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Orijinal araştırma (Original article)

Pathotype determination of the cereal cyst nematode, *Heterodera avenae* (Wollenweber, 1924) in the Eastern Mediterranean Region in Turkey

Tahıl kist nematodu, *Heterodera avenae*'nın Doğu Akdeniz Bölgesi patotipinin belirlenmesi

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Summary

Karlik (Adana-Sarıcam), Imece (Hatay-Kırıkhan) and Besaslan (Hatay-Reyhanlı) populations were used to determine the pathotype of the cereal cyst nematode, *Heterodera avenae,* in the Eastern Mediterranean Region of Turkey. The pathotypes of *H. avenae* were investigated by using "The International Test Assortment of Cereal Cultivars". The test was conducted on twelve barley, six oat, six wheat and four control lines (*milan, seri, silverstar* and *croc*). Test materials were grouped by three the nematode populations' virulence on resistance (*Rha*"E", *Rha*1, *Rha*2, *Rha*3, *Cre*1) and nonresistance genes, varieties and lines. According to results, *Rha*1 and *Rha*3 genes gave a resistance response but *Rha*2 and *Cre*1 did not. As a result, all populations demonstrated similar reactions and the three nematode populations were consistent with reactions for the Ha21 pathotype of the Ha1 group. This result is the first report on determination of the *H. avenae* pathotype in Turkey.

Key words: Nematode, pathotype, resistance, susceptible, virulence

Özet

Doğu Akdeniz Bölgesi'nde Tahıl kist nematodu, *Heterodera avenae* patotiplerinin belirlenmesi amacıyla Karlik (Adana-Sarıcam), İmece (Hatay-Kırıkhan) ve Besaslan (Hatay-Reyhanlı) popülasyonları kullanılmıştır. *H. avenae*'nin patotipleri "Uluslararası konukçu tahıl test ayırım materyalleri" kullanılarak araştırılmıştır. Bu materyaller içerisinde bulunan, yirmi adet arpa, altı adet yulaf ve altı adet buğday çeşit ve hatları ile dört kontrol hattı (*milan, seri, silverstar* ve *croc*) denemeye alınmıştır. Test materyalleri içerisinde dayanıklılık genleri (*Rha*"E", *Rha*1, *Rha*2, *Rha*3, *Cre*1) taşıyan ve dayanıklı geni taşımayan çeşit ve hatlara karşı, denemeye alınan 3 popülasyonun gösterdiği reaksiyon esas alınarak gruplandırılmıştır. Çalışma sonuçlarına göre, *Rha*1 ve *Rha*3 genleri taşıyan materyaller dayanıklılık göstermesine karşın, *Rha*2 ve *Cre*1 genleri taşıyanlar aynı tepkiyi göstermemiştir. Sonuç olarak konukçu test ayrım hatları genellikle benzer reaksiyonlar göstermiş ve denemeye alınan 3 popülasyonun da aynı patotip, Ha1 grubundan Ha21 olduğu saptanmıştır. Bu sonuçlar Türkiye'de *H. avanea* patotipinin belirlenmesi açısından ilk kayıt niteliğindedir.

Anahtar sözcükler: Nematod, patotip, dayanıklılık, hassas, virülens

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Introduction

The genus *Heterodera* includes 80 species (Subbotin et al., 2010) and, among them, twelve species in the *H. avenae* group include a subgroup of species called cereal cyst nematodes (CCN) that damage cereals and grasses. Three species, *H. avenae* (Wollenweber, 1924), *H. filipjevi* (Madzhidov, 1981) Stelter and *H. latipons* (Franklin, 1969) are economically the most important cyst nematodes (Rivoal & Cook, 1993; McDonald & Nicol, 2005; Smiley & Nicol, 2009). These nematodes are a major constraint to cereal production and one of the most important groups of nematodes in many parts of world. *Heterodera avenae* has wide distribution in temperate wheat-producing regions throughout the world, including North and South Africa, East and West Asia, Australia, Europe, the Indian subcontinent, the Middle East, and North America (Meagher, 1977; Sturhan & Rumpenhorst, 1996; Smiley & Nicol, 2009).

Heterodera avenae was also reported from wheat fields in Turkey (Yüksel, 1974). It has been increasingly detected and has become recognized as a damaging pathogen of wheat (*Triticum* spp.) and barley (*Hordeum vulgare* L.) cultivars, especially in the spring wheat-producing areas in the Aegean, Thrace and Eastern Mediterranean regions (Sturhan & Rumpenhorst, 1996; Gözel, 2001; Subbotin et al*.,* 2003; Abidou et al*.,* 2005; Imren et al*.,* 2010). For species identification, biochemical and morphological methods were used by Sturhan & Rumpenhorst, (1996) on numerous *H. avenae* and *H. avenae*-resembling populations from several geographic origins in Eastern Europe and west Asia. In Turkey, namely in the Central Anatolia Plateau, the *Heterodera* population's protein pattern indicated that the population was *H*. *filipjevi*, rather than *H. avenae*, which had previously been commonly understood (Rumpenhorst et al*.,* 1996). Later, *Heterodera avenae* and *H*. *filipjevi* were reported from Cukurova and the Central Anatolia Plateau, respectively, by using PCR-RFLP (Subbotin et al*.,* 2003; Imren et al*.,* 2012a).

Heterodera avenae is a severe pest of wheat that causes a great yield loss in wheat producing areas all around world (Smiley & Nicol*,* 2009). Resistant varieties, crop rotation and nematicides can reduce soil-borne nematode populations. Using resistant and tolerant wheat varieties offers the most effective, economic and environmentally friendly option for controlling nematode damage. However, resistance genes against the cereal nematodes are still not well incorporated into the local cultivars having high yield potential. Wheat varieties have different ability to resist nematodes. Virulence and reproductive capacity on the same host is highly variable among CCN species and among *H. avenae* and *H. filipjevi* pathotypes (Rivoal et al*.,* 2001; Mokabli et al*.,* 2002).

The *H. avenae* group is highly heterogeneous with respect to virulence to specific host genotypes (Cook & Noel, 2002; McDonald & Nicol, 2005). The *H. avenae* pathotype description was first given by Andersen (1959) and classifications such as Arabic numerals (1, 2, 3, etc.), letters (A, B, C, etc.) and Roman numerals (I, II, III, etc.), were developed to indicate the diversity (Andersen & Andersen, 1982). This diversity was described and categorized by using test cultivars in what is called "The International Test Assortment" for defining cereal cyst nematode pathotypes (Table 1). The test uses 12 barley, six oat (*Avena sativa* L.), and six wheat differential cultivars to define selected pathotypes of *H. avenae*. Initially, the pathotypes are broadly classified within one of three groups; 1, 2, 3. Pathotype groups 1 and 2 are widely distributed in Europe, North Africa and Asia (Al-Hazmi et al*.,* 2001; Cook & Noel, 2002; Mokabli et al., 2002; McDonald & Nicol, 2005) and group 3 is mostly found in Australia, Europe, and North Africa (Rivoal & Cook, 1993; Mokabli et al*.,* 2002). Additional pathotypes that do not fit this test assortment scheme have also been identified (Smiley et al., 2011). Characterization of the cereal cyst nematode species and pathotype is essential for developing resistance in breeding and nematode management programs.

The objective of the present study was to characterize the pathotype of three populations of *H. avenae* collected from the two major spring wheat-producing provinces (Adana and Hatay) in the Eastern Mediterranean Region of Turkey.

Material and Methods

Soil sampling

Cereal cyst nematode populations were collected from three different locations; Karlik (Adana-Sarıcam; 37º 10' 44 N-35º 28' 18 E), İmece (Hatay-Kırıkhan; 36º 27' 31 N- 36º 18' 16 E) and Besaslan (Hatay-Reyhanlı; 36º 13' 27 N-36º 80' 18 E).

Nematode extraction and identification from field samples

Soil samples for *H. avenae* were taken from infested wheat fields at the end of growing season, Cysts were extracted from soil by flotation technique (Kort, 1960). The cysts were identified using molecular markers (PCR-RFLP) and morphological features (Subbotin et al*.,* 2000; Tanha Maafi et al*.,* 2004).

Nematode hatching

About 1,000 cysts were hand collected under a stereoscopic microscope from each population. Hatching of *H. avenae* cysts was studied at two different incubation stages. For the first stage, all cysts were exposed to 4ºC for 66 days. For the second stage, each population was transfered and stored at 10 ºC for 4 months (Imren et al*.,* 2012b).

In vitro pathogenicity testing

To determine the *H. avenae* pathotypes, "The International Test Assortment" (Andersen & Andersen, 1982) for barley, oat and wheat entries was applied, plus two extra susceptible wheat cultivars (*Seri, Milan*) and two extra wheat cultivars (*Croc_1/Ae. squ, Silverstar*) that carry the *Cre*1 resistance gene (Table 1).

Table1. List of barley, oat and wheat cultivars in the "The International Test Assortment" of cereal cultivars to define pathotypes of *Heterodera avenae*

Cereal Cultivars and resistance gene, if known		Origin	Nord Gen Seeds Codes
Barley	Varde	Norway	NGB 2081
	Emir (*Rha* "E")	Netherlands	NGB 6957
	Ortolan (*Rha*1)	Germany	NGB 11085
	Morocco(*Rha*3)	Denmark	NGB 11086
	Siri (*Rha*2)	Denmark	NGB 9637
	KVL 191 (*Rha*2)	Denmark	NGB 8802
	Bajo Aragon (*Rha*2)	Denmark	NGB 11092
	Herta	Sweden	NGB 5083
	Martin 403-2 (*Rha*3)	Denmark	NGB 11093
	Dalmatische		NGB 11096
	La Estanzuela	Denmark	NGB 11094
	Harlan 43	Denmark	NGB 11095
Oat	Sun II	Denmark	NGB 11087
	Pusa Hybrid BS1	Denmark	NGB 11088
	Silva	Germany	NGB 8778
	MK. H. 72-646	Denmark	NGB 11097
Wheat	Capa		NGB 4823
	Aus 10894 (*Cre*1)	Denmark	NGB 11099
	Loros x Koga (*Cre*1)	Denmark	NGB 11090
	Psathias	Australia	NGB 11098
	Iskamish K-2 Light	Afghanistan	NGB 11091

Plastic tubes 13 cm long and 3 cm in diameter were filled with autoclaved soil mixture (70% sand, 29% clay, 1% organic matter). Each tube was filled with 80 g of the soil mixture and one pre-germinated seed was planted in it.. Immediately after planting, 200 freshly hatched second stage juveniles (J_2) of *H. avenae* were dispensed onto the soil surface of each tube (Imren et al*.,* 2012b). Plants were placed under controlled conditions in a growth room at 21 ºC, 16 hrs of artificial light and 60-70% of relative humidity. Twelve weeks after inoculation, plants were harvested and roots washed gently under a stream of tap water to free adhesive soil particles from swollen white females and brown-colored cysts attached to the roots. Slightly wet soil in the bucket was then stirred for 10 s and left for about 30 s to allow the heavy sand and soil debris to settle out while cysts and organic matter were poured through sieves of 850µm and 250µm aperture. The process was repeated 3 times to ensure all white females and brown cysts were extracted. Cysts were collected from the lower sieve (250µm) and the soil in the bucket and the roots were examined to determine if cysts or white females had not been extracted. Extracted cysts were then counted under a stereoscopic microscope.

Statistical analysis

Resistance and susceptibility were defined on the basis of cyst availability and the number on roots and in soil. Host plant reaction upon infection was defined as resistant when less than three white females were found in plant roots, and as susceptible if more than three were found (Mathur et al., 1974; Ireholm, 1994). Evaluation was based on cyst number within each resistant and susceptible group. The experiment was set up in a randomized complete block design and each treatment was replicated 3 times. Responses for the tested differentials were compared with those given by Andersen and Andersen (1982) and Rivoal and Cook (1993) to characterize the pathotype.

The results were analyzed according to standard analysis of variance procedures with the SPSS 14 program for Windows 2000X. Differences among treatments were tested using one way analysis of variance (ANOVA) followed by the Tukey test for comparison of means if the F-value was significant. Statistical differences were considered significant if $P \leq 0.05$.

Results and Discussion

Quantitative test results for pathotype identification of the Imece, Besaslan and Karlik populations are presented as the average number of swollen white females and brown cysts extracted from each plant and the soil in which that plant grew (Table 2). Plants in the International Test Assortment had similar reactions to each *H. avenae* population. The Karlik population had the highest nematode numbers when compared to Imece and Besaslan populations. Our results indicated that all nematode populations induced a resistance response when exposed to the resistance genes from barley cultivars, Ortolan (*Rha*1) and Morocco (*Rha*3). Although having the *Rha*2 gene, Siri and KVL 191 showed a resistance response, and Bajo Aragon showed reactions for susceptibility. Moreover, Emir, which contained the *Rha*E gene, was found to be susceptible (Table 2).

The oat cultivars, Sun II, Pusa Hybrid BS1 and Silva, showed a resistance reaction, whilst MK. H. 72-646 showed a susceptible' response (Table 2). The wheat cultivars, Capa and Aus 10894 (*Cre*1) mostly showed a susceptible response to nematode infection. Loros x Koga (*Cre*1), Psathias and Iskamish K-2 were resistant. However, Capa, Aus 10894 (*Cre*1) and Iskamish K-2 Light did not react the same to Imece, Karlik and Beasalan nematode populations. Furthermore, the *Cre*1 resistance genes induced different susceptible and resistance reactions on Aus 10894 and Loros x Koga (Table 2). Results were determined on the basis of Subbotin et al*.* (2010) and totally matched nematode populations categorized as the H21 pathotype. While 11 cultivars were divergent according to Subbotin et al*.* (2010), in our study, none of them was found to exhibit a divergent reaction.

Table 2. Total number of swollen white females and brown cysts extracted from roots and soil of plants of the International Test Assortment of cereal cultivars used to evaluate populations of *Heterodera avenae* collected near Imece, Karlik and Basaslan towns, Turkey

	Number of Female			Reaction
	Imece	Karlık	Besaslan	
Barley				
Varde	10,0±3,6	17,7±1,5	16,0±1,0	S
Emir	14,7±2,5	22,3±1,5	17,7±1,5	S
Ortolan	&	13,0±2,6	13,3±0,6	R
Morocco	4,3±2,5	10,7±3,1	7,0±1,0	R
Siri	3,0±3,0	9,0±1,5	&	R
Kvl 191	7,7±4,3	13,7±2,5	15,0±1,0	R
Bajo Aragon	5,3±5,3	10,0±1,0	12,3±0,6	R
Herta	19,3±1,5	19,7±8,7	18,0±3,0	S
Martin 403-2	4,0±2,6	8,7±1,5	8,3±0,6	R
Dalmatische	15,7±3,5	22,3±1,5	20,7±3,1	S
La Enstuanzuela	17,0±3,0	&	21,0±2,0	S
Harlan 43	14,0±1,0	20,3±2,5	17,7±1,5	S
Oats				
Sun II	&	&	7,7±0,6	R
Pusa Hybrid Bsi	8,0±1,7	&	&	R
Silva	4,3±1,0	10,0±2,0	5,7±1,5	R
Mk H. 72-646	13,0±4,6	23,3±2,5	23,0±1,0	S
Wheat				
Capa	17,7±2,1	17,7±1,5	&	S
Aus 10894	13,0±5,6	&	23,0±1,0	S
Loro x Koga	4,7±2,5	9,0±3,6	11,0±2,6	R
Psathias	7,7±0,6	11,0±1,0	10,7±1,2	R
Iskamish K-2 Light	&	12,0±2,0	&	R
Check Lines (Wheat)				
Milan	11,0±2,0	19,3±3,5	16,7±1,5	(S)
Silverstar	3,0±1,5	8,0±2,0	7,0±3,6	R
Seri	18,7±2,5	25,7±2,1	21,0±2,6	S
Croc_1/Ae. Squ	8,7±1,5	16,7±3,1	14,7±0,6	(R)

*. S= Susceptible, R= Resistance, ( ) = Moderate, &: Divergent.

Nematode reproduction and host reaction of the International Test Assortment to three Turkish *H. avenae* populations confirmed that the inoculum sources were virulent. Responses for the tested differential were compared, mainly on the basis of Subbotin et al. (2010) and were also compared to the findings of Romero et al. (1996), Al-Hazmi et al. (2001), McDonald & Nicol (2005) and Turner & Rowe (2006) to characterize the pathotype. According to the scheme of Subbotin et al. (2010) and its subsequent revisions, three primary groups of pathotype are distinguished by the reactions of barley, oats and wheat differentials. The similar responses of the differentials indicated that the tested Turkish populations of *H. avenae* are predominately the same pathotype. Based on Subbotin et al. (2010), the Turkish populations were in the Ha1 group and, according to the reactions of barley, oats and wheat they were classified as the Ha21 pathotype.

The response of the barley cultivars, Varde, Martin 403-2, Dalmatische, La Enstuanzuela and Harlan 43, to the nematode was response was variable (McDonald & Nicol, 2005; Turner & Rowe, 2006; Subbotin et al., 2010) but these differentials were constant in our study. Varde, Martin 403-2, La Enstuanzuela results showed similarities with Al-Hazmi et al., (2001) and Romero et al., (1996). While Dalmatische and Harlan 43 are resistant to the Spanish Torralba de Calatrava (P2) *H. avenae* population (Romero et al., 1996), they are susceptible to the Turkish nematode population.

Although *Heterodera avenae* prefers oats as a host, there could not find any *H. avenae* virulence in some Asia and Europe countries. The pathotype specific reaction on oats could be valuable for improving

the classification and it would be desirable to test more spring and winter oats. In this study, Oat Silva and MKH 72-646 were resistant and susceptible, respectively, but they were indicated as divergent (McDonald & Nicol, 2005; Turner & Rowe, 2006; Subbotin et al*.,* 2010). Moreover, Oat Silva showed the same host reaction as reported in Al-Hazmi et al*.* (2001) and Romero et al*.* (1996).

The partial susceptibility of the three wheat varieties and the new combination of virulence in the studied populations supports the proposition that populations of *H. avenae* may be highly heterozygous For wheat, Capa, Aus 10894 and Iskamish K-2 Light were also indicatative of a divergent response (McDonald & Nicol, 2005; Turner & Rowe, 2006; Subbotin et al*.,* 2010), whereas, in this study, these differentials gave different responses. Capa showed a susceptible response to Imece and Karlik populations, as reported by Al-Hazmi et al*.* (2001) and Romero et al*.* (1996). Although, Capa wheat showed resistance response to the Besaslan population, Aus 10894 showed susceptible response to Imece and Besaslan populations not for Karlik populations that these results shared similarities with Al-Hazmi et al*.,* (2001) and Romero et al*.,* (1996) studies. Iskamish K-2 Light was resistant to Imece and Karlik populations but it was susceptible to the Besaslan population; the reaction to the Besaslan population showed similarities to the findings of Al-Hazmi et al*.* (2001) and Romero et al*.* (1996).

In this study, most of the cultivars of barley and wheat with resistance genes gave a resistance response to all three populations and this is in agreement with previous studies (Romero et al*.,* 1996; Al-Hazmi et al*.,* 2001; McDonald & Nicol, 2005; Turner & Rowe, 2006; Subbotin et al*.,* 2010). *Heterodera avenae* reactions by hosts to the nematode populations were grouped on the basis of their virulence to resistance genes in six barley differentials, Emir (*Rha*"E"), Ortolan (*Rha*1), Morocco (*Rha*3), Siri (*Rha*2), Kvl191 (*Rha*2), La Enstuanzuela (*Rha*2) and two wheat differentials Aus 10894 (*Cre*-1) and Loros x Koga (*Cre1*) without resistance genes. *Rha*1 and *Rha*3 genes responded as resistant but *Rha*2 and *Cre*1 were determined not only resistant but also susceptible in the differentials. In conclusion, this study was carried out to determine the pathotype of *H. avenae* in Turkey. Furthermore, this study demonstrated that the studied Turkish *H. avenae* populations (Imece, Karlik and Besaslan) belong to Ha21 pathotype. However, comprehensive studies should be conducted with more populations to check their pathogenic and biochemical characteristics.

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