizosphere of *Brassica napus* were collected from three localities (Bílá Podolí, Prague, Kylešovice). All three localities were positive for the presence of *Aphelenchus avenae*. Morphological and molecular features of *A. avenae* are presented in this research note.

Keywords: *Aphelenchus avenae*; Ribosomal DNA; Czech Republic; Nematode

Introduction

Aphelenchus avenae Bastian, 1865 is primarily fungivorous and occurs commonly in soil, but also in leaf sheaths, plant crowns and the cortex of some roots (Hooper, 1974; Hunt, 1993). Records of pathogenicity on higher plants are relatively few (Barker and Darling, 1965). The pathogenicity of the species is insignificant but it might well be the carrier of other causative agents of diseases such as bacteria and fungi. It is not clear whether it is capable of penetrating healthy roots or whether it simply attacks those already damaged by other causes, or those of weakened plants (Decker, 1988). It is a cosmopolitan species. In the neighbouring countries of the Czech Republic it was recorded from Slovakia (Lišková & Renčo, 2007), Germany (Decker, 1988), Poland and Austria (Andrássy, 2007). In the Czech Republic it was recorded by Háněl (2000a; 2000b). The objective of this study was to describe *A. avenae* morphologically and molecularly.

Materials and methods

The occurrence of *A. avenae* associated with *Brassica napus* was examined from three localities (Bílá podolí, Prague, Kylešovice) in the Czech Republic. Specimens for morphological and molecular analysis from the soil were

extracted by Baermann funnel method. Small amount of soil (25 – 50g) was placed on a tissue paper on a Baermann funnel for 24 – 48 hours. Nematodes for morphological study were heat killed, fixed in TAF, processed in slow glycerin process and mounted in anhydrous glycerin on slides. Photomicrographs were recorded with a digital camera linked to a computer and measurements were made with the aid of imaging software (Olympus DP-soft).

Few alive specimens were preserved in IM NaCl for sequencing. Temporary mounts of six individual nematodes from three populations were made in a drop of IM NaCl containing glass beads and after taking photomicrographs the slides were dismantled, individual nematodes removed, and added to digest in 0.25M NaOH overnight. Total genomic DNA was extracted from these six single individuals with a rapid technique (Stanton *et al.*, 1998). 18S gene of ribosomal DNA was amplified in two overlapping fragments and primer combination was 988F + 1912R for the first fragment and 1813F + 2646R for the second fragment (Holterman *et al.*, 2006). D2/D3 expansion segments of 28S gene were amplified using D2A and D3B primers (De Ley *et al.*, 2005). PCR reactions were performed in a 25 µl volume with the following master mix: one PCR bead (GE Healthcare, Buckinghamshire, UK), 20.5 µl double distilled sterile water, 2.0 µl each primer (10 pmol/µl) and to this 0.5 µl of DNA was added as a template for PCR. The cycling conditions were: first denaturation for 3 min at 94°C, 40 cycles with 30 s at 94°C, 30 s at 55°C, 30 s at 72°C and a final elongation step was run at 72°C for 10 min. DNA was purified using High Pure Product Purification kit (Roche Diagnostics GmbH, Mannheim, Germany) and directly sequenced in both directions (Macrogen, Korea). Sequencher™ 4.8 (Genes Codes Corp., Ann Arbor, MI, USA) software was used to assemble and view sequences and check for base-calling errors.

Table 1. Morphometrics of females of *Aphelenchus avenae* Bastian, 1865. Measurements in μm (in form): mean $\pm$ standard deviation (range)

Locality	Bilé Podolí	Prague	Kylešovice
n	9	10	15
L	605 $\pm$ 52 (517 – 663)	654 $\pm$ 59 (596 – 772)	657 $\pm$ 80 (512 – 807)
a	35.5 $\pm$ 3.75 (32.0 – 44.2)	33.1 $\pm$ 2.59 (28.1 – 36.3)	35.5 $\pm$ 3.22 (27.8 – 42.0)
b	4.4 $\pm$ 0.87 (3.1 – 5.8)	–	–
c	30.9 $\pm$ 6.94 (26.5 – 45.4)	28.6 $\pm$ 2.57 (23.3 – 31.4)	29.9 $\pm$ 3.50 (26.5 – 38.3)
c'	1.75 $\pm$ 0.68 (0.75 – 3)	2.01 $\pm$ 0.37 (1.53 – 2.75)	1.81 $\pm$ 0.49 (1.21 – 2.67)
vulva	76 $\pm$ 0.81 (75 – 77)	76 $\pm$ 1.27 (74 – 77)	76 $\pm$ 1.47 (73 – 78)
stylet length	15 $\pm$ 0.88 (14 – 17)	15 $\pm$ 1.33 (14 – 18)	16 $\pm$ 1.73 (12 – 18)
Tail length	20 $\pm$ 4.21 (12 – 25)	23 $\pm$ 2.25 (19 – 27)	22 $\pm$ 3.48 (16 – 29)
Body diameter at lip region	6 $\pm$ 0.67 (5 – 7)	6 $\pm$ 0.70 (5 – 7)	6 $\pm$ 0.63 (5 – 7)
at mid body	17 $\pm$ 1.27 (15 – 19)	20 $\pm$ 2.85 (17 – 25)	19 $\pm$ 3.04 (14 – 25)
at anus	12 $\pm$ 2.92 (8 – 17)	12 $\pm$ 2.40 (8 – 15)	11 $\pm$ 2.28 (8 – 16)

Results and discussion

Morphometrics of females are presented in Table 1. Female cylindrical body more or less straight to slightly ventrally arcuate when killed by heat. Posterior part of the body narrows behind the vulva. Head bluntly rounded to flattened; slightly offset from body. Vulva a transverse slit the lips of which tend to protrude a little. Tail bluntly rounded. Stylet short without basal knobs. Procorpus cylindrical, metacarpus well developed, rectangular to oval with large valve. Metoacoprus fills whole body diameter. Metacoprus is a typical distinguishable character of the genus. Lateral fields wide in mid-body region with 12 – 14 incisures, decreasing in number toward the extremities. Nerve ring surrounds oesophageal isthmus half a bulb-length behind the median bulb. Esophagus lumen widens into intestine. Dorsal lobe of esophagus usually well developed. Monodelphic ovary anteriorly outstretched. Post-vulval sac reaching about half way from the vulva to anus. Morphometrics of females of *A. avenae* from three localities are similar to each other. They are in close agreement with the topotypes described by Hooper (1974) except ratio 'b' which is shorter in the Czech population (4.4 vs 5.9).

Six individuals (two per populations) from three localities were used for sequencing 28S and 18S genes of ribosomal DNA. Identical sequences were obtained from six individual. The representative sequences were deposited in National Center for Biotechnology Information (NCBI) and their accession numbers are JQ348399 (18S gene) and JQ348400 (28S expansion segments). Head and tail shape

of the individual nematodes used for sequencing from locality Prague is given in Figure 1. Sequences of this population were deposited in GenBank. The obtained sequences were compared by Basic Local Alignment Search Tool (BLAST) and the 18S sequence showed 1692/1697

Fig. 1. Anterior and posterior regions of specimens from locality Prague used for sequencing. A, B: first specimen, C, D: second specimen

nucleotides identity to *A. avenae* accession number AB368918 (Okada and Kadota, 2003) and 28S gene showed 721/728 identities also to *A. avenae* accession number AB368536 (Kanzaki *et al.*, 2008).

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