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Orijinal araştırma (Original article)

Pathotype characterization of the cereal cyst nematode *Heterodera filipjevi* (Madzhidov, 1981) Stelter in Turkey

Tahıl kist nematodu, *Heterodera filipjevi* (Madzhidov, 1981) Stelter'nin patotipinin belirlenmesi

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Summary

Turkey is ranked among top ten wheat producing countries in the world. Cereal cyst nematodes are considered one of the most damaging plant parasitic nematodes on wheat worldwide. Up to date, the most effective method of controlling this group of nematodes is the use of crop rotation and resistant varieties. The successful development of disease-resistant plants is contingent on knowledge of the pathotypes of the causal agent. In this study, three populations of *Heterodera filipjevi* (Madzhidov, 1981) Stelter were collected from different locations (Afsin, Elbistan and Yozgat) in Turkey in order to identify their pathotype using "The International Test Assortment of Cereal Cultivars". The results showed that two populations gave a similar reaction to the resistant and susceptible varieties. These populations were determined as Ha 33 pathotype which is a new report for Turkey.

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

Özet

Türkiye dünya buğday üretiminde ilk on ülke arasında yer almaktadır. Tahıl kist nematodları dünya genelinde buğday yetiştiriciliğinde en zararlı bitki paraziti nematod gruplardan biri olarak bilinmektedir. Bugüne kadar yapılan çalışmalara göre bu nematod gurubu ile en etkin mücadele yöntemi münavebe ve dayanıklı çeşit kullanımıdır. Nematodlara karşı dayanıklı çeşit geliştirmede en önemli unsur hedeflenen alanlardaki nematodların popülasyonlarına ait patotiplerin belirlenmesidir. Bu çalışmada Orta Anadolu Bölgesi'nde buğday alanlarında yaygın bulunan ve önemli zarara neden olan *Heterodera filipjevi* (Madzhidov, 1981) Stelter' nin patotipini belirlemek amacıyla üç farklı lokasyondan (Afsin, Elbistan and Yozgat) alınan popülasyonlar "Uluslararası test materyalleri" kullanılarak denemeye alınmıştır. Bu araştırma sonucunda her üç populasyonun *H. filipjevi*'nin Ha3 gurubu Ha 33 patotipine ait olduğu belirlenmiştir.

Anahtar sözcükler: Nematod, buğday, dayanıklı, hassas, virülens

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Introduction

World crop production is thought to be reduced 10% by damage from plant-parasitic nematodes (Whitehead, 1997). The most important plant-parasitic nematode species affecting wheat are in the genera of *Heterodera*, *Pratylenchus*, *Meloidogyne*, *Ditylenchus*, *Tylenchorhynchus* and *Merlinius*, *Paratrichodorus*, and *Anguina* (Rivoal and Cook, 1993; McDonald and Nicol, 2005; Nicol and Rivoal, 2008; Bockus et al., 2009). Cyst nematodes are the most studied plant-parasitic nematodes on wheat (Cook and Noel 2002; Nicol 2002; Nicol et al., 2003). The *Heterodera avenae* group is a complex of 12 species and intraspecific pathotypes that invade roots of cereals and grasses (Handoo, 2002; Subbotin et al., 2010). The most studied and economically important species in wheat growing areas of Turkey are *H. filipjevi*, *H. avenae* and *H. latipons* (Rumpenhorst et al., 1996; Gözel, 2001; Subbotin et al., 2003; Nicol, 2002; Şahin et al., 2009; İmren et al., 2010; 2012a; Toktay et al., 2012) These three species have been also reported from many locations around the world Cereal cyst nematodes (CCN) cause up to 89% yield losses in winter wheat (Rivoal& Cook, 1993; Evans & Rowe, 1998, Nicol et al.,2003). The damage can be higher, especially in rain-fed wheat producing areas.

The most acceptable and economically effective method of managing CCN is through the use of resistant wheat germplasm. Resistance is defined as the capacity of a plant to prevent nematode reproduction (Cook and Evans, 1987). However, the use of resistance requires a sound knowledge of the virulence spectrum of the targeted species and pathotypes. The major challenge to control CCN is occurrence of individuals within species and also among populations from different regions which are highly variable in virulence and multiplication (fitness) characteristics on the same host (Rivoal et al., 2001; Mokabli et al., 2002). Pathotypes are differentiated by testing unknown populations against a matrix of cereals in “The International Cereal Test Assortment” (Andersen and Andersen, 1982; Cook and Noel 2002; McDonald and Nicol 2005; Turner and Rowe, 2006; Subbotin et al., 2010). The test distinguishes three primary groups based on host resistance reactions of three barley cultivars carrying the resistance genes Rha1, Rha2, and Rha3. Additional barley, oats and wheat differentials are used to define pathotypes within each group. Characterization of the CCN species and pathotypes is essential for resistance breeding and nematode management programs. To date, there is little or no information available to distinguish the differences among the *H. filipjevi* pathotypes of Turkey.

Therefore, the objective of this study was to characterize the pathotypes of three populations of *H. filipjevi* collected from major wheat-producing regions in Turkey.

Materials and Methods

Soil Sampling and Identification

Heterodera filipjevi populations were collected at the end of the growing season from three different wheat producing locations; Elbistan (Büyükyapalak; 38º 19' 02 N-37º 16' 34 E), Afşin (Merkez; 38º 16' 13 N- 37º 01' 09 E) and Yozgat (Yerköy; 39°39' 74 N - 34°25' 42 E) in Turkey.

The cysts were extracted from soil by the flotation technique (Kort, 1960). Nematodes were identified using molecular markers (PCR-RFLP) and morphological features (Subbotin et al*.,* 2000; Tanha Maafi et al*.,* 2003; Subbotin et al., 2010; Imren et al., 2012a).

Nematode Inoculum

An average of 1000 cysts was selected under a stereomicroscope from each population. Hatching of *H. filipjevi* cysts was carried out in two different incubation stages. In the first stage, all cysts were exposed to 4ºC for 66 days. In the second stage, each population was transferred and stored at 10 ºC for 4 months (Şahin et al., 2010; Imren et al*.,* 2012b).

Test cultivars

The growth room tests were conducted to determine the pathotype of three Turkish *H. filipjevi* populations using “The International Test Assortment” (Andersen & Andersen, 1982) of barley, oats and wheat entries; two extra susceptible wheat cultivars (*Seri, Milan*) and two extra wheat cultivars (*Croc_1/Ae. squ, Silverstar*) carrying the *Cre*1 resistance gene were included in the test (Table 1).

Table1. List of the cereal cultivars in the “The International Test Assortment” used to define pathotypes of *Heterodera filipjevi* (Andersen and Andersen, 1982)

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

A mixture of sterilized soil consisting of sand, field soil, organic matter (70: 29:1 %) was placed in plastic tubes (13 cm long and 3 cm in diameter) containing 80 g of the soil mixture and one germinated seed was planted.

A total of 200 freshly hatched J_2 of *H. filipjevi* were inoculated per screening tube immediately after plantings. Plants were grown in a controlled conditions room at 21 ºC, 16 hrs of artificial light and 70% relative humidity. Plants were bottom watered as needed to maintain soil moisture. Twelve weeks after inoculation, plants were removed from the tubes and roots washed in a gentle stream of tap water to free adhesive soil particles. The cysts were extracted using a Kort Elutriator device (Kort, 1960). The cysts and organic matter were filtered through sieves of 850µm and 250µm aperture. Extracted cysts were then counted under a stereomicroscope.

Evaluation

Resistance and susceptibility were defined on the basis of cyst availability and the numbers on roots and soil. Seven replicates of each line were used. ANOVA was done using SPSS 10.0 (SPSS Inc., Chicago, IL, USA) program test. The evaluation was carried out according to Nicol et al. (2009) and İmren et al. (2012c), resistance categories were based on the number of cereal cyst nematode females per individual root system, according to resistant and susceptible check lines. Responses of the tested differentials were compared with those given by Subbotin (2010).

Results and Discussion

The development of *H. filipjevi* populations from Elbistan, Afşin and Yozgat on the international test cultivars is shown in Table 2. All three populations infected the cultivars and completed their life cycle on these cultivars.

Table 2. Reproduction of three Turkish *Heterodera filipjevi* populations on the International Test Assortment of Cereal Cultivars

Cereal cultivars and resistance gene, if known	Number of Female per plant		
	Afşin	Elbistan	Yozgat
Barley			
Varde	16,3±1,5	15,3±0,9	18,6±1,2
Emir	7,6 ±0,8	13,0±0,6	9,66±0,9
Ortolan	10,0±0,5	12,3±0,3	8,66±1,2
Morocco	4,3±0,8	7,6±0,3	5,3±0,9
Siri	11,3±0,8	14,3±0,9	10,3±0,9
Kvl 191	11,3±0,8	13,0±0,6	9,0±1,1
Bajo Aragon	12,3±0,8	13,0±0,6	15,0±0,6
Herta	8,0±0,6	13,0±0,6	7,0±1,1
Martin 403-2	4,3±0,9	8,3±0,3	5,3±1,7
Dalmatische	16,3±1,4	17,6±0,6	16,0±1,1
La Enstuanzuela	14,0±1,1	15,0±0,6	15,3±1,4
Harlan 43	12,0±1,1	17,6±0,6	12,3±0,9
Oats			
Sun II	5,0±0,6	10,0±0,6	13,6±0,9
Pusa Hybrid Bsi	13,0±1,5	14,0±0,6	16,3±1,2
Silva	6,0±0,9	8,0±0,6	6,0±1,1
Mk H. 72-646	12,3±1,2	16,0±0,9	6,0±1,1
Wheat			
Capa	15,6±1,8	16,0±0,6	16,0±1,1
Aus 10894	13,0±0,6	17,0±1,5	10,3±0,9
Loro x Koga	10,0±0,6	15,0±0,6	19,30,9
Psathias	4,0±0,6	7,6±0,9	6,60,9
Iskamish K-2 Light	8,3±0,3	13,0±0,6	15,0±1,1
Check Lines (Wheat)			
Milan	11,3±0,9	14,0±0,6	13,6±0,9
Silverstar	3,66±,3	8,0±0,6	6,0±1,1
Seri	14,6±0,9	17,6±0,9	19,3±0,9
Croc_1/Ae. Squ	9,0±0,6	12,3±0,9	9,3±0,9

The pathotype identification of three populations according to scheme of Subbotin (2010) is shown in Table 3. Three primary groups of pathotype (Ha1, Ha2 and Ha3) were distinguished by the reactions of barley differentials. Within each group, additional pathotypes were identified by the reactions of additional barley, oats and wheat cultivars. The present study confirmed that the nematode populations belong to the Ha3 group and Ha33 pathotype. The Yozgat population seems more virulent than the Elbistan and Afsin populations of *H. filipjevi*. Similar responses of the differentials indicate that the three tested Turkish populations of *H. filipjevi* are predominately the same pathotype (Table 3).

The results for the resistance genes of the barley (*Hordeum vulgare* L.) cultivars, Emir (Rha E) Ortolan (Rha 1) Siri , KVL 191, La Enstuanzuela (Rha 2), indicated that all nematode populations induced a susceptible response. Although, Morocco (Rha 3) and Herta, mostly showed resistance reactions. The oats cultivars, Sun II, Pusa Hybrid Bsi and MkH. 72-646, showed a susceptible reaction, whilst Silva gave a resistance response. From all the differential wheat cultivars, Capa, Aus 10894, Loro x Koga and Iskamish K-2 mostly proved to be susceptible to infection. But, Psathias showed resistance to all populations and Iskamish K-2 Light also gave a resistance reaction to the Afsin population.

Table 3. Pathotype groups of three *Heterodera filipjevi* populations from Turkey defined on the International Test Assortment of Cereal Cultivars

Cereal cultivars and resistance gene, if known	*Heterodera filipjevi* pathotype (Subbotin, 2010)		Populations in this study		
			Afsin	Elbistan	Yozgat
	Ha23	Ha33	Ha33	Ha33	Ha33
Barley					
Varde	+	+	+	+	+
Emir (*Rha "E"*)	(+)	+	+	+	+
Ortolan (*Rha1*)	+	+	(+)	(-)	+
Morocco (*Rha3*)	-	-	-	-	-
Siri (*Rha2*)	+	+	+	+	+
Kvl 191 (*Rha2*)	"	"	+	+	-
Bajo Aragon(*Rha2*)	+	+	+	+	+
Herta	"	"	+	+	-
Martin 403-2 (*Rha3*)	+	+	-	-	-
Dalmatische	(-)	+	+	+	+
La Enstuanzuela	(-)	"	+	+	+
Harlan 43	-	+	+	+	+
Oats					
Sun II	+	+	-	-	+
Pusa Hybrid Bsi	-	+	+	+	+
Silva	(-)	+	-	-	-
Mk H. 72-646	+	+	+	+	-
Wheat					
Capa	+	+	+	+	+
Aus 10894 (*Cre1*)	+	+	+	+	+
Loro x Koga (*Cre1*)	+	+	+	+	+
Psathias	+	-	-	-	-
Iskamish K-2 Light	+	+	-	+	+
Check Lines (Wheat)					
Milan (Susceptible)			+	+	+
Silverstar (Resistant)			-	-	-
Seri			+	+	+
Croc_1/Ae. Squ.			(-)	(-)	(-)

+=susceptible;-=resistant; ()=intermediate; "=no observations.

Resistance or susceptibility in barley, oat and wheat determined in this study is similar to that in other related studies by Ireholm (1994), Cook & Rivoal (1998), McDonald & Nicol (2005), Turner & Rowe (2006) and Subbotin et al*. (*2010). Ozarslandan et al. (2010) studied only one nematode population from Turkey which did not provide adequate evidence to determine pathotype. In the present study, *Heterodera filipjevi* populations were classified according to their virulence against resistance genes in six barley differentials, Emir (*Rha*"E"), Ortolan (*Rha*1), Morocco (*Rha*3), Siri (*Rha*2), Kvl191 (*Rha*2) and La Enstuanzuela (*Rha*2), and two wheat differentials, Aus 10894 (*Cre*-1) and Loro x Koga (*Cre*-1), and compared to other germplasms without resistance genes. None of the resistance genes resisted the three Turkish populations *of H. filipjevi,* except *Rha 3 (Morocco and Martin 403-2 cultivars).* Also in this study*,* the *Cre-1* gene was susceptible to all Turkish populations which was also reported by Ozarslandan et al. (2010), Sahin et al. (2010) and Toktay et al. (2012). So far, only two pathotypes of *H. filipjevi*, Ha23 and Ha33, have been reported (Subbotin et al. 2010). All three populations tested in this study belonged to the Ha33 pathotype. A similar study was carried out for the determination of *H. avenae* populations from the eastern Mediterranean region of Turkey and the Ha21 pathotype was identified (İmren et al., 2012c).

In conclusion, the pathotype results from the present study should be considered when breeders conductprograms to develop resistant varieties in Turkey. Nevertheless, further studies are needed on CCN pathotypes to identify their pathogenicity against to cereal cultivars cereal cultivars in Turkey which ultimately benefit the breeding programs in Turkey and the region as well.

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