Prevalence, distribution and intraspecific variation of *Heterodera schachtii* populations from semiarid environment

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**Abstract** A two-year survey study was carried out to identify and determine the distribution, community characteristics and intraspecific variation of the sugar beet cyst nematode, *Heterodera schachtii* populations collected from the six major sugar beet-producing governorates in Syria, west Asia. A total of 178 composite rhizosphere soil samples were collected from the six governorates during 2009 and 2010 growing seasons as follows: Aleppo (26), Ar Raqqah (17), Dayr az Zawr (33), Idlib (34), Hamah (41) and Homs (27 samples). The nematode was originally identified on the basis of morphometrics and morphological features of the cyst vulvar cones and second-stage juveniles (J2). The communities of *H. schachtii* were then analyzed using the criteria of frequency of occurrence (FO%), mean population density (PD) and prominence value (PV). Results showed that *H. schachtii* was the most frequent and prominent in Homs (FO% = 70.37% and PV = 23472.37) and the least frequent and prominent in Ar Raqqah (FO% = 5.88% and PV = 28.86). The highest nematode population density was also recorded in Homs in 2009 (50,545 J2 + eggs/100 g soil) while the least was recorded in Ar Raqqah in 2010 (119 J2 + eggs/100 g soil). The morphometric data of cyst vulvar cone, second-stage juveniles (J2) and eggs were also subjected to a multivariate principal component analysis to analyze the relationships between the studied populations, and to identify the variables that show the highest multiple correlations with these populations. Results showed that at the high order of hierarchical cluster analysis, four populations (Idlib, Hamah, Homs and Aleppo) formed one main cluster, while Dair az Zawr and Ar Raqqah failed to form clusters and separated individually. However, at low distance value, the main cluster further subdivided to separate the Aleppo population from Idlib, Hamah.
1. Introduction

Sugar beet, Beta vulgaris L., is the second largest crop used for sugar production all over the world. About 40% of the world sugar is extracted from this crop which is grown in about 42 countries located between the 30 latitudes (Asadi, 2007). The total world cultivated area of sugar beet reached up to 4.45 million ha in 2013, which produced up to 250.19 million tons of sugar beet roots with an average of 56.25 tons/ha (FAO, 2013). In Syria, Sugar beet is ranked the third most important economical crop after wheat and cotton, and is considered the only crop used for sugar production in the country. The total cultivated area in 2013 was about 22,593 ha which produced about 1.03 million tons of sugar beet roots with an average of 45.49 tons/ha (Ann. Stat. Group, Syria, 2013).

Many biotic and abiotic stresses can affect the growth of sugar beet plants in the field. Plant-parasitic nematodes are one of the most important biotic stress factors that affect these plants causing serious yield losses. A total of 16 nematode species were reported to be associated with the rhizosphere of sugar beet plants (Hussein, 2001). Of these nematodes, the sugar beet cyst nematode, Heterodera schachtii (Schmidt, 1871) is the most important and damaging (Greco et al., 1982). Yield losses caused by this nematode species in sugar beet yield might reach up to 80% (Curto, 2008). This nematode species is widely distributed, and has been reported from about 39 countries worldwide (Smith et al., 2004). The nematode has also been reported on sugar beet from several Arabic countries including: Iraq (Stephan, 1986), Libya (Edongali, 1986), and Syria (Albalkhi et al., 2006). It has also been reported on cabbage in Egypt (Ibrahim and Handoo, 2007), and cauliflower in Jordan (Saleh, 1987).

Disease symptoms of H. schachtii on sugar beet plants in the field first appear as scattered circular or ovoid spots of yellow weak plants. The nematode second-stage juveniles infect the apical meristematic tissues of the rootlets preventing these roots to grow and absorb water and nutrients. As a result, the plants tend to form new rootlets which also become infected with the nematodes, leading to the formation of the hairy root shape. The nematode-infected roots are also dwarfed and diminished in size, and their functions are negatively affected. The growth of the shoot system also greatly retarded, showing the symptoms of yellowing and wilting, especially on hot days (Dawabah and Al-Yahya, 2015). The damage could be severe on the young seedlings, which could easily die after emergence (Gründler et al., 1991).

Females and cysts of H. schachtii are lemon-shaped, and eggs are protected inside the dead cysts for about 5–7 years, which presents a great obstacle when trying to manage this nematode. The posterior end of the cyst (vulvar cone) is the most important structure for the identification of this nematode species (Cordero and Baldwin, 1991).

Information on the distribution, community characteristics and morphological variation of H. schachtii associated with sugar beet in Syria is very limited. So, this study aimed to identify and determine the distribution, community characteristics and the intraspecific variation of H. schachtii populations collected from six governorates in Syria, west Asia.

2. Materials and methods

In a two-year survey study (2009–2010), a total of 178 rhizosphere soil samples were collected from the six major sugar beet producing governorates in Syria, west Asia (Fig. 1). These governorates and the related total number of samples collected from each governorate in the two seasons were: Aleppo (26), Ar Raqqah (17), Dair az Zawr (33), Idlib (34), Hamah (41) and Homs (27 samples). Each governorate was visited twice (in the summer season of 2009 and autumn season of 2010). Soil samples were collected directly before and after plant harvest @ 10–15 samples/ha, using sampling augers at a depth of 10–30 cm (Soomro et al., 1997). Sampling sites were selected in the fields following a zig-zag pattern. Samples of each ha were thoroughly mixed, and a 2 kg composite sample was taken to represent this area. Root samples were also collected to be examined for the presence of white cysts and/or females on the roots. Soil samples were kept in plastic bags while, root samples were kept in paper bags. All samples were labeled, and sent in cool insulated chests to our laboratory in Damascus for nematode extraction, identification and community analysis.

In the laboratory, soil samples were air dried at room temperature, and sieved to exclude stones. Samples were then thoroughly mixed, and three 200 g sub-samples were selected from each sample to extract cysts using a modified Fenwick can having an extra water supply near the base of the apparatus (Kort, 1960). Cysts on the second sieve (250 μm) were transferred to a filter paper, and were examined and counted using a magnification lens. Root samples were also washed with a gentle stream of tap water on a 250 μm sieve, and cysts on the sieves were collected and counted. For each sample, the total number of cysts per soil and roots were accumulated, and expressed as the number of cysts/100 g soil. Cysts of several random samples were crushed in water, and the mean number of eggs + J2/100 g soil was also calculated.

Community analysis of H. schachtii in the surveyed governorates included: absolute frequency (FO%), mean population density (PD) (Norton, 1978) and prominence value (PV) (Beals, 1960).

The specimens used for the morphological and morphometric studies were obtained from fresh materials. Brown cysts...
were extracted from the roots and soil at the end of each growing season. Eggs were obtained by squashing the cysts in water and incubated to obtain the newly hatched second-stage juveniles (J2s). Other eggs without incubation were used for morphometric studies. The posterior ends (vulvar cones) of cysts which were collected from the roots and soil were cut-off in sterile water, and were cleaned in 30% H2O2 for 3–4 min. Cones were then transferred to clean drops of sterile water for 2–3 min, then to drops of ethyl alcohol to get-rid of air and water, and mounted in glycerin on glass slides (Hooper, 1970). Cones were finally examined under a compound microscope, and certain morphological and morphometric features were determined. Eggs and J2s were also mounted in TAF (7 ml formalin 40%, 2 ml triethanolamine, 91 ml distilled water) on glass slides, and certain morphometric criteria (Tables 2 and 3) were measured using a compound microscope with a digital camera and a computer system. The obtained morphological and morphometric data of the six populations were compared to each other and referenced to related published data (Subbotin et al., 2010).

Based on Euclidian distance coefficients, a phonological dendrogram was constructed to evaluate the level of phenotypic variation among the six tested *H. schachtii* populations. Data were then run through multivariate principal component analysis using SPSS 22 (SPSS Inc., 2012) to analyze the relationships between the studied populations and to identify the variables that show the highest multiple correlations with these populations.

### 3. Results

The sugar beet cyst nematode, *H. schachtii* was recorded in all of the surveyed sugar beet producing governorates in Syria (Table 1). Based on the mean values of frequency of occurrence (FO%) and prominence value (PV) in 2009 and 2010, the nematode was most frequent and dominant in Homs governorate (FO = 70.37% and PV = 23472.37), then in Aleppo, Hamah, Dair az Zawr and Idlib, in a descending order. However, the nematode was least frequent and dominant in Ar Raqqah governorate (FO = 5.88%, and PV = 28.86) (Table 1).

Levels of nematode infestation also varied among the surveyed governorates. Homs again recorded the highest infestation with average nematode population density (PD) of 27,981 J2 + eggs/100 g soil (50,545 and 5416 J2 + eggs/100 g soil in 2009 and 2010, respectively) (Table 1).

Symptoms on the sugar beet plants caused by *H. schachtii* in the surveyed fields were characterized by uneven patches of stunted and poor growing plants. Plant leaves were pale yellowish green in color. The tap roots of infected plants also were stunted and appeared highly branched with a hairy root appearance.

All the tested sugar beet cyst nematode populations collected from the six governorates were found to be belonging to one species, *H. schachtii*, based on the features and morphometrics of the cyst vulvar cone structures and the second-stage juveniles (J2) (Tables 2 and 3).
The multivariate principal component analysis, based on 14 morphometric characters of the cyst vulvar cones, 
J$_2$ and eggs showed that, at the high order of hierarchical cluster analysis, four populations formed one main cluster encompassing; Idlib, Hamah, Homs and Aleppo, while the Dair az Zawr and Ar Raqqah populations failed to form a cluster, and separated individually. However, at low distance value, the main cluster further subdivided to separate Aleppo population from Idlib, Hamah and Homs populations. The morphometric distance values ranged from 0.07 to 0.61. Hamah and Homs were the closest populations while Dair az Zawr showed the most diverse population (Fig. 2).
The fourteen morphometric characters of the cyst vulvar cones, J2 and eggs discriminated the populations, and 99.74% of the total variation was shown in two principle components (PC) (Table 4). The first and second PCs represented 79.33% and 20.41% of the variation, respectively. The first PC was strongly influenced by J2 body length with loading coefficient (LC) of 0.99 and egg width (LC = 0.09). The second PC was strongly influenced by fenestral length with LC = 0.71, egg length (LC = 0.45), semi-fenestral width (LC = 0.40) and number of bullae inside the cyst vulvar cone (LC = 0.18) (Table 4). The graphical representation of the distribution of the six populations in the space of the two PC functions (Fig. 3) showed that function 1 has clearly separated Ar Raqqah from Aleppo, Idlib and Dair az Zawr populations. However, the second function separated Dair az Zawr and Ar Raqqah populations from Idlib, Hamah, Homs and Aleppo populations.

These results revealed the value of some characters that can be utilized for separation of the different populations within H. schachtii species, and determining the intraspecific variations between these populations. These characters include, in a descending order of importance; J2 body length, egg width, fenestral length, egg length, semi-fenestral width and number of bullae inside the vulvar cone structure.

4. Discussion

The sugar beet cyst nematode, H. schachtii was found to be common in the sugar beet fields of all the Syrian sugar beet producing governorates except for Ar Raqqah governorate where it was, comparatively, least frequent and dominant. There has been a considerable increase of awareness regarding the occurrence and economic importance of H. schachtii worldwide (Nielsen et al., 2003). This study, however, showed that the distribution of H. schachtii in Syria has increased considerably, and the nematode which has been previously

| Table 3 | Morphometrics of second-stage juveniles (J2) and eggs of six Heterodera schachtii populations collected from six Syrian governorates (expressed in μm). |
|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Governorate (population) | Aleppo | Dair az Zawr | Idlib | Hamah | Homs | Ar Raqqah | All governorates |
|------------------|------------------|------------------|------------------|------------------|------------------|------------------|

Second-stage juveniles (J2)

| Character | Population | Mean ± SD (Range) |
|-----------|------------|-------------------|
| Body length (L) | 8 501.50 ± 6.98 (483.11–519.42) | 10 481.92 ± 4.88 (458.16–519.53) | 10 492.49 ± 4.99 (422.86–511.36) | 8 496.94 ± 6.93 (443.94–513.74) | 8 485.63 ± 5.03 (455.45–462.58) | 1 549.93 ± 4.13 (422.86–519.53) | 44 490.06 ± 5.11 (422.86–519.53) |
| Stylet length | 8 25.23 ± 0.41 (24.49–26.14) | 10 25.31 ± 0.86 (24.69–26.12) | 10 25.17 ± 0.43 (24.41–26.06) | 8 25.22 ± 0.55 (24.51–25.73) | 8 25.30 ± 0.44 (24.89–25.82) | 1 25.24 ± 0.41 (24.41–25.73) | 45 25.25 ± 0.42 (24.41–25.73) |
| Tail length | 8 43.19 ± 0.66 (42.69–43.38) | 10 42.84 ± 0.77 (41.69–43.26) | 10 42.94 ± 0.66 (41.98–43.93) | 8 42.91 ± 0.71 (41.24–43.07) | 8 42.64 ± 0.93 (42.25–52.81) | 1 42.96 ± 0.84 (41.25–52.81) | 45 42.87 ± 0.99 (41.25–52.81) |
| Hyaline tail length | 8 26.36 ± 0.77 (25.61–26.98) | 10 26.53 ± 1.11 (24.79–26.87) | 10 25.77 ± 1.23 (24.63–26.31) | 8 25.30 ± 0.97 (24.11–25.86) | 8 25.01 ± 0.84 (24.21–25.41) | 1 24.79 ± 1.16 (24.11–26.98) | 45 25.61 ± 0.49 (24.11–26.98) |
| Hyaline tail length/stylet length | 8 1.05 ± 0.04 (1.01–1.07) | 10 1.01 ± 0.03 (1.00–1.06) | 10 1.02 ± 0.04 (0.99–1.06) | 8 1.00 ± 0.03 (0.98–1.02) | 8 0.99 ± 0.02 (0.97–1.00) | 1 0.98 ± 0.02 (0.97–1.00) | 45 1.01 ± 0.03 (0.97–1.07) |

Eggs

| Character | Population | Mean ± SD (Range) |
|-----------|------------|-------------------|
| Length (L) | 8 106.22 ± 4.86 (100.32–112.32) | 10 108.45 ± 6.11 (102.45–113.42) | 10 96.44 ± 4.56 (91.85–101.32) | 8 100.05 ± 4.66 (96.93–102.35) | 8 105.65 ± 4.96 (93.84–114.03) | 1 105.85 ± 4.11 (93.07–109.53) | 45 105.37 ± 3.53 (91.85–114.03) |
| Width (W) | 8 53.42 ± 2.71 (51.65–55.97) | 10 53.48 ± 2.99 (50.32–58.98) | 10 58.78 ± 3.45 (51.98–63.98) | 8 58.67 ± 2.37 (55.87–60.54) | 8 56.67 ± 2.76 (51.74–63.61) | 1 55.89 ± 2.37 (53.75–57.87) | 45 57.04 ± 2.13 (50.32–63.98) |
| L/W ratio | 8 1.99 ± 0.44 (1.79–2.16) | 10 2.04 ± 0.21 (1.79–2.25) | 10 1.65 ± 0.29 (1.51–1.79) | 8 1.71 ± 0.26 (1.62–1.83) | 8 1.88 ± 0.36 (1.48–2.20) | 1 1.93 ± 0.26 (1.38–1.97) | 45 1.81 ± 0.31 (1.48–2.25) |

n = number of samples; each sample consists of 3 subsamples.
reported to be found only in Homs and Hamah governorates in 2003–2005 (Albalkhi, 2006), has reached another extra four governorates within only 5–6 years (2009–2010). This rapid dissemination of *H. schachtii* in the sugar beet production areas in Syria, has also been previously noted in 1920s in the western areas of the United States of America, and was attributed to poor management practices (Thorne, 1961), which might be the same case in Syria.

*Heterodera schachtii* is a biotrophic plant parasite, and can cause significant economic losses on sugar beet. However, the nematode can also complete its life cycle on a variety of other economic plants from the family Amaranthaceae and Brassicaceae (Dawabah and Al-Yahya, 2015; Ibrahim and Handoo, 2007; Saleh, 1987). Second-stage juvenile (J2) is the infective stage which invades the roots of the susceptible host plants and induce a syncytium on which the nematode feeds throughout its life cycle. The syncytium develops from a single cell inside the central cylinder through incorporation of neighboring cells (Grymaszewska and Golinowski, 1998). Secondary roots of sugar beet, invaded by the second-stage juveniles of *H. schachtii* cease to grow, causing proliferation of tertiary roots, which in turn, giving a bushy appearance to the root system. The tap (storage) root is stunted and the foliage is reduced. In hot weather, like in Syria and other arid and semiarid regions, chlorosis and wilt may also occur. This crop injury reduces root yield increasingly as the nematode initial population density in the soil exceeds the damage threshold level (Cooke and Thomason, 1979).

The world economic threshold level of *H. schachtii* is ranging between 1 and 8 eggs + J2/g soil (Cooke and Thomason, 1979; Fatemy et al., 2007; Greco et al., 1982). However, the mean population density of *H. schachtii* in Homs was so high, and greatly exceeded the world economic threshold level. Consequently, we could consider that *H. schachtii* now represents a serious threat to sugar beet production in Syria.

Unfortunately, the ability of *H. schachtii* to infect and reproduce on sugar beet and other host plants such as; cabbage (Ibrahim and Handoo, 2007), cauliflower (Saleh, 1987), rapeseed (Nielsen et al., 2003), spinach, radish and white mustard (Dawabah and Al-Yahya, 2015) which are also grown in Syria, makes it very difficult to effectively manage this nematode. Hence, search for resistant or tolerant plant cultivars is of great necessity. However, it seems that the nematode has reached Ar Raqqah governorate lately, and didn’t present a serious problem there yet. So, sanitation and strict quarantine regulations inside and outside this governorate could be of great importance to effectively combat the problem (Nielsen et al., 2003). Species within the genus *Heterodera* are mostly identified and separated using the cyst vulvar cone structures (Subbotin et al., 2010). The principal structures used in cyst identification are within the vulvar cone itself (Cordero and Baldwin, 1991). Second-stage juveniles are also of great importance in differentiating *H. schachtii* from the other related species within *H. schachtii* group (Ambrogioni and Irdani, 2001). Based on the 14 morphometrics and morphological features of the cyst vulvar cone, second-stage juveniles

Table 4 Standardized PC function coefficients for six *Heterodera schachtii* populations collected from six Syrian governorates, based on 14 morphometric characters.

| Cyst vulvar cone                      | PC1  | PC2  |
|--------------------------------------|------|------|
| Number of bullae                     | -0.01| 0.18 |
| Fenestral length                     | 0.03 | 0.71 |
| Semi-fenestral width                 | 0.01 | 0.40 |
| Distance between the two semifenestra| 0.01 | -0.09|
| Vulva slit length                    | -0.01| -0.09|
| Vulva slit width                     | -0.05| -0.12|
| Eggs                                 |      |      |
| Egg length                           | -0.13| 0.45 |
| Egg width                            | 0.09 | -0.26|
| Egg length/egg width                 | 0.00 | 0.01 |
| Second-stage juveniles (J2)          |      |      |
| J2 body length                       | 0.99 | 0.05 |
| J2 stylet length                     | -0.01| -0.01|
| J2 tail length                       | 0.03 | 0.04 |
| J2 hyaline tail length               | 0.01 | 0.00 |
| J2 c ratio                          | 0.01 | -0.02|
| J2 b ratio                          | 0.00 | 0.00 |
| Total variance                      | 79.33%| 20.41%|

*PC = principal component.

Figure 3 The two main principal components between the six *Heterodera schachtii* populations collected from six Syrian governorates (Aleppo, Idlib, Hamah, Homs, Ar Raqqah and Dair az Zawr), based on 14 morphometric characters. Components 1 and 2 accounted for 79.33% and 20.41%, respectively, in the total variation between the studied populations.
(J2) and eggs, all the sugar beet cyst nematode populations collected from the six Syrian governorates, in this study, were identified as *H. schachtii* (Ambrogioni and Irdani, 2001; Cordero and Baldwin, 1991; Subbotin et al., 2010). Nevertheless, some minor variations were observed in some morphometric characters between the tested populations. Accordingly, Hamah and Homs were the closest populations, while Dair az Zawr population was the most diverse one. These minor differences between the tested populations in some morphometric traits might be due to a genetic variation arising from an interaction between environmental and genetic factors (Ambrogioni and Irdani, 2001; Dawabah et al., 2012). Actually, Hamah and Homs governorates, which have the most similar populations, are adjacent governorates located in the center of the Syrian Republic. They also have similar climatic and environmental conditions. In addition, *H. schachtii* populations in these two governorates might have originated from the same parents. On the other hand, Dair az Zawr which had the most diverse population, is located in the far eastern part of the country, where the climate is hotter and drier. Beside, this governorate is located on the borders of Iraq where *H. schachtii* has also been reported on sugar beet (Stephan, 1986) earlier than Syria. This could suggest that the *H. schachtii* population in Dair az Zawr might be imported from Iraq along with commercial and agricultural exchange between the two countries, or originated from a hybridization between the Syrian and Iraqi populations, since this nematode species has a bisexual reproduction capability (Ambrogioni and Irdani, 2001).

This study revealed that the body length of second-stage juveniles (J2) as well as egg width, fenestral length, egg length, semi-fenestral width and number of bullae inside the vulvar cone structure are very useful traits in determining the semi-fenestral width and number of bullae inside the vulvar in the semiarid environment. With additional molecular studies to improve the evolutionary in differentiating the nematode species within *H. schachtii* populations 299 *H. schachtii* group. These preliminary results of this study need to be extended with additional molecular studies to improve the evolutionary status of the intraspecific variation within *H. schachtii* species in the semiarid environment.

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