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Orijinal araştırma (Original article)

Molecular characterization of cereal cyst nematodes from the South Anatolian Region in Turkey using ITS-rDNA sequences

Güney Anadolu Bölgesi Tahıl kist nematodu popülasyonlarının ribozomal DNA'nın ITS bölgesi sekanslanarak moleküler karakterizasyonu

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Summary

The *Heterodera avenae* group includes 12 species feeding on roots of cereals. Three species, *Heterodera avenae* Wollenweber, 1924, *Heterodera filipjevi* (Madzhidov, 1981) Stelter, 1984 and *Heterodera latipons* Franklin, 1969 are among the most economically important cyst nematode pests of cereals cultivated in different parts of Turkey. In this study, forty seven cereal cyst nematode isolates collected from cereal growing areas of the South Anatolian Region of Turkey (Hatay, Kahramanmaraş, Gaziantep, Kilis, Mardin and Adıyaman), were identified by using sequence analysis of the Internal Transcribed Spacer region of the ribosomal DNA (ITS-rDNA). Based on phylogenetic analysis using ITS-rDNA sequences, *H. avenae*, *H. filipjevi*, *H. latipons* and *Heterodera ciceri* Vovlas, Greco & Di Vito, 1985 were identified. 76,5 % of the isolates were characterized as *H. latipons*, 13 % *H. filipjevi*, 8,5 % *H. avenae* and 2 % *H. ciceri*.

Key words: *Heterodera avenae, Heterodera filipjevi, Heterodera latipons,* phylogenetic analysis

Özet

Tahıl kist nematodlarının (*Heterodera avenae* group) buğday köklerinde beslenen 12 farklı türü bilinmektedir. Türkiye'de buğday yetiştiriciliğinin yapıldığı farklı alanlarda ekonomik olarak önemli üç tür, *Heterodera avenae* Wollenweber, 1924, *Heterodera filipjevi* (Madzhidov, 1981) Stelter, 1984 ve *Heterodera latipons* Franklin, 1969 bulunmaktadır. Bu çalışmada Güneydoğu Anadolu Bölgesi (Hatay, Kahramanmaraş, Gaziantep, Kilis ve Mardin) buğday alanlarından toplanan 47 adet Tahıl kist nematodu popülasyonuna ait ribozomal DNA'nın transkripte olmayan (ITS-rDNA) bölgesi sekanslanarak moleküler teşhisi yapılmıştır. Filogenetik analiz sonuçlarına göre, *H. avenae*, *H. filipjevi*, *H. latipons* ve *H. ciceri* Vovlas, Greco & Di Vito, 1985 tanımlanmıştır. Çalışmada kullanılan nematod popülasyonunun % 76,5'i *H. latipons*, %13'ü *H. filipjevi*, % 8,5'i *H. avenae* ve % 2'si *Heterodera ciceri* olarak saptanmıştır.

Anahtar sözcükler: *Heterodera avenae, Heterodera filipjevi, Heterodera latipons,* filogenetik analiz

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Introduction

The sedentary cereal cyst nematodes (*Heterodera avenae* group) have a global distribution and cause significant economic yield losses in many countries of the world, in particular where rainfed cereal production is practised (Nicol et al., 2004). The cereal cyst nematode (CCN) complex is a group of 12 valid species, with *Heterodera avenae* Wollenweber, 1924*, Heterodera filipjevi* (Madzhidov, 1981) Stelter, 1984 and *Heterodera latipons* Franklin 1969 considered as the most economically important species in West Asia, North Africa and Mediterranean countries (Nicol & Rivoal, 2008). *Heterodera avenae* is the most studied CCN species. It has several pathotypes and is found predominately on temperate cereals (Nicol & Rivoal, 2008). Recent surveys of cereal fields in the Southeast Anatolian Plateau (SEA) and the Central Anatolian Plateau (CAP) of Turkey showed that the three species are widely distributed in the major wheat and barley cultivating areas, with *H. filipjevi* being common in CAP, while *H. avenae* and *H. latipons* are dominant in the SEA (Abidou et al., 2005; İmren et al., 2010). Yield losses caused by CCN have been reported by Nicol (2002) ranged from 15 to 20 % in wheat in Pakistan, 40 to 92 % in wheat and 17 to 77 % in barley in Saudi Arabia, and 20% in barley and 23 to 50 % in wheat in Australia. Preliminary yield loss studies indicated yield losses due to *H. filipjevi* reaching 50 % in common in Central Anatolian Region in Turkey (Nicol et al., 2005).

The identification of *Heterodera* species using morphological and morphometrical characteristics is time consuming and requires great skill and training by the observer. However, the analysis of coding and non-coding regions of ribosomal DNA (rDNA) has become a preferred way for nematode identification (Vrain et al., 1992; Wendt et al., 1993; Zijlstra et al., 1997). The internal transcribed spacer region (ITS) is variable and therefore useful for nematode identification and phylogenetic studies at species level. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) and sequencing based on ITS-regions of the rDNA repeat units have proven to be reliable tools for quick and precise identification of many cyst nematode species and subspecies (Bekal et al., 1997; Subbotin et al., 1999, 2000; Rivoal et al., 2003; Madani et al., 2004; Abidou et al., 2005; Smiley et al., 2008).

The objective of this study was to identify cereal cyst nematode populations (*Heterodera avenae* group species) originating from the South Anatolian Region (Hatay, Kahramanmaras, Gaziantep, Kilis, Mardin and Adıyaman) of Turkey. This was done based on sequencing of the ITS-rDNA region, by comparing the sequences obtained from the populations with those available in the GenBank database, and by determination of the phylogenetic relationship between these populations and reference populations.

Materials and Methods

CCN populations

Forty six populations of the *H. avenae* group and *Heterodera ciceri* were analyzed in this study from major barley and wheat-growing areas in six provinces in different locations in the South Anatolian region of Turkey (Table 1). Two kg of soil was randomly collected from each visited field; and soil samples were processed using a Kort elutriator (Kort, 1960). Extracted cysts were caught on a 250-µm-pore sieve, picked up with a brush while using a stereomicroscope, air dried and kept at room temperature for molecular work.

DNA extraction

Of each *Heterodera* population, one to five juveniles from a single cyst were transferred to a drop of water on a glass slide. Juveniles were cut into two or more pieces using a sterilized scalpel. A suspension of 8 µl consisting of the nematode fragments in water was transferred to a 0.2 ml Eppendorf tube containing 10 µl worm lysis buffer (final concentration: 50 mM KCl, 10 mM Tris-Cl pH 8.3, 1.5 mM $MgCl_2$, 1 mM DTT, 0.45% Tween 20) and 2 µl of proteinase K (600 µg/ml). The tubes were stored at -70ºC for at least 10 min. After deep freezing, the lysate was incubated in a thermocycler (PTC-200 Biozym, Landgraaf, the Netherlands) at 65ºC for 1 h. The tubes were incubated for 10 min at 95ºC to denature the proteinase K and subsequently centrifuged for 2 min at 13,000 rpm to collect the supernatants (extracted DNA). Finally, the extracted DNA was cooled to 4ºC and stored at -20ºC. Alternatively, DNA was extracted by transferring 1 cyst to a 0.5 ml Eppendorf tube containing 40µl ddH_2O. The cyst was broken manually with a pestle and the contents were further crushed using a micro-vibromixer for 30 s. Before incubation, as for the DNA extraction for juveniles, 50µl of worm lysis buffer (see earlier) and 10µl proteinase K (600 µg/ml) were added to each tube for a total volume of 100 µl. Incubation and further procedures were the same as for juveniles (Subbotin et al., 2003; Tanha et. al., 2003).

PCR amplification

PCR amplification of the ITS-rDNA region was done in 50µl reaction volume containing 23 µl ddH2O, 25µl 2X DreamTaq PCR Master Mix (Fermentas Life Sciences, Germany), 1µM of each forward primer (5'-CGTAACAAGGTAGCTGTAG-3') and reverse primer (5'-TCCTCCGCTAAATGATATG-3') (Ferris et al., 1994), and 1µl of DNA-extract. The PCR thermal cycler program consisted of 5 min at 95°C; 40 cycles of 94°C for 30 s, 48 °C for 45 s and 72°C for 45 s; followed by a final extension step of 8 min at 72 °C. After PCR amplification, 5 µl of each PCR product was mixed with 1 µl of 6X loading buffer (Fermentas Life Sciences, Germany) and loaded on a 1.5% standard TAE buffered agarose gel. After electrophoresis (100 V for 40 min), the gel was stained with ethidium bromide (0.1 µg/ml) for 15 min, and visualized and photographed under UV-light. The remaining PCR product was stored at –20°C until used for electrophoresis (Subbotin et al., 2003; Tanha et. al., 2003).

Purification and cloning of the PCR products

The remainder of the PCR-product was loaded on a 1 % agarose gel in 1X TAE buffer. Electrophoresis was done until the DNA band of interest separated from adjacent contaminating fragments, primer-dimers and leftovers. The bands were visualized by staining the gel with 0.003% ethidium bromide (0.02 µg/ml) for 10 min. Agarose gel slices with the DNA bands of interest were cut from the gel on a UV light box by using a sterile scalpel and placed in 1.5 ml microcentrifuge tubes. The PCR-product was purified from the gel slice according to the manufacturer's instructions (Wizard SV Gel and PCR Clean-Up System, Promega). Before cloning, the concentration of the purified PCR-product was determined using a UV-spectrophotometer (Nanodrop-1000) (Subbotin et al., 2003; Tanha et. al., 2003).

Fifty nanograms of PCR-product was ligated into pGEM-T vectors and cloned into JM109 high efficiency competent cells, according to the instructions provided by the manufacturer (Protocols and Applications Guide, The Source for Discovery, 3th edition, Promega). After cloning, 3 individual white colonies were removed and re-cultured at 37ºC in tubes containing liquid LB at 37ºC while shaking (150 rpm) overnight. PCR was done using pGEMT-vector primers (SP6 and T7) on each cultured colony to check whether the PCR-product was ligated into the vector and transformation was successful. The PCR

products were checked on an agarose gel after electrophoresis, as described before. Only the colonies which produced a PCR band of approximately 1.2 kb (PCR product of approximately 1 kb + part of the vector approximately 200 bp) were used for sequencing. Before sequencing, the plasmid DNA was extracted from the transformed bacteria cells by following the manufacturer's instructions as described in the PureYield Plasmid Miniprep System (Promega). The micro centrifuge tube containing the eluted DNA was stored at -20°C. The concentration of the purified plasmid-DNA was determined using a UV-spectrophotometer (Nanodrop-1000) (Subbotin et al., 2003; Tanha et. al., 2003).

Sequencing

All samples were sent to a sequencing service (Macrogen, South-Korea). Due to cycle sequencing and electrophoresis limitations, only part of the PCR-product can be sequenced. Therefore, from all the samples, both DNA-strands were sequenced by using one of the vector primers, to be able to construct the complete sequence of the cloned PCR products which are longer than 1 Kb. The software-package Chromas version 1.45 (Technelysium, Helensvale, QLD, Australia) was used to visualize the sequences on the computer. Both sequences from the same sample (forward sequence and inverted complementary of the reverse sequence) were exported together in one text file. After removing the overlapping part in one of the sequences and the sequence parts belonging to the plasmid in both sequences, one complete sequence was generated for each.

Phylogenetic analysis

For identification purposes, an alignment using ClustalX 2.0 (Thompson et al., 1997) was constructed of all our sequences, together with representatives of all species of the *H. avenae* group, plus some other *Heterodera* groups available in GenBank. As with Madani et al. (2004), *Cryphodera brinkmani* (AF274418) and *Meloidodera alni* (AF274419) were also incorporated in the phylogenetic analysis. For phylogenetic analysis; *H. latipons* (Iran, AF498382), *H. hordecalis* (Estonia, AY692356), *H. filipjevi* (Iran, AY148404), *H. ustinovi* (Belgium, AY148407), *H. pratensis* (Russia, AY148351), *H. australis* (Australia, AY148396), *H. mani* (United Kingdom, AY692357), *H. avenae* (India, AF274397), *H. aucklandica* (United Kingdom, AY148380), *H. arenaria* (United Kingdom, AF274396), *H. humuli* (Iran, AF498384), *H. schachtii* (Morocco, AY166436), *H. ciceri* (Syria, AY045758), *H. goettingiana* (Iran, AF498374) and *H. filipjevi* (Turkey) were used. The alignment was imported into MEGA5 and after checking 24 different nucleotide substitution models, the model with the lowest BIC score (Bayesian Information Criterion) and HKY+G (Hasegawa-Kishino-Yano with Gamma distribution), was retained for constructing a 60% consensus Maximum Likelihood tree, and bootstrap values were calculated with 100 replicates (Figure 2). The analysis involved 64 nucleotide sequences with a total of 771 positions in the final dataset.

Results and Discussion

Forty seven populations of cysts collected from six provinces in the South Anatolian Region of Turkey were identified, based on sequences of their ITS-rDNA region (Table 1).

Results showed that 76,5 % of the isolates could be characterized as *H. latipons*, 13 % as *H. filipjevi*, 8,5 % as *H. avenae* and 2 % as *H. ciceri*. *Heterdera avenae*, *H. filipjevi* and *H. latipons* samples contained cysts belonged to the *H. avenae* group, except *H. ciceri* (sample 28). Cysts in this sample were identified as *H. ciceri,* which represents a first record on chickpea in Adıyaman province in the South Anatolian region. *H. filipjevi* was found in a limited area (Elbistan-Kahramanmaras) and in one sample in

Şenyurt-Mardin province. *H. latipons* was predominantly found in Hatay, Gaziantep, Kilis and Mardin provinces in the same region. *H. avenae* was found in only Kilis, Hatay and Mardin.

Table 1. Origin and host plant of populations of *Heterodera* spp. in South Anatolia, Turkey, as well as their identification to species level, based on sequencing of the ITS-rDNA region

Samples	Location	Province	Host	Species
1	Karkamış- Soylu	Gaziantep	Barley	*H. latipons*
2	Karkamış- Kıvırcık	Gaziantep	Barley	*H. latipons*
3	Karkamış- Türkyurdu	Gaziantep	Wheat	*H. latipons*
4	Karkamış- Sınır	Gaziantep	Wheat	*H. latipons*
5	Karkamış- Akçaköy	Gaziantep	Wheat	*H. latipons*
6	Karkamış- K.Eşme	Gaziantep	Wheat	*H. latipons*
7	Karkamış- Arıkdere	Gaziantep	Barley	*H. latipons*
8	Oğuzeli - Yeniköy	Gaziantep	Barley	*H. latipons*
9	Oğuzeli – Devehöyüğü	Gaziantep	Barley	*H. latipons*
10	Oğuzeli – Beskılıc	Gaziantep	Barley	*H.latipons*
11	Oğuzeli - Karaman	Gaziantep	Barley	*H.latipons*
12	Elbeyli- Akıncı	Kilis	Barley	*H. latipons*
13	Elbeyli- İnanlı	Kilis	Barley	*H. latipons*
14	Elbeyli- Yağızköy	Kilis	Wheat	*H. latipons*
15	Elbeyli- Ardıçlı	Kilis	Wheat	*H. latipons*
16	Elbeyli- Beşiriye	Kilis	Wheat	*H. avenae*
17	Elbeyli- Çıldıroba	Kilis	Wheat	*H. latipons*
18	Elbeyli- Alahan	Kilis	Wheat	*H. latipons*
19	Elbeyli- Sınır	Kilis	Wheat	*H. latipons*
20	Reyhanlı-Beşaslan	Hatay	Wheat	*H. latipons*
21	Reyhanlı-Sınır	Hatay	Barley	*H. avenae*
22	Reyhanlı-Hacıpaşa	Hatay	Barley	*H. latipons*
23	Reyhanlı-Av suyu	Hatay	Barley	*H. latipons*
24	Reyhanlı-Bohşin	Hatay	Wheat	*H. latipons*
25	Merkez-İmece	Hatay	Wheat	*H. latipons*
26	Merkez -1	Adıyaman	Chickpea	*H. latipons*
27	Merkez -2	Adıyaman	Wheat	*H. latipons*
28	Kahta	Adıyaman	Chickpea	*H. ciceri*
29	Elbistan Büyükyapalak-1	Kahramanmaras	Wheat	*H. filipjevi*
30	Elbistan Büyükyapalak-2	Kahramanmaras	Wheat	*H. filipjevi*
31	Elbistan Küçükyapalak-1	Kahramanmaras	Wheat	*H. filipjevi*
32	Elbistan Küçükyapalak-2	Kahramanmaras	Wheat	*H. filipjevi*
33	Elbistan Merkez	Kahramanmaras	Wheat	*H. filipjevi*
34	Çağlayancerit	Kahramanmaras	Wheat	*H. latipons*
35	Nusaybin-Akıncı	Mardin	Barley	*H. avenae*
36	Nusaybin-Sulak	Mardin	Wheat	*H. latipons*
37	Nusaybin-Kızıltepe-1	Mardin	Wheat	*H. latipons*
38	Nusaybin- Kızıltepe-2	Mardin	Barley	*H. latipons*
39	Nusaybin- Kızıltepe-3	Mardin	Barley	*H. avenae*
40	Nusaybin- Kızıltepe-4	Mardin	Wheat	*H. latipons*
41	Afşin-Merkez	Kahramanmaras	Wheat	*H. filipjevi*
42	Şenyurt-Ataköy	Mardin	Wheat	*H. latipons*
43	Şenyurt-Sınır	Mardin	Wheat	*H. latipons*
44	Şenyurt-Şenyurt Batı	Mardin	Wheat	*H. latipons*
45	Şenyurt – Merkez	Mardin	Wheat	*H. latipons*
46	Şenyurt – Merkez	Mardin	Wheat	*H. latipons*
47	Karkamış- Öncüler	Gaziantep	Wheat	*H. latipons*

DNA extraction from some of the juveniles of the cyst resulted in useful DNA l for PCR. This first method has the advantage of using only part of the cyst's content. In this way, juveniles from the same cyst can still be used for other purposes like morphological identification or culturing. The second method is preferable when no experiments, except molecular ones, are planned; it uses the whole cyst and produces a higher amount of DNA. Moreover, the latter method is faster, as no juveniles need to be separated from the cyst. Either way, PCR resulted in fragments of approximately 1kb (Figure 1) which could be purified and used for cloning and sequencing.

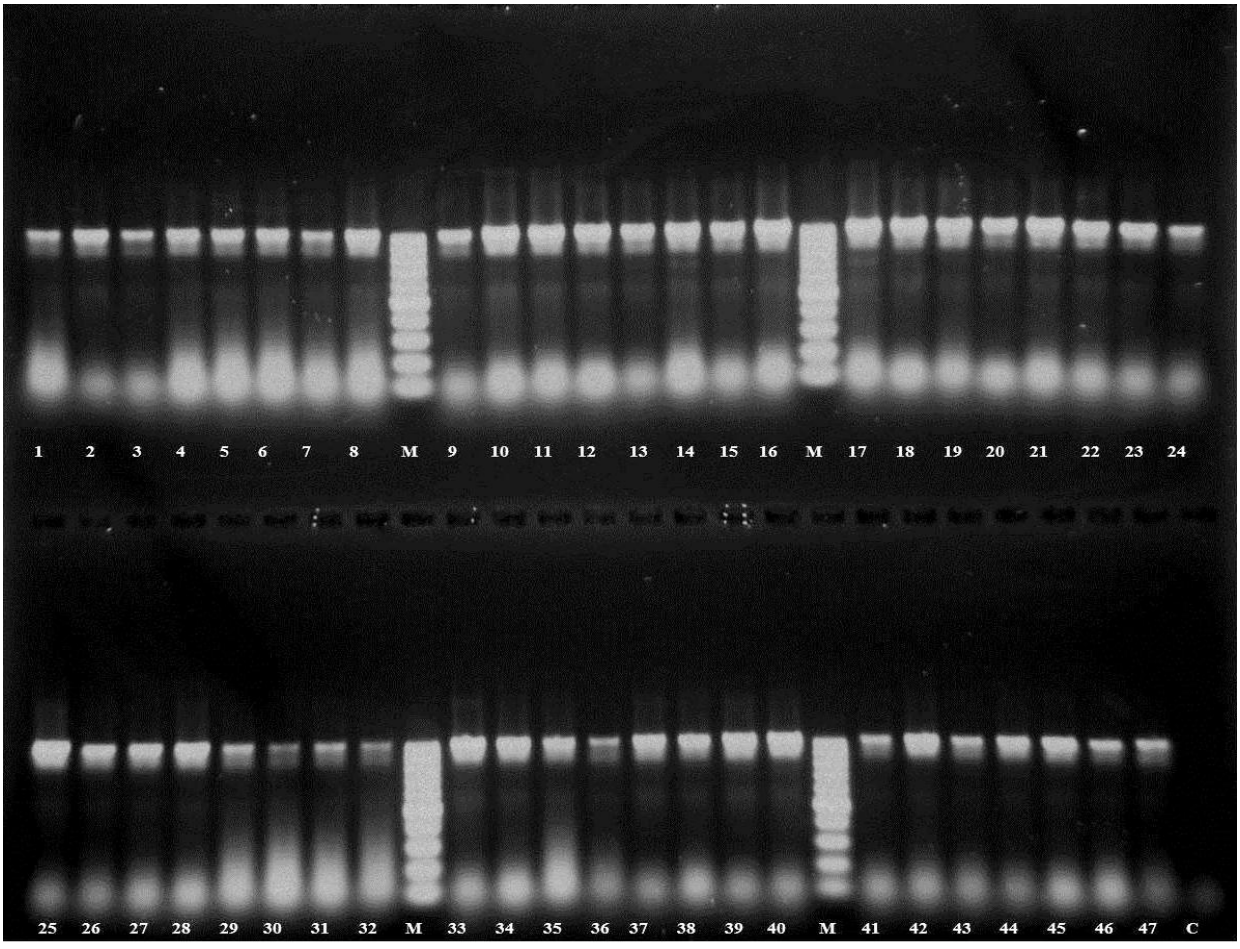

Figure 1. The PCR products of juveniles of 47 populations with 1200 bp DNA bands. M: 100 bp DNA ladder, C: Negatif Control. The codes correspond with those in Table 1.

Based on phylogenetic analysis using ITS-rDNA sequences, the populations from Hatay were identified as *H. avenae* and *H. latipons,* those from Gaziantep as *H. latipons,* those from Kilis as *H. avenae* and *H. latipons,* from Kahramanmaras as *H. filipjevi* and *H. latipons*; from Mardin as *H. avenae* and *H. latipons,* and from Adıyaman as *H. latipons* and *H. ciceri*.

Most of the samples formed distinct groups with one of the *Heterodera* sequences included from GenBank, supported by a moderate to high bootstrap value. Population 28 was most closesly related to *H. ciceri* (bootstrap value 67%). Populations 31, 32, 33, 34 and 44 grouped together with *H. filipjevi* (bootstrap value 97%). Populations 16, 21, 35, 38, 39? and 42 clustered together with *Heterodera arenaria* Cooper, 1955, *H. avenae, Heterodera aucklandica* Wouts & Sturhan, 1995 and *Heterodera mani* Mathews, 1971 (bootstrap value 62%). All other samples were grouped together with *H. latipons* (bootstrap value 96%).

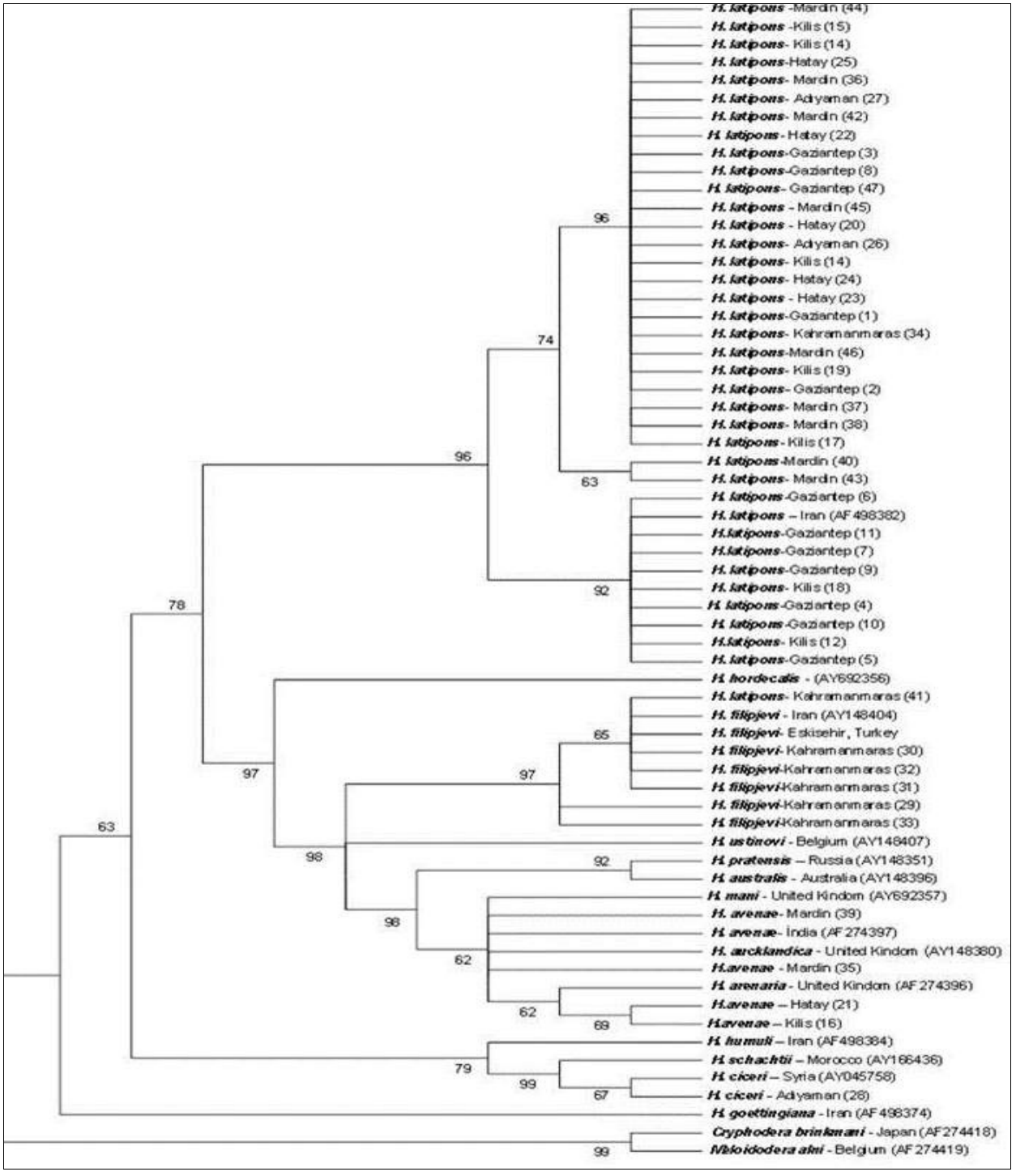

Figure 2. Phylogenetic relationships between nematodes inferred from partial nucleotide sequences of the acting gene using maximum parsimony. Populations from South Anatolia are designated with a code described in Table 1.

The 'latipons'-group was distinct from the other *H. avenae* group representatives, supported by bootstrap values of 96 and 97%, respectively. Wouts and Sturhan (1995) considered *H. latipons* and *Heterodera hordecalis* Andersson, 1975r as a separate species complex within the *H. avenae* group. Our findings suggest a separate 'latipons'-group as *H. hordecalis* seems to be more closely related to the

other members of the avenae group than to the 'latipons'- group. Adding more GenBank populations of *H. latipons* and *H. hordecalis* that were originally isolated from different geographical sources did not change the overall topology of the tree (data not shown). The 'latipons'-group further displays three subgroups supported by bootstrap values of 96, 63 and 92%. However, these groups do not correspond with host or geographical location. Some of the *H. latipons* and *H. filipjevi*, samples could not be identified; indisputably 16, 21, 38 and 42 grouped together with different *Heterodera* species. These species, with the exception of *H. avenae,* have never been reported from the South Anatolia region of Turkey. As expected, most of the samples belong to the *H. avenae* group and it is clear that *H. latipons* is the dominant species. Only one sample (28) falls outside the avenae group and is probably *H. ciceri*. This would be the first record on chickpea in Adıyaman province in the South Anatolian region although morphological studies should also be performed.

CCN is the most important pathogen of wheat and other cereals in Turkey and it has adverse effects on the production and quality of wheat. Three species of cyst-forming nematodes belonging to the *Heterodera avenae* group were identified from cereal fields in Turkey; *H. avenae*, *H. filipjevi* and *H. latipons. H. filipjevi* and *H. latipons* are the most prevalent species in cereal fields (Abidou et al., 2005; İmren et al., 2010; 2011). *H. avenae* was reported from Turkey by Yüksel (1973), Subbotin et al. (2003) and Abidou (2005) by using only single samples for identifications. *H. latipons* is widespread in the South Anatolian region, while *H. filipjevi* is present in most wheat growing areas of the Central Anatolian Plateau (İmren et al., 2011; Şahin et al., 2009). This study highlights the ecoregional distribution of CCN in the South Anatolian region. *H. filipjevi* was predominantly found at higher altitudes in these regions, *H. avenae* was predominantly found in the eastern Mediterranean region, while *H. latipons* was the most prevalent species in the spring wheat area of the South Anatolian region of Turkey. Further comprehensive surveys in these regions are needed, such as mapping population densities in order to define potential economic damage rates and determining more accurately the distribution patterns of CCN.

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References

Abidou, H., A. El-Ahmed, J. M. Nicol, N. Bolat, R. Rivoal & A. Yahyaoui, 2005. Occurrence and distribution of species of the *Heterodera avenae* group in Syria and Turkey. Nematology Mediterranean, 33: 197-203.

Bekal, S., J. P. Gauthier & R. Rivoal, 1997. Genetic diversity among a complex of cereal cyst nematodes inferred from RFLP analysis of the ribosomal internal transcribed spacer region. Genome, 40: 479–486.

Ferris, V. R., J. M. Ferris, J. Faghihl & M. Ireholm, 1994. Comparisons of isolates of *Heterodera avenae* using 2-D PAGE protein patterns and ribosomal DNA. Journal of Nematology, 26: 144-151.

İmren, M., A. Özarslandan, H. Toktay, A. Öcal, İ. H. Elekçioğlu, A. Dababat & J. Nicol, 2010. Preliminary survey study on *Heterodera* species in some provinces of Southeast Anatolia region of Turkey (19-23 September, Vienna, 2010). Proc. of 30th European Society of Nematologist, 68 pp.

İmren, M., H. Toktay, A. Özarslandan, A. Öcal & İ. H. Elekçioğlu, 2011. Doğu Akdeniz Bölgesi buğday alanlarında Tahıl kist nematodu (*Heterodera avenae* group) türlerinin belirlenmesi. Türkiye IV. Bitki Koruma Kongresi 28-30 Haziran 2011- Kahramanmaraş. s 10.

Kort, J., 1960. A technique for the extraction of *Heterodera* cysts from wet soil and for the estimation of their egg and larval content. Verslagen en Medelingen Plantenziektenkundige Dienst. Wageningen. Netherlands, 233: 3-7.

Madani, M., N. Vovlas, P. Castillo, S. A. Subbotin & M. Moens, 2004. Molecular characterization of cyst nematode species (*Heterodera* spp.) from the Mediterranean basin using RFLPs and sequences of ITS-rDNA. Journal of Phytopathology, 152: 229-234.

Nicol J. M., 2002. Important nematode pests. Pp. 345–366 *in* B. C. Curtis, S. Rajaram, and H. Gomez Macpherson, eds. Bread wheat: Improvement and production. Rome: FAO Publisher.

Nicol, J., R. Rivoal, S. Taylor & M. Zaharieva, 2004. Global Importance of Cyst (*Heterodera spp*.) and Lesion Nematodes (*Pratylenchus spp*.) on Cereals: yield loss, population dynamics, use of host resistance and integration of molecular tools. Nematology Monographs and Perspectives 2: 233-251.

Nicol J. M., N. Bolat, A. Bağcı, R. T. Trethowan, M. William, H. Hekimhan, A. F. Yıldırım, E. Şahin, İ. H. Elekcioğlu, H. Toktay, B. Tunalı, A. Hede, S. Taner, H. J. Braun, M. Van Ginkel, M. Keser, Z. Arısoy, A. Yorgancılar, A. Tülek, D. Erdurmuş, O. Büyük & M. Aydoğdu, 2005. The international breeding strategy for the incorporation of resistance in bread wheat against the soil borne pathogens (Dryland Root Rot and Cyst and Lesion Nematodes) Using Conventional and Molecular Tools. 7th International Wheat Congress, 2005, Mardel Plata, Argentina.

Nicol J. M & R. Rivoal, 2008. Global knowledge and its application for the integrated control and management of nematodes on wheat. In 'Integrated management and biocontrol of vegetable and grain crops nematodes.' (Eds A Ciancio, KG Mukerji) pp. 243-287. (Springer Academic Publishing: The Netherlands)

Rivoal R., S. Valette, S. Bekal, J. P. Gauthier & A. Yahyaoui, 2003. Genetic and phenotypic diversity in the graminaceous cyst nematode complex, inferred from PCR-RFLP of ribosomal DNA and morphometric analysis. European Journal of Plant Pathology, 109: 227–241.

Smiley R. W, G. P Yan & Z. A. Handoo, 2008. First record of the cyst nematode *Heterodera filipjevi* on wheat in Oregon. Plant Disease, 92: 1136.

Subbotin, S. A., L. Waeyenberge, I. A. Molokanova & M. Moens, 1999. Identification of *Heterodera avenae* group species by mophometrics and rDNA-RFLPs. Nematology, 1: 195-207.

Subbotin, S. A., L. Waeyenberge & M. Moens, 2000. Identification of cyst forming nematodes of the genus *Heterodera* (Nematoda: Heteroderidae) based on the ribosomal DNA-RFLP. Nematology, 2: 153-164.

Subbotin, S. A., D. Sturhan, H J. Rumpenhorst & M. Moens, 2003. Molecular and morphological characterization of the *Heterodera avenae* species complex (Tylenchida: Heteroderidae). Nematology 5: 515-538.

Şahin, E., J. M. Nicol, İ. H. Elekcioğlu, Ö. Yorgancılar, A. F. Yıldırım, A. Tülek, H. Hekimhan, A. Yorgancılar, A. T. Kılınç, N. Bolat & G. Erginbaş-Orakçı, 2009. Frequency and diversity of cereal nematodes on the Central Anatolian Plateau of Turkey. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT. Riley, J. M. Nicol, A. A. Dababat) pp. 100-105.

Tamura K., D. Peterson, N. Peterson, G. Stecher, M. Nei & S. Kumar, 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution (In Press).

Tanha, M.T., S. A. Subbotin, M. Moens., 2003. Molecular identification of cyst-forming nematodes (Heteroderidae) from Iran and a phylogeny based on ITS-rDNA sequences. Nematology 5, 99–111.

Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin & D. G. Higgins, 1997. The Clustal X Windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research, 25: 4876-4882.

Vrain, T. C., D. A. Wakarchuk, A. C. Le´vesque & R. I. Hamilton, 1992. Intraspecific rDNA restiction fragment length polymorphism in the *Xiphinema americanum* group. Fundamental and Applied Nematology, 15: 563–573.

Yüksel, H., 1973. Studies on the morphological and biological differences of *Heterodera* species (Nematoda: Heteroderidae) in Turkey. Atatürk Üniversity Ziraat Fakültesi Dergisi 4, pp. 53–71.

Wendt, K. R., T. C. Vrain & J. M. Webster, 1993. Separation of three species of *Ditylenchus* and some host races of *D. dipsaci* by restriction fragment length polymorphism. Journal of Nematology, 25: 555–563.

Wouts, W. M & D. Sturhan, 1995. *Heterodera aucklandica* sp. n. (Nematoda: *Heteroderidae*) from a New Zealand native grass, with notes on the species of the *H. avenae* group. New Zealand. Journal of Zoology 22: 199-207.

Zijlstra, C., J. B. Uenk & C. H. Silfhout, 1997. A reliable, precise method to differentiate species of root-knot nematodes in mixtures on the basis of ITS-RFLPs. Fundamental and Applied Nematology, 20: 59–63.