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Genetic and pathogenic variation in *Heterodera latipons* populations from Turkey

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Summary – The cereal cyst nematode, *Heterodera latipons*, is an important plant parasite causing substantial yield losses in wheat throughout the world. This study aimed to determine genetic and pathogenic variation in *H. latipons* populations obtained from the southern part of Turkey. The populations were identified as *H. latipons* by sequencing the ITS-rDNA region and further sequence analysis showed an intraspecific genetic variation in *H. latipons* populations, which were clustered into different groups. The International Test Assortment materials were used to determine pathogenic variation (pathotypes) in these populations. The results showed that 'Ortolan', 'Morocco', 'KVL191', 'Bajo Aragon 1-1', 'Herta', 'Martin 403-2', 'Sun II' and 'Pusa Hybrid Bsi' cultivars were resistant or moderately resistant to the tested nematode populations. 'Emir', 'Dalmatische' and 'Capa' were susceptible to *H. latipons* populations. The Hatay population of *H. latipons* was detected as the most virulent nematode population because ten out of 20 cultivars were susceptible or moderately susceptible to this population. The least virulent population was the Kilis population, which caused susceptible reaction on six out of all cultivars with different levels. Based on this scheme, the Turkish populations were in the Ha1 group: the reactions of barley, oats and wheat classified them as either Ha41 or Ha51. Barley 'KVL191' was resistant to all nematode populations but susceptible to Ha51, and the reactions of the other barley cultivars were also consistent with the Turkish populations being Ha51. 'AUS10894' was susceptible to three nematode populations but resistant to Ha41, and the reaction of 'Capa' was also consistent with the Turkish populations being Ha51. However, the degree of susceptibility of all wheat differentials distinguishes the Turkish populations from other pathotypes in the Ha1 group.

Keywords – cereal cyst nematode, pathotypes, Turkish populations.

The cereal cyst nematode, *Heterodera latipons* Franklin, is classified in the phylum Nematoda, order Tylenchida and family Heteroderidae (Siddiqui *et al*., 2000). A typical marked sex dimorphism is observed within isolates of this cyst nematode (Handoo, 2002). Unlike the males, the females are spherical and live in the root system of cereal plants (Siddiqui *et al*., 2000; Handoo, 2002). In the absence of the host, the nematode survives for a few years in soil in the form of cysts (expanded dead bodies of females) that contain dormant unhatched second-stage juveniles (J2), serving as a source of new infections (Dababat & Fourie, 2018). It is a damaging soil-borne pathogen of cereals that can cause a serious economic threat to crop production worldwide with stunting of the haulm and the root system, leaf yellowing and ultimately yield losses (Scholz, 2001; Dababat & Foruie, 2018). *Heterodera latipons* has been reported frequently from several countries located in the Mediterranean basin, such

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as Syria, Cyprus, Iran, Italy, Libya and Morocco (Philis, 1988; Scholz & Sikora, 2004; İmren *et al*., 2012, 2015; Mokrini *et al*., 2012, 2017; Dababat & Fourie, 2018). The yield losses caused by this nematode in Syria and Cyprus, for example, ranged from 15 to 70% (Philis *et al*., 1988; Scholz & Sikora, 2004). It is also widely distributed in Turkey and has been found in several provinces including Adana, Elazığ, Osmaniye, Hatay, Yozgat, Gaziantep, Kilis and Mardin (Abidou *et al*., 2005; Dababat *et al*., 2014; İmren *et al*., 2015, 2018; Toktay *et al*., 2015).

Nematode populations from different geographic areas might show some morphological similarities or divergences (Dababat & Fourie, 2018). The range of inherited variability among cereal cyst nematode (CCN) populations has not yet been completely identified (Subbotin *et al*., 2010). Understanding these genetic and morphological differences plays a crucial role in selecting the appropriate control methods against CCN. The use of resistant cultivars is one of the most effective methods to control CCN (Smiley *et al*., 2008; Dababat *et al*., 2015). Resistance and tolerance are genetically independent variables. The cultivars providing resistance or tolerance to one nematode species may not necessarily provide resistance or tolerance to another species. Therefore, precise identification of a nematode at species level and determination of whether different pathotypes exist within the species are fundamental for developing resistant cultivars (Smiley & Nicol, 2009; Dababat & Fourie, 2018).

The pathotype identification is primarily based on the reproductive ability of the cyst nematodes on wheat, barley and oat cultivars. The differentiation scheme called the 'International Test Assortment' was derived from the existence of 7-11 virulent phenotypes resulting from previous extensive selection pressure (Andersen & Andersen, 1982; Rivoal & Cook, 1993; Cook & Rivoal, 1998). Among the cyst nematode species, *H. avenae*, *H. filipjevi* and *H. latipons* have been reported from different wheat- and barley-producing areas of Turkey (Dababat *et al*., 2015; İmren *et al*., 2015, 2018; Toktay *et al*., 2015). Moreover, two pathotypes, Ha21 and Ha31, have been identified in the Turkish populations of *H. avenae* and *H. filipjevi*, respectively (İmren *et al*., 2013; Toktay *et al*., 2013; Dababat *et al*., 2015). However, information about *H. latipons* pathotypes in Turkey is still lacking. Therefore, the objectives of this study were to characterise *H. latipons* populations from Hatay, Gaziantep, Kilis and Mardin provinces, Turkey, by employing sequence analyses of the internal transcribed spacer (ITS) region, and to determine the pathotypes using the International Test Assortment set.

Materials and methods

Sources of *Heterodera latipons* populations

A total of 25 soil samples were collected from Hatay, Gaziantep, Kilis and Mardin provinces during the 2015-2016 growing seasons (Table 1). The four sampled sites were identified to be historically infested with a high density of *H. latipons* (106 cysts (250 g soil)$^{-1}$) (İmren *et al*., 2012, 2018). The modified sieving-decanting method (Fenwick, 1940) was used to extract cysts from soil samples. The cysts were identified at the genus level under a Zeiss V20 binocular microscope. For each population, a minimum of ten cysts were collected and stored at 4°C to be used for the molecular analysis.

Genetic diversity of *Heterodera latipons* populations

The genomic DNA was extracted from a single mature cyst as described by Subbotin *et al*. (2000). The cyst was crushed in 10 μl of double-distilled water in a microhomogeniser, then the entire suspension was transferred into a 1.5 ml Eppendorf tube. Then, 10 μl of nematode lysis buffer (125 mM KCl, 25 mM Tris-HCl pH 8.3, 3.75 mM $MgCl_2$, 2.5 mM DTT, 1.125% Tween-20 and 0.025% gelatine) and 2 μl of proteinase K (600 μg ml^{-1}; Qiagen) were added to the homogenate. The tube was then centrifuged at 20 000 *g* for 1 min. The supernatant was carefully removed without disturbing the pellet, transferred into another Eppendorf tube, and stored at –20°C until further use.

The ITS regions of rDNA of the populations were amplified with TW81 (5′-TCCTCCGCTAAATGATATG-3′) and AB28 (5′-CGTAACAAGGTAGCTGTAG-3′) primers (Subbotin *et al*., 2003) using a T100 thermal cycler (Bio-Rad). The amplification steps consisted of an initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 60°C for 45 s, and extension at 72°C for 1.5 min. The program was finalised with an extension step at 72°C for 10 min. Negative control (no template DNA) was used to ensure that there was no contamination in the reaction mix. The amplification products were evaluated on 1.5% agarose gel using the G: BOX F3 gel doc system (Syngene) after ethidium bromide staining.

Table 1. List of *Heterodera latipons* populations from different wheat fields in the southern part of Turkey.

Isolate	Province	District	Location	Latitude	Longitude	GenBank Acc. No.
CCN01	Gaziantep	Karkamış	Soylu Kuzey	36°50′06″N	37°50′06″E	MT393902
CCN02	Gaziantep	Karkamış	Soylu Güney	36°49′31″N	37°55′14″E	MT393903
CCN03	Gaziantep	Karkamış	Türkyurdu	36°48′37″N	37°55′05″E	MT393904
CCN04	Gaziantep	Karkamış	Akçaköy Sınır	36°49′32″N	37°52′32″E	MT393905
CCN05	Gaziantep	Karkamış	Akçaköy Güney	36°48′27″N	37°52′33″E	MT393906
CCN06	Gaziantep	Karkamış	Kıvırcık	36°49′36″N	37°57′20″E	MT393907
CCN07	Gaziantep	Karkamış	Arıkdere Güney	36°49′14″N	37°50′54″E	MT393908
CCN08	Gaziantep	Karkamış	Arıkdere Sınır	36°48′52″N	37°50′40″E	MT393913
CCN09	Gaziantep	Karkamış	Yeniköy	36°51′21″N	37°34′01″E	MT393909
CCN10	Gaziantep	Karkamış	Merkez	36°22′58″N	37°64′28″E	MT393910
CCN11	Gaziantep	Karkamış	Sınır Kapı Doğu	36°49′54″N	37°55′14″E	MT393911
CCN12	Gaziantep	Karkamış	Sınır Kapı Batı	36°49′29″N	37°59′26″E	MT393912
CCN13	Gaziantep	Karkamış	Karaman	36°50′19″N	37°39′25″E	MT393914
CCN14	Gaziantep	Oğuzeli	Devehöyüğü	36°45′18″N	37°43′37″E	MT393915
CCN15	Hatay	Reyhanlı	Acarköy	36°18′17″N	36°35′04″E	MT393916
CCN16	Hatay	Kırıkhan	Mazmanlı	36°40′17″N	36°32′17″E	MT393917
CCN17	Kilis	Musabeyli	Haydarlar	36°50′14″N	36°55′70″E	MT393918
CCN18	Kilis	Musabeyli	Kocabeyli	36°48′35″N	36°54′42″E	MT393919
CCN19	Kilis	Musabeyli	Karaçavuş	36°49′18″N	36°55′50″E	MT393920
CCN20	Kilis	Merkez	Topdağı	36°45′42″N	37°11′49″E	MT393921
CCN21	Kilis	Elbeyli	Doğanlı	36°42′25″N	37°20′49″E	MT393922
CCN22	Kilis	Elbeyli	Çıldıroba	36°39′03″N	37°15′19″E	MT393923
CCN23	Mardin	Nusaybin	Serçe	37°06′18″N	41°05′18″E	MT393924
CCN24	Mardin	Nusaybin	Duruca	37°07′31″N	41°01′40″E	MT393925
CCN25	Mardin	Kızıltepe	Güneyli	37°05′07″N	41°18′30″E	MT393926

The bands were cut and eluted from the gel and purified using a PCR purification kit (Qiagen). The purified ITS products were sent to Macrogen, for bidirectional sequencing. The resultant sequences were aligned with Clustal W (Thompson *et al*., 1994), a multiple sequence alignment method, and manually Blasted to identify the closest available reference sequences in the complete NCBI nucleotide collection. Phylogenetic analyses of 25 *H. latipons* populations from this study and corresponding populations representing species from GenBank were performed with MEGA X software (Kumar *et al*., 2018). The neighbour-joining tree was constructed using the Tamura & Nei (1993) model with 1000 bootstrap replicates.

Pathotype characterisation of *Heterodera latipons* populations

Four samples belonging to different groups formed on the phylogenetic tree were selected to evaluate pathotype characterisation. The identification of pathotypes of the populations was determined by differential host materials, named as the International Test Assortment set in Cook & Rivoal (1998), with international cultivars and susceptible wheat 'Seri-82' (Dababat *et al*., 2015). At least 50 kg soil including plant roots for each *H. latipons* population was collected to prepare inoculum from wheat fields of Hatay, Gaziantep, Kilis and Mardin provinces at the end of the growing season in 2016 (Table 2).

The soil samples were washed and then the cysts were collected using the modified Fenwick's (1940) extraction method as mentioned above. At least 10 000 cysts were collected from each sample and incubated at 4°C to be used as the nematode inoculum. After an incubation period at 4°C for 60 days, the cysts were exposed to 16°C to enhance hatching of second-stage juveniles (J2). The seeds of the standard cereal cultivars (12 barley cultivars, three oat cultivars and five wheat cultivars) shown as in Table 3 were pre-germinated in Petri dishes and then transplanted into plastic tubes (3 cm diam. × 15 cm length) filled with 100 cm^3 sterile soil mixture of compost and sand (7:3, v/v). There was one seed per tube with four replications (each pot represented one replicate). The

Table 2. *Heterodera latipons* populations from the studied provinces in Turkey used in the pathotype test.

Province	District	Location	Altitude (m a.s.l.)	Latitude	Longitude
Hatay	Kırıkhan	Mazmanlı	220	36°40′17″N	36°32′17″E
Gaziantep	Karkamış	Akçaköy Sınır	430	36°49′32″N	37°52′32″E
Kilis	Elbeyli	Çıldıroba	500	36°39′03″N	37°15′19″E
Mardin	Kızıltepe	Güneyli	409	37°05′07″N	41°18′30″E

Table 3. Reaction status of cultivars in the international differential set for cereal cyst nematode species against *Heterodera latipons* population from Turkey.

Entry (resistance gene)	Interaction with populations[1,2]							
	Hatay		Gaziantep		Kilis		Mardin	
Barley								
‘Varde’	5.67 ± 2.20	(+)	15.73 ± 1.33	+	2.60 ± 0.92	(–)	21.60 ± 2.03	+
‘Emir’(*Rha*E)	5.70 ± 1.32	(+)	5.53 ± 0.74	(+)	10.73 ± 1.65	+	6.03 ± 1.99	(+)
‘Ortolan’(*Rha*1)	0.13 ± 0.23	–	0.70 ± 0.75	–	0.77 ± 0.67	–	1.57 ± 0.81	–
‘Morocco’(*Rha*3)	0.53 ± 0.55	–	1.30 ± 0.92	–	2.53 ± 0.95	(–)	0.93 ± 0.38	–
‘Siri’(*Rha*2)	2.70 ± 1.31	(–)	5.47 ± 0.93	(+)	5.47 ± 1.42	(+)	5.07 ± 0.93	(+)
‘KVL191’(*Rha*2)	0.27 ± 0.31	–	2.77 ± 0.74	(–)	2.73 ± 0.35	(–)	2.90 ± 1.22	(–)
‘Bajo Aragon 1-1’	2.40 ± 0.79	(–)	1.57 ± 0.47	–	0.67 ± 0.47	–	1.60 ± 0.75	–
‘Herta’(*Rha*2)	0.93 ± 0.64	–	1.47 ± 0.65	–	0.17 ± 0.15	–	0.00 ± 0.00	–
‘Martin 403-2’	1.43 ± 0.32	–	2.50 ± 0.89	(–)	2.67 ± 1.27	(–)	2.80 ± 0.40	(–)
‘Dalmatische’	12.93 ± 1.55	+	12.50 ± 1.51	+	10.5 ± 0.70	+	6.33 ± 0.81	(+)
‘La Estanzuela’(*Rha*2)	6.10 ± 1.08	(+)	5.40 ± 0.82	(+)	2.60 ± 0.70	(–)	2.87 ± 1.16	(–)
‘Harlan 43’	6.13 ± 1.10	(+)	1.50 ± 0.66	–	1.53 ± 0.31	–	2.90 ± 1.82	(–)
Oats								
‘Sun II’	0.27 ± 0.15	–	1.30 ± 0.44	–	2.50 ± 0.96	(–)	0.37 ± 0.32	–
‘Pusa Hybrid Bsi’	0.40 ± 0.20	–	0.30 ± 0.26	–	1.57 ± 0.58	–	2.73 ± 1.17	(–)
‘Silva’	2.20 ± 0.17	(–)	0.93 ± 0.21	–	0.93 ± 0.67	–	10.27 ± 1.14	+
Wheat								
‘Capa’	28.20 ± 3.94	+	18.63 ± 0.91	+	15.67 ± 2.21	+	6.43 ± 1.75	(+)
‘AUS10894’(*Cre-1*)	21.13 ± 2.50	+	14.77 ± 3.18	+	17.17 ± 3.07	+	3.03 ± 1.36	(–)
‘Psathias’	8.33 ± 1.33	+	0.43 ± 0.31	–	1.60 ± 0.70	–	2.27 ± 0.50	(–)
‘Iskamish K-2-light’	4.60 ± 0.66	(+)	0.23 ± 0.25	–	1.70 ± 1.23	–	2.43 ± 1.46	(–)
‘Seri-82’(Susceptible)	62.53 ± 8.31	+	61.33 ± 1.53	+	67.00 ± 2.65	+	70.00 ± 9.64	+

[1]Data are the means of ten replicates.
[2]Phenotypic reaction, adopted from Cook & Rivoal (1998): –, resistant (<3% of white females produced on ‘Seri-82’(local)); (–), moderately resistant (3-5%); (+), moderately susceptible (5-10%); +, susceptible (>10%).

experiment was repeated twice. Just after transplanting, each tube was inoculated with 100 J2 of *H. latipons*. To obtain a final inoculum density of *ca* 400 J2, another two inoculations of 150 J2 of *H. latipons* were performed at 3-day intervals (Cui *et al*., 2016).

The inoculated plants were left to grow in a glasshouse for 2 weeks at 12-15°C, followed by 4 weeks at 16-18°C, and 6 weeks at 19-21°C with an artificial photoperiod of 16 h (Cui *et al*., 2015). Normal plant growth conditions of irrigation, fertilisation, and disease and insect control were routinely applied. Ninety days after the last nematode inoculation, roots of each plant and soil were gently washed with tap water over 800 μm and 250 μm sieves. White and brown cysts were collected from the 250 μm sieve and counted under the binocular microscope, then the mean number of cysts per plant was calculated. The number of new cysts produced was calculated, and the final density of *H. latipons* cysts was determined per tube.

All experiments were evaluated by counting the newly formed cysts on the root surface under the microscope.

The phenotypic reaction of the cultivars were categorised into four resistance reaction groups based on the mean number of white females per plant: '–' = resistant (<3% of white females compared to the number on 'Seri-82'); '(–)' = moderately resistant (3-5%), '(+)' = moderately susceptible (5-10%), '+' = susceptible (>10%) (Smiley *et al*., 2011) and taking into the account the reaction of the known control lines used in the study. All experiments were repeated twice. Data were analysed with analysis of variance (ANOVA) using SPSS 17.0 for Windows (SPSS). Differences among treatments were tested using one-way ANOVA followed by the Tukey test for comparison of means if the F-value was significant at $P < 0.05$.

Results

Genetic diversity of *Heterodera latipons* populations

The PCR amplification of all 25 cyst populations with TW81 and AB28 primers produced a single fragment of approximately 1040 bp. Populations from Hatay, Gaziantep, Kilis and Mardin were identified as *H. latipons* by BLAST analysis of the ITS sequences. All sequences of populations derived from this study were deposited in GenBank with the accession numbers, as listed in Table 1. The sequences were compared based on the ITS loci of the *H. latipons*, *H. filipjevi* and *H. avenae* populations as shown in Figure 1.

The relationship of the sequences of the ITS region of *H. latipons* from the four studied provinces was measured using neighbour-joining analysis, revealing intraspecific polymorphism among *H. latipons* populations. Based on the topology of the calculated majority role, the neighbour-joining tree, all populations were differentiated and grouped according to their province (Fig. 1). *Heterodera latipons* populations from different countries were grouped with the populations obtained in this study. However, *H. filipjevi* and *H. avenae* populations clustered separately from *H. latipons* populations.

Pathotype characterisation of *Heterodera latipons* populations

The numbers of white and brown cyst per plant were counted 12 weeks after inoculation. The average number

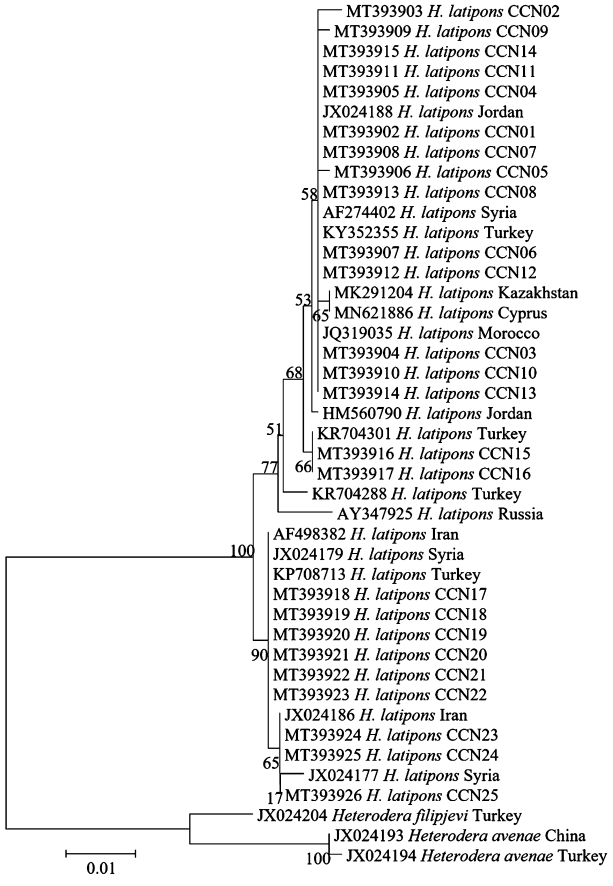

Fig. 1. Phylogenetic tree (neighbour-joining) constructed through the ITS sequence alignment from the 25 *Heterodera latipons* populations from this study and corresponding populations representing species from GenBank. Numbers on the branches represent bootstrap values obtained from 1000 bootstrap replications.

of cysts of *H. latipons* populations in different cultivars resulted in variable numbers of cysts on the root of the barley, oats and wheat cultivars. The control line 'Seri-82' was the most susceptible to the tested *H. latipons* populations, with a mean cyst number of 36.3.

The barley cultivars 'Ortolan', 'Morocco', 'KVL191', 'Bajo Aragon 1-1', 'Herta' and 'Martin 403-2' gave resistant or moderately resistant reactions to all studied *H. latipons* populations, whereas 'Emir' and 'Dalmatische' were susceptible or moderately susceptible to nematode populations (Fig. 2). 'Varde', 'Siri', 'La Estanzuela' and 'Harlan43' showed different reactions depending on *H. latipons* populations. 'Varde' was moderately resistant to Kilis population and susceptible or moderately susceptible to other populations. 'Siri' was moderately susceptible

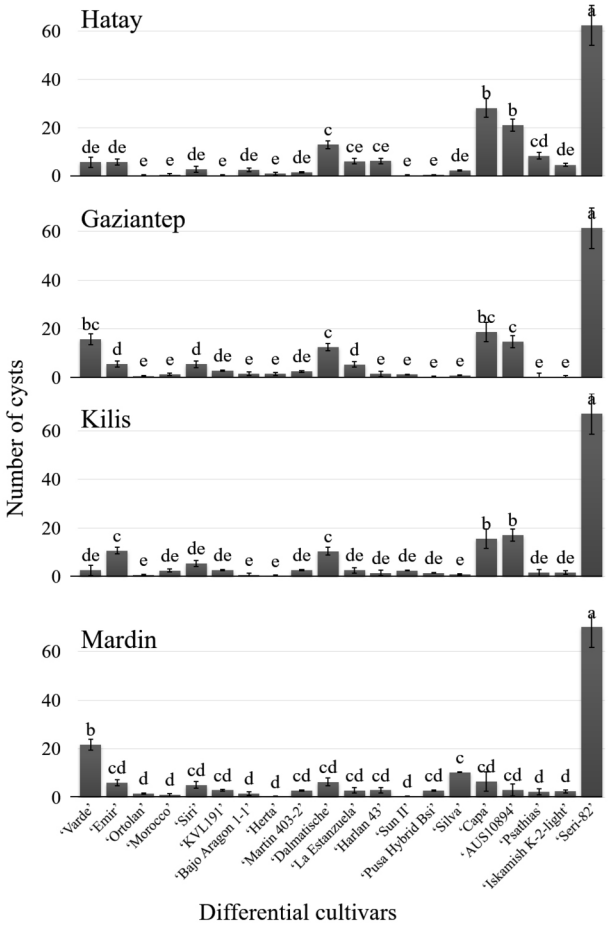

Fig. 2. The reaction of international standard differential hosts to the tested populations of *Heterodera latipons*. (Means with the same letter, in the same column, are not significantly different at $P = 0.05$, using the Tukey test.)

against Gaziantep, Kilis and Mardin populations, whereas it was moderately resistant to the Hatay population. 'La Estanzuela' was found to be moderately resistant to Kilis and Mardin, while it was moderately susceptible to the Hatay and Gaziantep populations. 'Harlan43' was moderately susceptible to the Hatay population, but it was resistant or moderately resistant against the Gaziantep, Kilis, and Mardin populations (Fig. 2). 'Emir', having the *Rha*E gene, showed susceptible reactions to all nematode populations at different levels. Some of the barley cultivars carrying resistance genes such as *Rha*1, *Rha*2 and *Rha*3 were detected as resistant or moderately resistant to Hatay, Gaziantep, Kilis and Mardin populations. 'Ortolan' (*Rha*1) and 'Morocco' (*Rha*3) were resistant to all nematode populations. 'KVL191' and 'Herta', having the *Rha*2 gene, were found resistant or moderately resistant to these nematode populations, but 'La Estanzuela', also with the *Rha*2 gene, was moderately resistant to Kilis and Mardin populations and susceptible or moderately susceptible to Hatay and Gaziantep populations. Similarly, 'Siri', with the *Rha*2 gene, was moderately resistant to the Hatay population and susceptible to Gaziantep, Kilis and Mardin populations (Table 3).

Three oats entries, 'Sun II', 'Pusa Hybrid' and 'Silva', were resistant or moderately resistant to the tested nematode populations, except that the Mardin population caused a susceptible reaction on 'Silva'. This crop was damaged by *H. latipons* in all experiments. Resistant entries could be used as donor parents to provide a source of resistance.

The wheat 'Capa' was susceptible or moderately susceptible to all nematode populations. 'AUS10894', containing the *Cre*1 gene, was not consistently effective against these populations in repeated trials under growth room conditions. The single-dominant *Cre*1 gene did not entirely halt the reproduction of the populations, as illustrated by the results of a recent test with selected entries (Fig. 2). 'AUS10894' showed only a moderate resistant reaction to the Mardin population, but susceptible reactions to the other populations. 'Psathias' and 'Iskamish K-2-light' were moderately resistant or resistant to Gaziantep, Kilis and Mardin populations and susceptible to the Hatay population.

Based on these results, Turkish populations were in the Ha1 group: the reactions of the barley, oats and wheat classified them as either Ha41 or Ha51. Barley 'KVL191' was resistant to all nematode populations but susceptible to Ha51, and the reactions of the other barley cultivars were also consistent with the Turkish populations being Ha51. Wheat 'AUS10894' was susceptible to all nematode populations but resistant to Ha41, and the reactions of 'Capa' and 'Loros' were also consistent with the Turkish populations being Ha51. The wheat 'Psathias' and 'Iskamish K-2-light' showed resistant and susceptible reactions, respectively, depending on the nematode populations, which were inconsistent with the Turkish populations being Ha41. However, the degree of susceptibility of all the wheat differentials distinguishes the Turkish populations from other pathotypes in the Ha1 group.

Discussion

The genera of cyst nematodes have been the most studied plant-parasitic nematodes on wheat, barley and

oats (Cook & Noel, 2002; Nicol, 2002; Nicol *et al*., 2003). Several studies have shown intraspecific variation concerning nematode behaviour on the host (Cook & Noel, 2002; Dababat & Fourie, 2018). 'Host races' or biological races of a nematode species were distinguished by an inherited ability or inability to parasitise certain host species (Subbotin *et al*., 2010). Pathotypes are known as physiological races distinguished by inherited ability or inability to reproduce on certain cultivars or lines of a host plant species (Cook & Rivoal, 1998; Cook & Noel, 2002; Mc Donald & Nicol, 2005). Pathotypes have become an important part of intraspecific classification for some cyst nematode genera, such as *Globodera* and *Heterodera* (Subbotin *et al*., 2010). CCN attacking the roots of cereals and grasses contain a complex of 12 species and their intraspecific pathotypes (Rivoal & Cook, 1993; Mc Donald & Nicol, 2005).

The intraspecies variation occurs phenotypically either as pathotypes or as ecotypes, which are explained as a specific heritable adaptation to the different climates in which they evolved (Rivoal & Cook, 1993). In the present study, the populations of *H. latipons* showed an intraspecific polymorphism. The nematode populations were divided into different groups within the phylogenetic tree constructed by the neighbour-joining algorithm method based on the ITS sequences. Likewise, Rivoal *et al*. (2003) and Madani *et al*. (2004) demonstrated intraspecific variation among *H. latipons* populations based on molecular methods. Toumi *et al*. (2013) reported that intraspecific polymorphism occurred in the *H. latipons* populations of Syria. İmren *et al*. (2012) observed genetic variation in some specimens of *H. latipons* in the Mediterranean region of Turkey. However, Toktay *et al*. (2015) did not detect any genetic variation in three *H. latipons* populations from the East Anatolian region of Turkey. This grouping in the phylogenetic tree was not directly associated with pathotyping features. This intraspecific polymorphism within *H. latipons* populations collected from Turkey needs to be supported with comprehensive studies on more populations to check their pathogenic and biochemical characteristics.

There have been many global reports to determine pathotypes of CCN populations, especially those of *H. avenae*. Essentially, most pathotypes were found in European and South Asian populations of *H. avenae*. The common pathotype of *H. avenae* in south-east Australia is Ha13 (Brown, 1969; Yuan *et al*., 2010). Peng & Cook (1996) reported that pathotypes of *H. avenae* populations in China might differ considerably from those of other regions but they could not allocate the number of pathotypes using the scheme developed by Andersen & Andersen (1982). Al-Hazmi *et al*. (2001) demonstrated that *H. avenae* populations of Al-Kharj, Hail and Al-Gassim provinces in Saudi Arabia were Ha11 or Ha21 pathotypes. Yuan *et al*. (2010) reported a new pathotype (Ha43) in Xushui and Xingyang, China. In Turkey, there have been a few studies to identify pathotypes of cyst nematode species. İmren *et al*. (2013) stated that *H. avenae* populations in Adana (Sariçam) and Hatay (Reyhanlı and Kırıkhan) were classified as Ha21 pathotype. Özarslandan *et al*. (2010) showed that the reaction of the *H. filipjevi* population obtained from Yozgat province on the differential lines was different to the other five known *H. filipjevi* populations studied by Ireholm (1994). Toktay *et al*. (2013) reported that *H. filipjevi* populations of Kahramanmaraş (Afşin and Elbistan) and Yozgat (Çiçekdağı) were Ha33 pathotype.

There was only one report about the identification of *H. latipons* pathotypes by Scholz (2001). This is the second report for the world and the first report for Turkey on variation in virulence of *H. latipons* populations collected from Hatay, Gaziantep, Kilis and Mardin provinces. Based on the results, the Turkish populations were in the Ha1 group: the reactions of the barley, oats and wheat classified them as either Ha41 or Ha51.

Barley 'KVL191' was resistant to three nematode populations but susceptible to Ha51, and the reactions of the other barley cultivars were also consistent with the Turkish populations being Ha51. Wheat 'AUS10894' was susceptible to three nematode populations but resistant to Ha41, and the reaction of 'Capa' was also consistent with the Turkish populations being Ha51. The degree of susceptibility of certain wheat differentials discriminate the Turkish populations from other pathotypes in the Ha1 group.

Scholz (2001) reported that 'Ortolan' (*Rha*1), 'Morocco' (*Rha*3), 'KVL191' (*Rha*2), 'Bajo Aragon 1-1', 'Herta' and 'Martin 403-2' were resistant to the Syrian *H. latipons* populations. Likewise, the response of these cultivars was resistant to all tested nematode populations in the present study. Scholz (2001) informed that 'Siri' (*Rha*2) were resistant to the Syrian nematode populations. This genotype was moderately resistant against the Hatay population, but it was susceptible to Gaziantep, Kilis and Mardin populations in this study (Fig. 2).

All oat genotypes, 'Sun II', 'Pusa Hybrid Bsi' and 'Silva' were resistant to the nematode populations, except 'Silva' to the Mardin population. However, Scholz

(2001) stated that they were susceptible to Syrian populations. Wheat 'Capa' and 'AUS10894' (*Cre*-1) were susceptible to four and three nematode populations, respectively, from Turkey, and they were also susceptible to Syrian populations (Scholz, 2001). Moreover, 'Psathias' and 'Iskamish K-2-light' were moderately resistant or resistant to the populations of Gaziantep, Kilis and Mardin, but they were susceptible to the Hatay population (Fig. 2). Also, they were resistant or moderately resistant to Syrian populations.

Heterodera latipons causes a reduction of wheat yield, highly valued in Turkey (Dababat *et al*., 2014; İmren *et al*., 2015). Damage caused by nematodes and consequent yield reduction are known to be related to a range of factors including nematode pathotype and ecotype (Rivoal & Cook, 1993; Smiley, 2005). The use of resistant cultivars requires a sound knowledge of the virulence spectrum for the targeted species and pathotypes present in each region. However, with the use of resistant cultivars, the CCN virulence pathotypes could shift rapidly and population purity also affects the pathotypes (Cook & Rivoal, 1998). Further intraspecies diversity occurs as pathotypes with different inheritable capacities for reproducing on specific genotypes of a host plant species, and as ecotypes with specific heritable adaptation to the different climates in which they evolved. Integrated control options have been established with various rotation schemes and the use of resistant cultivars constitutes the most appropriate management option to maintain population densities below damaging levels. The results of the present work can provide reference data for breeding resistant wheat varieties in Turkey.

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