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RESEARCH ARTICLE

Occurrence, identification and phylogenetic analyses of cereal cyst nematodes (*Heterodera* spp.) in Turkey

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Abstract

Plant-parasitic nematodes are very common on cereal crops and cause economic losses *via* reduction in grain quality and quantity. During 2014, 83 soil samples were collected from wheat and barley fields in 21 districts of 13 provinces across five regions (Central Anatolia, Marmara, Aegean, Southeast Anatolia, and Black Sea Region) of Turkey. Cyst-forming nematodes were found in 66 samples (80%), and the internal transcribed spacer (ITS) sequencing and species-specific PCR identified the species in 64 samples as *Heterodera filipjevi*, *Heterodera latipons*, and *Heterodera avenae*. The predominant pathogenic cereal cyst nematode was *H. filipjevi*, which was found in all five regions surveyed. *H. avenae* was only detected in Southeast Anatolia whereas *H. latipons* was detected in Southeast Anatolia and Central Anatolia. ITS-rDNA phylogenetic analyses showed that *H. avenae* isolates from China clustered with *H. australis*, and Turkish isolates were closely related to European and USA isolates of this species. *H. filipjevi* from Turkey and China were clustered closely with those from the UK, Germany, Russia, and the USA. The density of many of these populations exceeded or approached the maximum threshold level for economic loss. To our knowledge, this is the first report of *H. filipjevi* in Diyarbakir, Edirne, and Kutahya provinces, and the first report of *H. avenae* in Diyarbakir Province. These results exhibit the most rigorous analysis to date on the occurrence and distribution of *Heterodera* spp. in Turkey's major wheat-producing areas, thus providing a basis for more specific resistance breeding, as well as other management practices.

Keywords: species specific PCR, cereal cyst nematode, molecular identification, ITS-rDNA, wheat pathogen

1. Introduction

The cereal cyst nematodes (CCNs, *Heterodera* spp.), first reported in 1874 in Germany, cause serious economic damage to cereal crops worldwide, especially in temperate regions (Rivoal and Nicol 2009). *Heterodera* species can reduce yields of wheat and barley, with *Heterodera avenae* Wollenweber, 1924, *Heterodera filipjevi* Madzhidov, 1981, *Heterodera latipons* Franklin, 1984, the three most

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economically important species (Smiley *et al.* 2009). The *H. avenae* complex group is comprised of eleven species: *H. arenaria*, *H. aucklandica*, *H. avenae*, *H. bifenestra*, *H. filipjevi*, *H. hordecalis*, *H. iri*, *H. latipons*, *H. mani*, *H. spinicauda*, and *H. turcomanica* (Subbotin *et al.* 1999; Subbotin *et al.* 2003). The most commonly reported species, *H. avenae*, was described as the oat cyst nematode, and is now found in more than 40 wheat-growing countries (Subbotin *et al.* 2010). In China, *H. avenae* has been shown to cause yield losses of 16–55% when population densities exceed 10 eggs g^{-1} of soil (Hou *et al.* 2012). *H. filipjevi* has been detected in 22 countries across Europe, Asia, the Middle East, and North America, whereas *H. latipons* has been detected in 27 countries throughout Europe, Asia, the Middle East, North America, and northern Africa (Subbotin *et al.* 2010). In Iran, the effect of *H. filipjevi* on wheat increased commensurately with increasing initial population (*Pi*) density: aerial shoot yields losses of 13, 21, 29, and 37%, and grain yield losses of 11, 31, 38, and 47% occurred at the *Pi* levels of 2.5, 5, 10, and 20 eggs and second-stage juveniles (J2s) g^{-1} of soil, respectively (Hajihasani and Hajihasani 2010).

Turkey is currently among the top ten wheat producers in the world, with a gross production of more than 22 million tons (http://faostat.fao.org/). *H. avenae* was first reported in Turkey by Yuksel (1973). *H. filipjevi* was subsequently also detected in Turkey in 1995 (Rumpenhorst *et al.* 1996), and found in 87% of the wheat growing areas of the Central Anatolian Plateau (CAP). Recent surveys of cereal fields in the CAP have shown that *H. filipjevi* is widely distributed in major areas of wheat and barley cultivation (Şahin *et al.* 2009), and causes average yield losses of 42% for rain-fed winter wheat grown in the CAP (Nicol *et al.* 2006). Sahin *et al.* (2009) reported that, in Turkey, the threshold density for economic impact by yield loss due to *H. filipjevi* may be in the range of 5 eggs g^{-1} of soil.

Heterodera species are traditionally identified by their cysts, as well as morphological and morphometric features of J2s, though this is difficult and requires experienced nematologists (Subbotin *et al.* 1999). The use of molecular techniques for identification is easier and more accurate, as DNA-sequence variation in the internal transcribed spacer (ITS) regions of ribosomal DNA can be used to clarify phylogenetic relationships and identify many nematode taxa (Subbotin *et al.* 2001). Similarly, the sequence characterized amplified region (SCAR) marker system can be used to rapidly detect *H. avenae* and *H. filipjevi* in mixed nematode samples with high accuracy and sensitivity (Qi *et al.* 2012; Peng *et al.* 2013). The primer set (Hlat-act), designed using Allele ID 7.73, was shown to be very specific to *H. latipons* (Toumi *et al.* 2013). SCAR-PCR amplification with species-specific primers has been used to identify several CCNs such as *H. glycines*, *H. latipons*, *H. schachtii*, *H. avenae*, and *H. filipjevi*, without requiring the subsequent restriction fragment length polymorphism (RFLP) procedure (Yan *et al.* 2013).

In this study, CCN populations from 13 provinces, over five regions in Turkey, were identified by the SCAR-PCR method, as well as by sequencing the ITS-rDNA regions. Using the ITS-rDNA sequence of the CCN populations — available in the GenBank — we performed a phylogenetic analysis comparing the Turkey CCN populations with the downloaded GenBank ITS sequences. This study aimed to: 1) survey wheat growing areas in Turkey to refine knowledge of the distribution of predominant CCN species; 2) identify CCN isolates to the species level using molecular tools; and 3) quantify cyst densities in each wheat growing area. Above all, this survey was designed to elucidate the current status of species distribution as a basis for developing CCN-management strategies.

2. Materials and methods

2.1. Nematode collection

Soil samples were taken from 83 sites in the major wheat and barley growing areas of 13 provinces (Konya, Uşak, Edirne, Kutahya, Eskisehir, Mardin, Diyarbakir, Sanliurfa, Kilis, Gaziantep, Bolu, Haymana, and Yozgat) of Turkey during August 2014. The areas surveyed covered the five major wheat-producing regions in Turkey: CAP, Marmara, Aegean, Southeast Anatolia, and the Black Sea region. One kilogram of soil was collected from each field by taking five samples in a zigzag pattern. Cysts were extracted from the soil using Cobb's sieving gravity method (Persmark *et al.* 1992). A 100-g subsample of soil was washed, and cysts were then collected with a brush. A stereomicroscope was then used to count the number of eggs from five gravid cysts. Five samples from the major wheat-producing areas in China (Beijing City and Henan, Qinghai and Shandong provinces) were used as controls.

The following equations were used to estimate the empty cyst rate and the frequency of the cyst occurrence:

Empty cysts rate (%)=Empty cysts/Total cysts×100

Frequency (%)=Samples with cysts/Total samples×100

Eggs in cysts of each sample are reported as the means and standard deviation of the mean. Data were evaluated using ANOVA to determine if number differed significantly among samples ($P \leq 0.05$).

2.2. DNA extraction

Nematode genomic DNA was extracted as described by Qi *et al.* (2012). Briefly, at least three full cysts from each subsample were transferred to a 0.2-mL microtube (one