RESEARCH/ INVESTIGACIÓN

MOLECULAR IDENTIFICATION AND PHYLOGENETIC DIVERSITY OF CEREAL CYST NEMATODES (*HETERODERA* SPP.) POPULATIONS FROM ALGERIA

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ABSTRACT

Mehalaine, K., M. Imren, G. Özer, M. Hammache, and A. A. Dababat. 2020. Molecular identification and phylogenetic diversity of cereal cyst nematodes (*Heterodera* spp.) populations from Algeria. Nematropica 50:134-143.

Cereal cyst nematodes (CCN), *Heterodera* spp., are the most devastating plant-parasitic nematodes of cereals causing serious global economic losses. In this study, surveys to investigate plant-parasitic nematodes associated with wheat were performed in twenty fields in twelve provinces of Algeria in 2018. Cereal cyst nematodes were found in 41.6% of the investigated wheat fields. Forty-eight CCN populations from twenty locations were obtained and morphologically classified. To confirm the morphological classification, the internal transcribed spacer (ITS) of rDNA was amplified with F194/F195 primers, sequenced, and analyzed using BLASTn searches of the NCBI database. Populations were classified as *Heterodera avenae*, *H. hordecalis*, *H. carotae*, and *H. cruciferae*. *Heterodera carotae* and *H. cruciferae* are reported in Algeria for the first time from two and three surveyed locations, respectively. *Heterodera carotae* and *H. cruciferae* were grouped into a well-supported clade and close to populations from Italy and the Netherlands in the phylogenetic tree, respectively. *Heterodera hordecalis* and *H. avenae* were found in ten and five fields, respectively. Based on phylogenetic analysis, *H. hordecalis* showed high similarity to the Israeli population, whereas *H. avenae* populations from Algeria were found to have high similarity to the Spanish population. Due to the variation among the Algerian populations of *H. hordecalis* and *H. avenae*, it can be assumed they have been introduced into Algeria multiple times.

Key words: Cereal cyst nematodes, *Heterodera* spp., phylogeny, wheat

RESUMEN

Mehalaine, K., M. Imren, G. Özer, M. Hammache, y A. Dababat. 2020. Identificación molecular y diversidad filogenética de poblaciones del nematodo del quiste de los cereales (*Heterodera* spp.) en Algeria. Nematropica 50:134-143.

Los nematodos del quiste de los cereales (NQC), *Heterodera* spp., son de los nematodos parasitos de plantas más devastadores en cereales, causando serias pérdidas económicas a nivel global. En este estudio, se investigaron los nematodos parásitos de plantas asociados a 20 campos de trigo en 12 provincias de Algeria en el 2018. Los nematodos del quiste de los cereales se encontraron en un 41,6% de los campos de

trigo investigados. Se obtuvieron 48 poblaciones de NQC procedentes de 20 localidades y se clasificaron morfológicamente. Para confirmar lo obtenido en la clasificación morfológica, la región espaciadora transcrita interna (ITS) del ADN ribosomal se amplificó con los imprimadores F194/F195, se secuenció y analizó por medio del BLASTn de la base de datos del NCBI. Por consiguiente, las poblaciones se clasificaron como *Heterodera avenae*, *H. hordecalis*, *H. carotae*, y *H. cruciferae*. *Heterodera carotae* y *H. cruciferae* se identificaron por primera vez en Algeria en dos y tres localidades, respectivamente. En el árbol filogenético, *Heterodera carotae* y *H. cruciferae* se agruparon en un clado con un alto respaldo, y cercano a poblaciones originarias de Italia y Holanda, respectivamente. *Heterodera hordecalis* y *H. avenae* se encontraron en diez y cinco campos de trigo, respectivamente. Basados en el análisis filogenético, *H. hordecalis* mostró una alta similitud con la población Israelí, mientras que las poblaciones de *H. avenae* de Algeria presentaron una alta similitud con la población Española. Debido a la variación entre las poblaciones de *H. hordecalis* and *H. avenae* procedentes de Algeria, se puede asumir que han sido introducidas al país multiples veces.
Palabras clave: Nematodo del quiste de los cereales, *Heterodera* spp., filogenia, trigo

INTRODUCTION

Cereal grains have been an integral part of human nutrition for thousands of years and have played an important role in establishing human civilization. Cereal crops account for more than 70% of the area used for food crops (Riley *et al*., 2009). In 2017, wheat (*Triticum aestivum* L.) was cultivated on more area than any other commercial crop with 218.4 million ha, and continues to be the most important food grain source for humans (FAOSTAT, 2020). Due to the increase in the world population, global demand for wheat is estimated at 860 million tons per year in 2025 (Marathee and Gomez-MacPherson, 2001). In Algeria, cereals are cultivated in semi-arid areas on over 3 million ha. In 2017, total cereal production yielded 3.5 million tons, which is 17% below the previous five-year average (2012-2016) and 5% above the 2016 average (FAOSTAT, 2020). Unfortunately, this amount is still insufficient to meet the demand of the growing population in Algeria. Cereal production is often faced with various biotic and abiotic constraints, such as soil nutrient deficiencies, insufficient irrigation facilities, as well as the incidence of diseases, insect pests, and plant-parasitic nematodes (PPNs) (Shroyer *et al*., 1990).

Among the biotic stress, PPNs generally are important factors associated with decreasing global agricultural yields (Brown, 1984; Nicol *et al*., 2002). The cereal cyst nematodes (CCN), of the genus *Heterodera*, are widespread and one of the most important groups of PPNs threatening global cereal production (Rivoal and Cook, 1993; Dababat and Fourie, 2018). The genus *Heterodera* includes 62 species attacking crops including cereals and grasses (Wouts and Baldwin, 1998; Yan and Smiley, 2009). Twelve species can affect the roots of cereal; however, three species (*Heterodera avenae*, *H. latipons*, and *H. filipjevi*) are the most economically important (Rivoal and Cook, 1993; Dababat and Fourie, 2018). *Heterodera avenae* is thought to be the most important nematode species limiting cereal production in North Africa (Rammah, 1994; Namouchi-Kachouri and B'Chir, 2005; Mokrini *et al*., 2009, 2017; Dababat *et al.*, 2015; Dababat and Fourie, 2018). This nematode was first reported in Algeria by Scotto La Massese (1962) and then by Lamberti *et al*. (1975). Moreover, the presence of this species in various cereal-growing areas of Algeria has also been noted in recent studies (Mokabli *et al*., 2001, 2002; Tirchi *et al*., 2016). More recently, *H. hordecalis* has also been shown to be present in cereal fields located in Batna, Setif, Tlemcen, and Tiaret provinces of Algeria for the first time (Smaha *et al*., 2018).

Morphometric and morphological characteristics are commonly used to identify *Heterodera* species, however, the determination of these characteristics is laborious, time-consuming and may be highly variable depending on conditions of culture and incubation. Molecular techniques based on the polymerase chain reactions (PCR) have been widely used to overcome these bottlenecks in nematode identification and have provided precise results (Toumi *et al*., 2013; Yan *et al*., 2013). Subbotin *et al*. (2001) indicated that the sequence variation in the internal transcribed spacer (ITS) region of ribosomal DNA provided useful information to identify many nematode taxa,

including CCN. To our knowledge, the description of Algerian CCN populations has only been conducted using morphological tools (Mokabli *et al*., 2001, 2002; Haddadi *et al*., 2013); consequently, there is no information on genetic traits of CCN populations in Algeria. Therefore, a comprehensive survey was performed in wheat-growing areas in northern Algeria to: i) determine the prevalence and distribution of CCN in the surveyed areas, ii) identify *Heterodera* species, and iii) evaluate the phylogenetic relationships among the *Heterodera* populations based on variation in ITS sequences.

MATERIALS AND METHODS

Nematode population collection

This study was conducted between mid-June and mid-August in 2018. Forty-eight soil samples from 12 provinces in Algeria were collected (Fig. 1). Soil samples were collected in a zigzag pattern with separation of 10-20 km distance from each location. At least 10 cores were taken per sample, then soil was mixed, and a representative of 1-kg sample was retained. The samples were taken with a soil auger to a depth of 20 cm. The Fenwick can technique was used to extract cysts from 250 g soil per sample (Fenwick, 1940). Cysts collected on a 250-µm sieve were picked by hand using a dissecting needle under a V20 stereo-binocular microscope (Zeiss, Jena, Germany). Collected cysts were surface disinfected with a 0.1% NaOCl solution for 5 min followed by triple rinsing with sterile ultra-pure water. Air-dried cysts were transferred into a 1.5 ml Eppendorf tube and stored at 4°C until further identification and molecular studies.

Molecular identification of nematodes

Cysts were crushed using a microhomogenizer (Vibro mixer El, Chemap AG, Switzerland) to release second-stage juveniles (J2) into 10 µl of double-distilled water. The J2 were homogenized using a micro-homogenizer, and then the entire lysate was transferred to a 1.5-ml Eppendorf tube. Ten µl of 1x PCR reaction mix [75 mM Tris-HCl (pH 8.8), 20 mM $(NH_4)_2SO_4$, 0.01% (v/v) Tween 20] and 2 µl of proteinase K (600

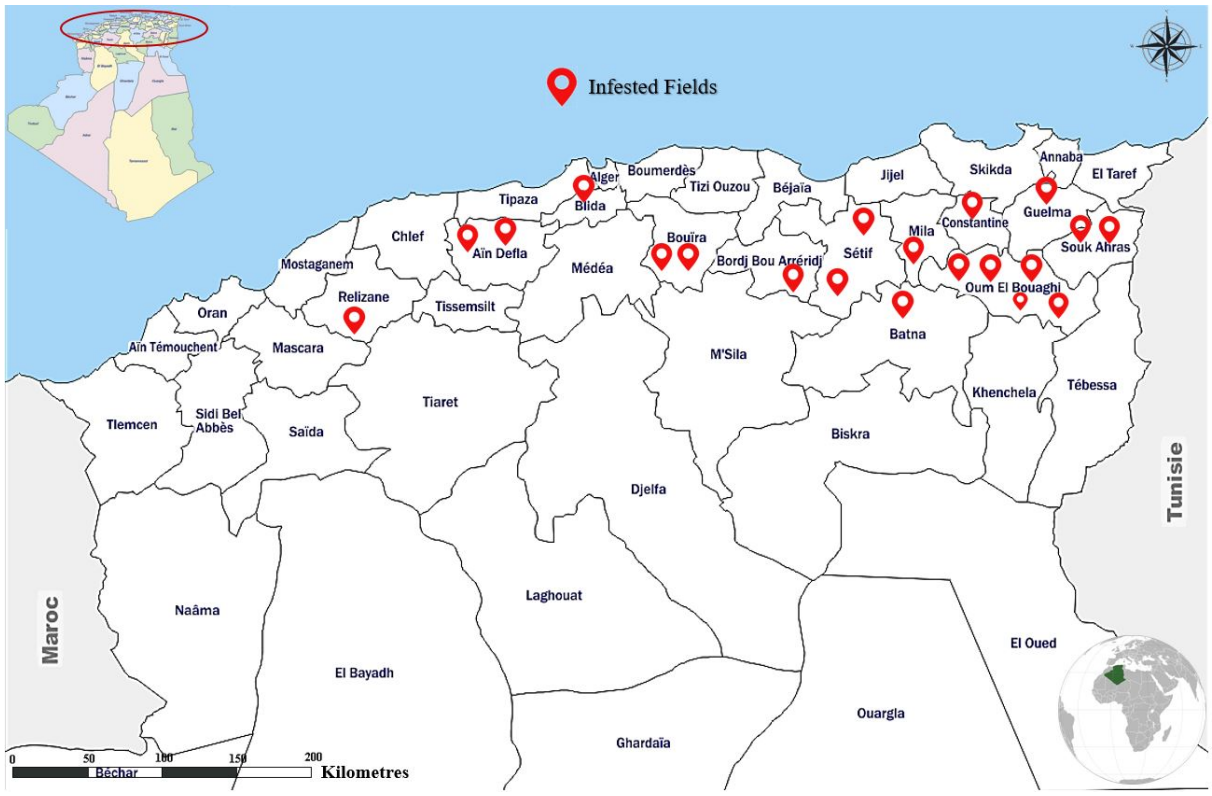

Figure 1. Map of northern Algeria showing the twelve provinces where the survey was conducted. The markers indicate the fields infested with *Heterodera* populations.

µg/ml; Qiagen GmbH, Hilden, Germany) were added to the lysate. The tube was incubated at 60°C for 30 min, and then 5 min at 95°C. The tube was centrifuged at 16,000 rpm for 1 min and the supernatant was carefully removed without disturbing the pellet, transferred to another Eppendorf tube, and stored at -20°C until further use.

PCR amplification was conducted with F194 (5′-CGT AAC AAG GTA GCT GTA G-3′) and F195 (5′-TCC TCC GCT AAA TGA TAT G-3′) primers described by Ferris *et al*. (1994) to amplify the ITS region including the ITS1, ITS2, and 5.8S ribosomal gene, as well as parts of the 18S and 28S ribosomal genes using a T100 thermal cycler (Bio-Rad, California, USA). The amplifications were carried out in a 25 µl final reaction volume including 2.5 µl of the supernatant, 200 µM of each dNTPs, 0.5 µM of each primer, 1x *Taq* reaction buffer, 1.5 mM $MgCl_2$ and 1 U Dream *Taq* DNA polymerase (Thermo Fisher Scientific, USA). The amplification conditions for the ITS region were 3 min for an initial denaturation step at 94°C, followed by 35 cycles with denaturation for 30 s at 94°C, annealing for 30 s at 60°C, an extension for 60 s at 72°C and a final extension at 72°C for 10 min. A negative control (no template DNA) was used to ensure that there was no contamination in the reaction mix. Following DNA amplification, 5 µl of each PCR product was mixed with 1 µl 6x loading dye and loaded in 1x TAE buffer (Sambrook *et al*., 1989) on a 1.2% agarose gel. After electrophoresis for 60 min at 100 V and 100 mA, the DNA fragments were stained with ethidium bromide (0.02 µg/ml) for 10 min. The gel was imaged using a G: BOX F3 gel doc system (Syngene, UK) after ethidium bromide staining to control for the presence of the fragments.

The amplified ITS fragments were purified using the QIAquick PCR purification kit (Qiagen GmbH, Hilden, Germany) and subjected to bidirectional sequencing by a commercial company (Macrogen, Inc., Seoul, Korea) using an ABI 3500xL Genetic Analyzer. The sequences were identified using the BLASTn algorithm on the NCBI website (https://www.ncbi.nlm.nih.gov/). The sequences derived from this study were deposited in the GenBank database with the Accession numbers shown in Table 1.

Phylogenetic analysis

The ITS sequences were used to explore intraspecific genomic variability of *Heterodera* species and to determine phylogenetic relationships among the collected populations and to representative *Heterodera* populations from different countries available in the GenBank

Table 1. Geographical locations of *Heterodera* species from northern Algeria with their accession numbers.

No.	Province	Location	Species	Accession No.
1	Oum Bouaghi	Oum Bouaghi	*H. hordecalis*	MK848400
2	Oum Bouaghi	Ain Melila 1	*H. hordecalis*	MK840641
3	Oum Bouaghi	Ain Melila 2	*H. hordecalis*	MK848401
4	Oum Bouaghi	Ain Melila 3	*H. hordecalis*	MK840638
5	Oum Bouaghi	Meskiana	*H. cruciferae*	MK848395
6	Batna	Seriana	*H. hordecalis*	MK840640
7	Constantine	Constantine a	*H. avenae*	MK848396
8	Bouira	Ain Bessem	*H. hordecalis*	MK848399
9	Bouira	El Asnem	*H. avenae*	MK840645
10	Bourdj Bou Ariridj	El Achir	*H. hordecalis*	MK840642
11	Ain Defla	Khmiss Meliana	*H. cruciferae*	MK848393
12	Ain Defla	Bordj Khaled	*H. hordecalis*	MK848402
13	Guelma	Guelma	*H. hordecalis*	MK840637
14	Mila	Tadjenanet 2	*H. carotae*	MK840644
15	Relizane	Relizane	*H. cruciferae*	MK848394
16	Souk Ahras	Merahna	*H. hordecalis*	MK840639
17	Souk Ahras	Merahna	*H. carotae*	MK840643
18	Algiers	Oued Smar	*H. avenae*	MK840646
19	Setif	Hammem Sokhna 1	*H. avenae*	MK848397
20	Setif	Hammem Sokhna 2	*H. avenae*	MK848398

database. A total of 24 nucleotide sequences were included in the phylogenetic analysis that was conducted using MEGA7 (Kumar *et al*., 2016). The evolutionary history was inferred using the neighbor-joining method (Saitou and Nei, 1987), based on evolutionary distances computed using the Tamura-Nei method (Tamura and Nei, 1993). Bootstrap support was calculated for all analyses using 1,000 replicates (Felsenstein, 1985).

RESULTS

Occurrence of Heterodera *spp. in Algeria*

Twenty fields (41.6%) out of the 48 cereal surveyed fields in 12 provinces were infested with cyst nematodes. The highest infestation rates were recorded from the following provinces; Bourdj Bou Ariridj (100%), Setif (67%), Oum Bouaghi, Relizane and Ain Defla (50%), Bouira (40%), Mila, Algiers, Batna, and Constantine (33.3%), and Souk Ahras (17%) (Fig. 1).

Full and empty cysts were found in several fields. CCNs were detected in most of the fields especially where wheat and barley monoculture is practiced such as in Setif, Oum Bouaghi, Relizane, Ain Defla, Bouira, Mila, Algiers, Batna, Constantine, and Souk Ahras provinces. The degree of infestation was variable depending on the field and the location within a field and ranged from 11 cysts/kg of soil in the field of Bordj Khaled in Ain Defla province to 128 cysts/kg of soil in the field of El Achir in Bourdj Bou Ariridj. The degree of infestation of soil samples for the surveyed location is shown in Figure 2. Bourdj Bou Ariridj was infested by *Heterodera* spp. with the highest infestation rate (100%), followed by Setif (67%), while the lowest infestation rate was recorded in Souk Ahras with 16%.

Molecular identification

The ITS regions of the 20 nematode populations were successfully amplified using the F194/F195 primers pair. For all populations, the primers yielded a single fragment of approximately 1,040 bp. No PCR products were obtained from the negative control, which lacked a DNA template. The results of sequencing and BLAST analysis indicated that the populations were identified as *H. avenae*, *H. hordecalis*, *H. carotae*, and *H. cruciferae*. Ten cyst populations collected from Oum Bouaghi, Ain Melila 1, Ain Melila 2 and Ain Melila 3, Seriana, Ain Bessem, El Achir, Bordj Khaled, Guelma b, and Merahna locations were identified as *H. hordecalis*. *Heterodera avenae* was detected from five fields from Constantine, El Asnem, Oued Smar, Hammem Sokhna 1, and Hammem Sokhna 2. *Heterodera carotae* and *H. cruciferae* are reported in Algeria, for the first time in this study. *Heterodera carotae* was discovered in Tadjenanet 2 and Merahna, while *H. cruciferae*

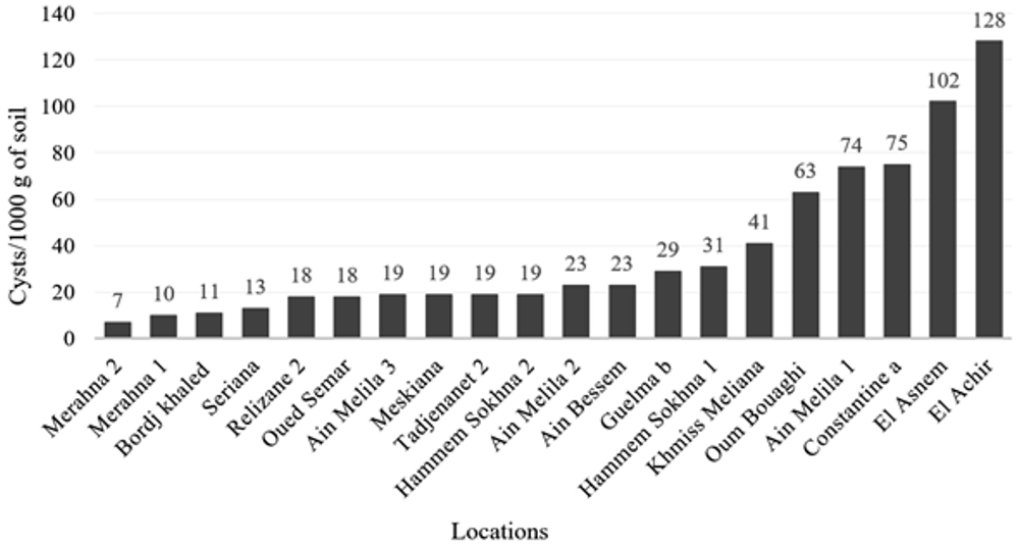

Figure 2. Number of *Heterodera* spp. cysts/kg of soil samples from the fields surveyed in Algeria.

was found in Meskiana, Khmiss Meliana, and Relizane (Table 1).

Phylogenetic analysis

The phylogenetic analysis based on ITS sequences clustered the populations from 12 geographically sites according to species (Fig. 3). The phylogenetic relationship of cyst nematode populations belonging to *H. avenae*, *H. hordecalis*, *H. carotae*, and *H. cruciferae* were compared to data from other geographical locations.

The *Heterodera* populations investigated in our study were divided into three groups in a phylogenetic tree based on the ITS1, ITS2, and 5.8S rDNA sequences. All groups formed distinct clades supported by high bootstrap values (99-100%) (Fig. 3). Group I was comprised of *H. hordecalis* populations (MK840637, MK840639, MK848402, MK840642, MK848399, MK840640, MK840638, MK848401, MK848400, and MK840641). Group II included the *H. avenae* populations (MK840646, MK848396, MK840645, MK848397, and MK848398). Within this group, the Algeria 46 population was distinct from the rest of the group (Algeria 03, 04, 47, and 48), indicating the existence of a subgroup formation in Group II. The sequences of Group II populations were 98% similar to the ITS sequence of the population from northern Algeria, suggesting that they might be the same pathotype. *Heterodera avenae* populations from Algeria had high similarity with a Spanish population (AY148354), whereas the *H. hordecalis* populations exhibited a high similarity with an Israeli population (AY347923). Group III included two *H. carotae* populations (MK840643 and MK840644) and three *H. cruciferae* populations (MK848393, MK848394, and MK848395). The *Heterodera carotae* and *H. cruciferae* populations were similar to populations from Italy (AY347917) and the Netherlands (AF274411), respectively. *H. carotae* and *H. cruciferae* formed a well-supported clade with the corresponding populations.

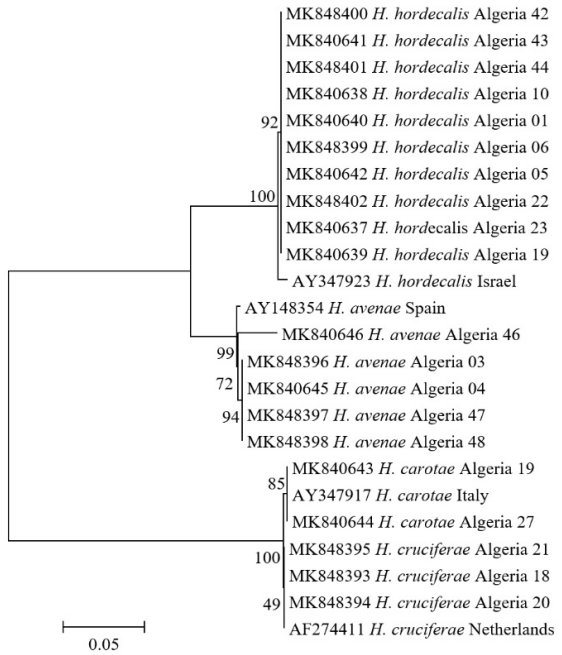

Figure 3. Phylogenetic tree of *Heterodera* spp. populations from north Algeria.

DISCUSSION

Wheat is an essential component in the Algerian diet, however, production in the country is insufficient to feed the population. Therefore, increasing wheat yield is a priority either by increasing the cultivated area or increasing yield per unit area by reducing abiotic and biotic stress. A survey was conducted in northern Algeria to determine which CCNs exist in the region. A total of 48 cereal fields in 12 provinces were sampled. The results showed that 41.6% of wheat fields in the provinces were infested with CCNs. The degree of CCN infestations varied widely depending on the cultivation system in the surveyed fields. Sharma *et al*. (2007) and Renčo and Cereukoua (2008) reported that the repeated cultivation of cereals in the same field contributes to the explosion of cyst nematode populations, which may explain the high infestations in Ain Melila-1, Oum Bouaghi, Constantine, Al Achir, and Khmiss Meliana. Fallow or non-host plant cultivation in fields infested with these nematodes have been recommended to reduce nematode population densities in soil, so the low infestation rate in the remaining provinces may have resulted from the previous crop (Brown, 1984; Griffin, 1988; Rivoal and Bourdon, 2005).

This study provides new information on the distribution and occurrence of *H. avenae*, *H. hordecalis*, *H. carotae*, and *H. cruciferae* in the main wheat-growing areas in northern Algeria. *Heterodera hordecalis* was detected in wheat fields in Batna, Tiaret, Tlemcen, Setif, and Sidi Bel Abbes for the first time (Smaha *et al*., 2018). The contamination of various cereal-growing areas of

Algeria with *H. avenae* was also reported in previous studies (Mokabli *et al*., 2002; Tirchi *et al*., 2016). Smaha *et al*. (2018) found that *H. avenae* was the most common species found in 23% of wheat fields in 16 locations in Algeria. Mokabli *et al*. (2002) reported the presence of both species *H. avenae* and *H. latipons* in other regions of Algeria such as Béjaia, Dar El Beida, Mascara, Relizane, and Setif. Assia *et al*. (2019) reported that most of the localities in the Relizane region were infested with *H. avenae*, *H. latipons*, *H. hordecalis*, *H. mani*, and *H. filipjevi*, with a dominance of *H. avenae* (93% of the municipalities surveyed). A recent study by Smaha *et al*. (2019) showed that 23% of samples studied were infested by *H. avenae*, while *H. hordecalis* was observed in 7% of the samples collected. *Heterodera filipjevi* was the least frequent cyst nematode detected in the sampled areas (2%). Our study revealed that *H. avenae* was more extensive than the finding of previous reports. *Heterodera latipons* was not found in the sampled fields.

The ITS region has been commonly used to discriminate nematode species, including in the genus *Heterodera*, and to reveal intraspecific polymorphism among certain isolates belonging to one species (Subbotin *et al*., 2003; Baklawa *et al*., 2015; Imren *et al*., 2015; Mokrini *et al*., 2017). In this study, phylogenetic analysis based on the ITS sequences provided useful information to separate species. Additionally, little variation among Algerian populations of *H. avenae* was observed, a result which is compatible with previous studies examining the polymorphism among *H. latipons* and *H. avenae* populations obtained from several cereal areas (Bekal *et al*., 1997; Subbotin *et al*., 1999; Rivoal *et al*., 2003; Madani *et al*., 2004; Imren *et al*., 2015). Subbotin *et al*. (2003) reported that *H. avenae* populations from Africa, Europe, and Asia formed several clusters based on the ITS region. Baklawa *et al*. (2015) showed a grouping of the *H. avenae* complex into one cluster, with a high bootstrap value. Additionally, Abidou *et al*. (2005) could not determine any intraspecific polymorphism among *H. avenae* populations from Syria and France. Imren *et al*. (2015) reported an intraspecific polymorphism based on the ITS sequences observed in *H. avenae* populations from the eastern Mediterranean region of Turkey. However, Mokrini *et al*. (2017) grouped *H. avenae* populations from different parts of Morocco into one cluster with high similarity.

No polymorphism among *H. hordecalis* populations was found in this study. Similarly, the study performed by Smaha *et al*. (2018) clustered *H. hordecalis* populations from Algeria into one group with a high bootstrap value. Madani *et al*. (2018) demonstrated the 100% nucleotide identity of the ITS sequences of *H. hordecalis* isolates from Italy and Russia. In this study, similarly, all Algerian *H. hordecalis* populations grouped in the same clade with a high bootstrap value.

Heterodera carotae is a highly specialized endoparasitic cyst nematode infecting both cultivated and wild carrots as well as the hedge parsley (*Torilis leptophylla* L.) and is responsible for carrot decline in several countries (Jones, 1950; Subbotin *et al*., 2010). Compared to other cyst nematodes, *H. carotae* has a very limited host range. The nematode is mainly restricted to cultivated and wild carrots (Jones, 1950) and other *Daucus* spp. within the Apiaceae. However, other plants, such as *Torilis arvensis* (Hudson), which is a species of flowering plant in the family Apiaceae and known by the common name "spreading hedgeparsley", may act as a reservoir for cyst nematode survival (Vallotton, 1980; Subbotin *et al*., 2010). *Heterodera carotae* caused serious yield loss ranging from 20 to 90% in carrots cultivated in Italy (Greco *et al*., 1994). Distribution of this pathogen has been determined in South Africa, Europe, Cyprus, India, USA, and Canada (Berney and Bird, 1992; Subbotin *et al*., 2010; Yu *et al*., 2017; Madani *et al*., 2018). This study detected *H. carotae* for the first time in Tadjenanet-2 and Merahna provinces in northern Algeria. Two populations of *H. carotae* grouped with a bootstrap value of 96%. These populations were similar to a *H. carotae* population from Italy (AY347917) in the phylogenetic tree. Therefore, *H. carotae* populations found in this study are thought to have originated from other plant roots in these fields such as weeds in the Apiaceae family.

Heterodera cruciferae is reported for the first time from Meskiana, Khmiss Meliana, and Relizane 2 province in northern Algeria. This nematode attacks different species of Cruciferae and causes losses in crop productivity (Subbotin *et al*., 2010). *Heterodera cruciferae* has been reported from cabbage-growing areas in the USA (California), Europe, South Australia, Iran, and Eastern Azerbaijan (Stone and Rowe, 1976; Whitehead, 1998; Sturhan and Liskova, 2004; Jabbari and Niknam, 2008). The length of ITS

fragments of *H. cruciferae* population obtained from this study corresponded to the fragment of a *H. cruciferae* population (AF274411) from the Netherlands. The ITS sequences of *H. cruciferae* populations were different in three nucleotides from those of the *H. carotae* populations in this study. Subbotin *et al*. (2001) and Madani *et al*. (2004) reported the high similarity between nucleotide sequences of *H. cruciferae* and *H. carotae*. Chizhov *et al*. (2009) indicating that the *H. cruciferae* population from Moscow grouped in a clade with a *H. carotae* population in GenBank. Shubane (2018) indicated a high nucleotide similarity of 99% between *H. cruciferae* and *H. carotae* in South Africa. *Heterodera cruciferae* has been reported from Europe, Russia, California, and South Australia (Stone and Rowe, 1976; Whitehead, 1998), as well as Turkey (Mennan *et al*., 2006; Mennan and Handoo, 2006). *Heterodera cruciferae* is not a major pest and its host range is limited to Cruciferae (nearly all genera), and some weeds and members of Labiaceae. Therefore, *H. cruciferae* samples found in the present study may have been associated with the roots of cruciferous weeds growing in wheat fields.

Surveys conducted over the past two decades have reported the presence of cyst nematodes of the genus *Heterodera* in almost all cereal zones in northern Algeria (Smaha *et al*., 2018). The presence of *Heterodera* species in wheat-growing regions could be a limiting factor to wheat production in Algeria. If we consider the number of eggs (more than 600) contained in a cyst (Siddiqui, 2000), the levels of infestation noted in most locations can be described as high with the potential to affect wheat yield. Wheat has an important socio-economic value for Algeria, further detailed nematode surveys in other wheat-growing areas are needed. The population densities of *Heterodera* species should be suppressed to keep their population densities below threshold levels by appropriate management measures using resistant wheat varieties and applying proper crop rotation.

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