Morphological and morphometrical analysis of *Heterodera* spp. populations in Jordan

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**Abstract** Phenotypic diversity of five Jordanian populations of cyst nematodes, *Heterodera* spp. collected from five regions from Jordan (Ar-Ramtha, Madaba, Dana, Al-Karak, and Jerash) was investigated. Soil samples were collected from one representative field in each region. Morphological and morphometrical characteristics revealed that *Heterodera latipons* is dominated in cereal fields at Ar-Ramtha, Madaba, Dana and Al-Karak regions and *Heterodera schachtii* in Jerash. Cysts populations from all cereal fields had bifenestrate vulval cone and a strong underbridge. Wherever, cysts of the cabbage population had ambifenestrate vulval cone with long vulval slit. The bullae were absent in Ar-Ramtha, Madaba and Dana populations, but present in Al-Karak and Jerash. Based on 12 morphometrical characters, the first three functions in canonical discriminant analysis accounted 99.3% of the total variation. Distance from dorsal gland duct opening to stylet base, underbridge length, \(a = L/W\) (body length/midbody width) and length of hyaline tail tip had strong and significant contributions in the first function. While the second function was strongly influenced by length of hyaline tail, fenestral length, fenestral width and tail length. However, the third canonical discriminant function was found to be influenced by stylet length, fenestral length, \(a = L/W\) (body length/midbody width) and underbridge width. The graphical representation of the distribution of the samples showed that the first canonical discriminant function clearly separated *H. schachtii* from Jerash from other populations. Whereas, *H. latipons* collected from

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1. Introduction

Cyst-forming nematodes (Heterodera spp.) are highly specialized and economically important soil-borne parasites attacking numerous agricultural crops (Greco et al., 2002). The Mediterranean cereal cyst nematode (MCCN), Heterodera latipons, occurs mainly in Mediterranean region but also in Asia and Europe (Abidou et al., 2005; Smiley and Nicol, 2009). In Jordan, the MCCN (H. latipons) was first detected in two irrigated wheat fields at low population densities at the Jordan Valley (Yousef and Jacob, 1994) and it was first noted in a rainfed field of barley at Ar-Ramtha region in the Northern part of Jordan in 1996 (Abu Gharbieh and Al-Banana, unpublished data). Later on, barley fields’ surveys revealed that Mediterranean cereal cyst nematode was recovered from Northern and Southern Mediterranean zones and Eastern Desert (Al Abed et al., 2004). Incidence and severity studies showed that these nematodes were dominant and severe in Ar-Ramtha and Mafraq areas whereas they were moderate in Al-Karak area and not found in newly introduced areas such as the south region at Al Mudawarra (Al Abed et al., 2004). Although, sugar beet is not cultivated in Jordan the sugar beet cyst nematode Heterodera schachtii Schmidt had been recorded on cabbage and cauliflower in Jordan (Saleh, 1987). Investigations on the nematode distribution had shown that it is restricted to a small area near the old Roman city of Jerash (Saleh and Qadri, 1989).

Nowadays, the genus Heterodera contains more than 60 species. Extensive studies have revealed the presence of several distinct species of Heterodera infecting cereals and grasses within studied populations primarily identified as Heterodera avenae. Presently, the H. avenae complex is considered to contain: H. avenae, Heterodera arenaria, Heterodera aucklandica, Heterodera australis, Heterodera filipjevi, Heterodera mani, Heterodera pratensis, and Heterodera ustinovi (Wouts and Sturhan, 1995; Gabler et al., 2000; Sturhan and Krall, 2002; Subbotin et al., 2002). The species H. latipons is considered to form a separate species complex within the H. avenae group (Subbotin et al., 2003). On the other hand, H. schachtii belongs to the H. schachtii sensu stricto group, which also contains Heterodera betae, Heterodera ciceri, Heterodera daverti, Heterodera galeopsidis, Heterodera glycines, Heterodera lespedezae, Heterodera medicaginis, Heterodera rosii and Heterodera trifoli (Subbotin et al., 2000). Knowledge and characterization of genetic variability among and within the cyst nematode populations can provide predictive estimates of genetic variation among these populations and for efficient management and selection of appropriate control strategies (Ganguly and Rao, 2003).

Several multivariate analysis methods are used to analyze the genetic variability and to investigate the differences between populations of a nematode species. Since published studies related to the morphological variability of Heterodera spp. in Jordan are limited, this study was carried out to assess the interspecific variability among Heterodera spp. using morphological and morphometrical analysis.

2. Materials and methods

2.1. Nematode populations

Five Jordanian populations of cyst nematodes, Heterodera spp. were collected from major rainfed barley and wheat producing regions (Ar-Ramtha, Madaba, Al-Karak and Dana) and from irrigated cabbage field in Jerash (Fig. 1). Soil samples were collected from one representative field in each region. Soil samples were collected in July and August 2006–2008 after harvesting. Each sample weighs about 250 g soil collected from the root zone system. Cysts were extracted using sieving and floatation methods (Shephered, 1986). Eggs were obtained by crushing the extracted cysts. Whereas, the second-stage juveniles (J2s) were obtained by subjecting eggs at 10 °C to hatch in tap water
(Al Abed et al., 2009). Vulval cones were prepared as described by Hesling (1965). J2s were fixed in hot buffered formalin (Humason, 1972). Permanent mounts of second juvenile stage were prepared following Seinhorst (1959).

2.2. Morphometrical and morphological characterization

For each population, both qualitative and quantitative morphological characteristics of J2s and cysts were tabulated and used to identify the species following the original descriptions and the diagnostic keys of H. Latipons (Franklin, 1969) and H. schachtii (Schmidt, 1871). The obtained morphological and morphometrical data of the five populations were compared to each other, and referenced to related published data.

Morphometrical data were run through discriminant multivariate analysis to investigate the separate ability of the five populations based on their morphometrical characters. Data were analyzed with reference H. latipons of Franklin (1969), reference H. latipons Jordanian population (Madaba region) of Al Abed (2004) and reference H. avenae of Handoo (2002). Canonical discriminant analysis (CDA) was performed using SPSS 15 (SPSS Inc., 2006).

3. Results and discussion

Morphological features of second-stage juveniles (J2) of the five Jordanian populations were almost similar (Fig. 2A).

Second stage juveniles are cylindrical in shape, with slightly offset anterior part (Fig. 2A–D), and a tapering round tail tip (Fig. 2D). Stylet is strong; with basal knobs shallowly concave anteriorly (Fig. 2B). However, the morphometrical data of J2 showed some variability (Table 1). Brown cysts of all populations were variable in size, and mostly lemon-shaped, with a protruding neck and vulvar cone structure (Fig. 2F). Cyst wall is dark brown in color, and bearing a zig-zag pattern. New cysts mostly enveloped with a chalk-like bloom. Vulval cones were either bifenestrate with short vulva slit (8.4–10.2 μm) (Fig. 2F) or ambifenestrate with long vulval slit (42.7–45 μm) (Fig. 2E). Underbridge was found in the vulval cone structures of all the examined populations, while crowded protruding bullae were present only in some populations (Fig. 2G).

Species of Heterodera were differentiated using morphological and morphometrical features including; seven characters of J2 (body length, midbody width, a, tail length, hyaline tail length, stylet length, and distance between dorsal gland duct opening to stylet base), and five characters of the brown cysts (underbridge length, underbridge width, fenestral length, fenestral width, vulva slit length, and presence of bullae) (Handoo, 2002).

Results based on these morphological and morphometrical characters for target cyst species are given in Table 1. The results showed that the bullae were absent in H. latipons populations from Ar-Ramtha, Madaba and Dana regions, however, it was present regularly in H. latipons population.

Figure 2 (A) The second stage Juvenile of Heterodera latipons. (B). Stylet-&-DGO of Heterodera latipons. (C) Excretory pore of Heterodera latipons. (D) Anus & Hyaline tail tip of Heterodera latipons. E. Female fenestra of Heterodera schachtii. F. Female underbridge & Vulval slit of Heterodera latipons. G. Bullae of Heterodera schachtii female. (Scale bars: A = 50 μm; B–G = 10 μm).
from Al-Karak and maral in *H. schachtii* population from Jerash (Table 1). All *Heterodera* cysts populations had a strong underbridge, with either bifenestrate vulval cone in *H. latipons* populations (Ar-Ramtha, Madaba, Dana and Al-Karak) or ambifenestrate one in *H. schachtii*. In the other hand, variations were existing in some morphometrics of these three populations (Table 1). Such variations exist in vulval cone measurements of the cysts. For example, the fenestral length
was the longest in Dana, and shortest in Madaba. There were also slight differences between the isolates in semi fenestral length. The J2s of *H. latipons* recovered from cysts collected from Madaba were the longest, furthermore, distance from anterior end to start of overlapping and distance from anterior end to end of overlapping were also the longest. Whereas, Dana isolate J2s had the shortest length of hyaline tail tip and distance from anterior end to start of overlapping (Table 1). Both qualitative and quantitative characters of J2s and vulval cones of the cyst nematode that recovered from the three barley geographic regions were agreed with the original *H. latipons* description of Franklin (1969) and Al Abed et al., 2004. Measurements of Al-Karak population also agreed with *H. latipons* original description. Sometimes bullae are present at the level of the underbridge (Franklin, 1969). The morphological characteristics of the Jerash isolate agreed with the original *H. schachtii* description of Schmidt (1871).

The canonical discriminant analysis, performed with the standardized canonical discriminant function coefficients for the 12 morphometrical traits, showed that the first three functions accounted 99.3% of the total variation (Table 2).

| Function | Eigen value | % of variance | Cumulative% |
|----------|-------------|---------------|-------------|
| 1        | 837.781     | 97.3          | 97.3        |
| 2        | 9.610       | 1.1           | 98.4        |
| 3        | 7.850       | 0.9           | 99.3        |
| 4        | 4.937       | 0.6           | 99.9        |
| 5        | 0.361       | 0.0           | 99.9        |

The second function (accounted 1.1%) was found to be strongly influenced by length of hyaline tail, fenestral length, fenestral width and tail length. The third canonical discriminant function (accounted 0.9%) was found to be influenced by stylet length, fenestral length, \( a = L/W \) (body length/midbody width) and underbridge width (Table 3). These results reveal the value of some characters that can be utilized for the separation of different species within *Heterodera* genus. Dawabah et al. (2012) reported some other characters that can be utilized for the separation of different populations within *H. avenae* and in determining the intraspecific variations between these populations. These characters include cyst body dimensions, J2 midbody width, J2 body width at the anus, J2 head height and J2 ratios like total body length/tail length and tail length/ body width at the anus.

The graphical representation of the distribution of the five and referenced populations in the space of the two discriminate functions (Fig. 3) showed a clear separation of *H. schachtii* population (Jerash) and *H. avenae* reference of Handoo from the four *H. latipons* populations collected in this study together with *H. latipons* references of Franklin and Al Abed. This separation was due to morphometrics differences in distance from dorsal gland duct opening to stylet base, underbridge length, \( a = L/W \) (body length/midbody width), length of hyaline tail tip, fenestral length, fenestral width and tail length. The cyst population distribution also agreed with the characteristics of bullae, underbridge and fenestra, the bullae were absent or slightly present in the *H. latipons* populations from Ar-Ramtha, Madaba, Dana and Al-Karak regions and *H. latipons* references of Franklin and Al Abed. Otherwise, it was present in *H. schachtii* population from Jerash (Table 1). With exception of the *H. avenae* reference of Handoo, all other *Heterodera* cysts populations had a strong underbridge, with either a bifenestrate vulval cone in *H. latipons* populations (Ar-Ramtha, Madaba, Dana and Al-Karak) or an ambifenes- trate one in *H. schachtii*. Abidou et al., 2005 showed specific differentiation between *H. latipons* and both *H. avenae* and *H. filipjevii*, which was based on strong and deep underbridge without bullae. Furthermore, Yan and Smiley (2009) reported a good discrimination of *H. filipjevii* and *H. avenae* from *H.

The table below shows the standardized canonical discriminant function coefficients for the five Jordanian populations of *Heterodera* spp. and the references populations of Franklin, Al Abed and Handoo, based on 12 morphometrical characters.

| Trait                     | Function | 1   | 2   | 3   | 4   | 5   |
|---------------------------|----------|-----|-----|-----|-----|-----|
| **Cyst**                  |          |     |     |     |     |     |
| Underbridge length        | 0.420    | 0.005 | -0.557 | -0.790 | -0.795 |
| Underbridge width         | 0.167    | 0.228 | 0.757  | 0.159  | 0.601 |
| Fenestral length          | 0.121    | 0.438 | 1.131  | 0.459  | 0.438 |
| Fenestral width           | -0.066   | 0.400 | -0.245 | 0.741  | 0.700 |
| Vulval slit               | -0.026   | -0.557 | -0.349 | -0.268 | -0.208 |
| **Second stage juvenile** |          |     |     |     |     |     |
| Body length               | -0.331   | -0.265 | -0.232 | -0.010 | -0.326 |
| Midbody width             | -0.138   | 0.125 | -0.050 | 0.011  | 0.594 |
| \( a \)                   | 0.383    | 0.313 | 0.883  | 0.509  | -0.679 |
| Tail length               | 0.000    | 0.345 | 0.157  | -0.691 | 0.765 |
| Length of hyaline tail tip| 0.329    | 1.264 | -0.944 | 0.318  | -0.168 |
| Stylet length             | -0.085   | -1.262 | 1.180  | 0.490  | 0.156 |
| Distance (dorsal gland duct opening to stylet base) | 1.171 | -0.026 | -0.001 | -0.023 | -0.027 |
and *H. schachtii* based on PCR-RFLP molecular assay utilizing six endonucleases.

Our study showed that morphological characters and morphometrical analysis can distinctly separate species and populations within the *Heterodera* genus. Furthermore, DNA observations, biological, ecological and biogeographical studies can be done to identify at which taxonomic level populations of cyst-forming nematodes can be separated. The creation of a catalog of morphological characters and morphometrical analysis of cyst forming nematode species would facilitate the identification of species and population.

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