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Orijinal araştırma (Original article)

Identification of genetic resistance to cereal cyst nematodes; *Heterodera avenae* (Wollenweber, 1924), *Heterodera filipjevi* (Madzhidov, 1981) Stelter and *Heterodera latipons* (Franklin, 1969) in some international bread wheat germplasms[1]

Uluslararası bazı ekmeklik buğday çeşitlerinin Tahıl kist nematodları, *Heterodera avenae* (Wollenweber, 1924), *Heterodera filipjevi* (Madzhidov, 1981) *Stelter* and *Heterodera latipons* (Franklin, 1969*)* karşı genetik dayanıklılığının belirlenmesi

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Summary

The cereal cyst nematodes, *Heterodera avenae* group, are known as parasites of cereals worldwide. In Turkey, the cereal cyst nematodes, *Heterodera filipjevi*, *H. avenae* and *H. latipons*, are the three known species infested wheat fields and cause high yield losses. The using of resistance varieties is one of the most effective methods for controlling cereal cyst nematodes. Recently, resistance genes (*Cre* genes) which are obtained from wild wheat genotypes have been transferred into bread wheat varieties to control the cereal cyst nematodes species. In this study, the efficiency of some sources of resistance (*Cre*R, *Cre*1, *Cre*2, *Cre*3, *Cre*7 and *Cre*8) in wheat against some Turkish *H. avenae*, *H. filipjevi* and *H. latipons* populations was investigated *in-vitro* conditions. According to results, the effect of resistance genes showed variation depending on different Cereal cyst nematode populations; *H.avenae*, *H. filipjevi* and *H. latipons*. Although *Cre1* gene was only found as completely resistant to all (three) nematode species, *Cre*3 and *Cre*7 were found resistant to *H. avenae* and *H. latipons*. *Cre* R was also determined as resistant to *H. filipjevi* and *H. latipons populations but Cre*8 was only found resistant against to *H. filipjevi* population. No resistance was found in *Cre 2* gene against to all nematode populations. Additionally, 2 resistance gene-free variety and lines were found resistant to *H. avenae*; 3 wheat lines to *H. filipjevi* and 11 wheat genotypes were found moderately resistant to *H. latipons*.

Key words: Cereal cyst nematodes, resistance, *Cre* genes, wheat

Özet

Tahıl kist nematodları, *Heterodera avenae* group, buğdayın önemli zararlılar arasında yer almaktadır. Türkiye'de buğday alanlarının Tahıl kist nematodları, *Heterodera filipjevi, H. avenae* ve *H. latipons*'la bulaşık olduğu bilinmektedir. Dayanıklı çeşit ve hatların Tahıl kist nematodları karşı kullanılması en önemli mücadele yöntemlerinden biridir. Son zamanlarda, Tahıl kist nematodlarına karşı buğdayın yabani formlarından elde edilerek ekmeklik buğday çeşitlerine aktarılmış birçok dayanıklılık (*Cre* genleri) geni geliştirilmiştir. Bu çalışmada dayanıklılık genlerinin (*Cre* R, *Cre*1, *Cre*2, *Cre*3, *Cre*7, *Cre*8) *H.avenae*, *H. filipjevi* ve *H. latipons*'un ülkemizdeki bazı popülasyonlara karşı etkinlikleri *in-vitro* koşullarda araştırılmıştır. Çalışmada sonucunda Tahıl kist nematodları, *H.avenae*, *H. filipjevi* ve *H. latipons* popülasyonlarına göre dayanıklılık genlerinin etkinliklerinin değişiği; bunla birlikte *Cre*1'in her üç nematod türüne karşı tam bir dayanıklılığa sahip olduğu, *Cre*3 ve *Cre*7'nin *H. avenae* ve *H. latipons*'a, *Cre* R'nin *H. filipjevi* ve *H. latipons*'a, *Cre*8'in ise sadece *H. filipjevi* popülasyonlarına karşı dayanıklı olduğu ve *Cre*2'nin ise her üç nematod türüne karşı dayanıklılığa sahip olmadığı saptanmıştır. Ayrıca, dayanıklılık geni içermeyen çeşit ve hatlardan *H. avenae* karşı 2 adet, *H. filipjevi* karşı 3 adet, *H. latipons*'a karşı 11 adet buğday genotipi orta dayanıklı bulunmuştur.

Anahtar sözcükler: Tahıl kist nematodları, dayanıklılık, *Cre* genleri, buğday

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Introduction

Turkey is among the ten largest wheat producers in the world with 18-20 million tons of grain production cultivated on 9.5 million ha area (Anonymous, 2010). In Turkey, Wheat production is highly affected by biotic and a biotic stresses. One of the biotic stresses affecting wheat production is the plant parasitic nematodes which cause economic yield losses. In particular, the cereal cyst nematode, *Heterodera avenae* group, caused crop losses in the areas of high initial population densities has been reported in different countries (Rivoal & Cook, 1993; Evans & Rowe, 1998; Nicol et al., 2004; Nicol & Rivoal, 2008). In Turkey, the main three important species of the cereal cyst nematodes attaching wheat are *Heterodera filipjevi* (Madzhidov, 1981) Stelter was found in the Central Anatolian Plateau and caused losses of up to 50 % in wheat (Nicol et al., 2004; Sahin et al., 2009); *Heterodera avenae* (Wollenweber, 1924) and *Heterodera latipons* (Franklin, 1969) were found in 45% of surveyed area in the Southeastern Anatolia Region (İmren et al., 2011).

Several attempts were implemented to control the cereal nematodes around the world. Crop rotation, using resistant varieties and lines with different tillage techniques are recommended to control nematodes in wheat production. Chemical methods can be applied but they are not preferred by the producers because of the high cost per unit area in wheat field in Turkey. Resistance sources around the world were obtained from wild wheat relatives through breeding programs (Ogbonnaya et al., 2001a). It is reported that 9 resistance genes were transferred to control the cereal cyst nematode *H. aveane* in bread wheat; *Cre*1 gene from *Triticum aestivum*; *Cre*2, *Cre*5 and *Cre*6 genes *Aegilops ventricosa*; *Cre*3 and *Cre*4 genes in *Triticum tauschii*, *Cre*7 in *Aegilops* species*; Cre*8 and *Cre*R from *Secale cereale* lines were transferred (Barloy et al., 2007). Among resistance genes, Cre1 and Cre3 genes were present as advantageous lines for using in wheat breeding (Ogbonnaya et al., 2001a). In this point, the reaction of resistance in some *Cre* genes of *H. avenae* Australia pathotype Ha13 were reported as *Cre* 6>*Cre*1>*Cre*F≥*Cre*5 (Ogbonnaya et al., 2001b). Similarly, *Cre*2 and *Cre*5 genes showed moderately resistance against *H. avenae* Spanish pathotype, Ha71 (Montes et al., 2003). The efficiency of the *Cre* genes against *H. avenae* group has not been studied comprehensively in Turkey. However it was reported that some bread wheat lines carrying *Cre* genes and wild wheat genotypes did not show completely resistance response against the Turkish *H. filipjevi* populations from both Haymana and Yozgat (Özarslandan et al., 2008; Nicol et al., 2009; Akar et al., 2009; Şahin, 2010).

In this study, the efficiency of some international bread wheat varieties and lines carrying *Cre* genes against the cereal cyst nematodes, *H. avenae*, *H. filipjevi* and *H. latipons,* have been investigated in Turkey.

Material and Methods

Twenty-five international wheat lines were used to investigate their resistance response against the three *Heterodera* species *H. avenae*, *H. filipjevi* and *H. latipons* populations obtained from Eastern Mediterranean region of Turkey. Among those lines, seven lines have *Cre* genes coming from wild relatives of wheat *Aegilops* genus (Nicol & Rivoal, 2008). The experiment was conducted at Biological Control Research Station in 2010 -2011.

In this study, different populations of *H. avenae* (Hatay-Besaslan, Imece and Adana-Karlik), *H. filipjevi* (Yozgat and Kahramanmaras–Elbistan, Afsin) and *H. latipons* (Gaziantep–Elbeyli, Karkamıs) species were used as coordinates listed in (Table 1). Nematode inoculums were collected from infested

wheat fields and cysts were extracted by Court apparatus (Kort, 1960; Shepherd, 1986). About 1000 cysts were collected for each population. Cysts were surface sterilized by 0.5% NaOCl. Then, cysts were stored at 4°C for two months before incubated at 10°C for 200 days and after then placed at room temperature to encourage hatching (Imren et al., 2012).

Seeds of each cultivar were germinated and planted in a mixture containing 70:29:1 of sand: field soil: organic matter (v/v). Each plant was inoculated with 250 second-stage juveniles (j_2) at the same day of planting. Tubes were placed in a randomized complete block design with 7 replications. Plants were grown at 23-25 °C for 16 h of artificial light and a relative humidity of 60-65%. After 12 weeks of nematode inoculation, plants were uprooted and washed under tap water. Cysts from both roots and soil were extracted and counted under the binocular microscope.

Table1. Populations of *Heterodera* species collected from different localities in Turkey

No	Province	Town	Species	Coordinates	
1	Adana	Saricam	*H. avenae*	37º 11' 49 N	35º 30' 05 E
2	Hatay	Besaslan	*H. avenae*	36º 13' 27 N	36º 80' 18 E
3	Hatay	Imece	*H. avenae*	36º 27' 31 N	36º 18' 16 E
4	K. Maras	Elbistan	*H. filipjevi*	38º 11' 14 N	36º 50' 05 E
5	K. Maras	Afsin	*H. filipjevi*	38º 15' 17 N	37º 57' 01 E
6	Yozgat	Merkez	*H. filipjevi*	39º 39' 74 N	34º 25' 42 E
7	Gaziantep	Karkamıs	*H. latipons*	36º 49' 14 N	37º 50' 54 E
8	Kilis	Elbeyli	*H. latipons*	36º 39' 03 N	37º 25' 19 E

The resistance reaction was evaluated according to Nicol et al. (2009) scale based on the number of cysts per root system; R= resistant (<5 females), MR = moderately resistance (5-10 females), MS= moderately susceptible (11-14 females), S= susceptible (15-25 females) and HS= highly susceptible (> 25 females). The results were analyzed according to standard analysis of variance procedures with the SPSS 10 program for Windows. Differences among treatments were tested using one way analysis of variance (ANOVA) followed by Tukey test for mean comparison if the F-value was significant. Statistical differences were measured at ($P \leq 0.05$).

Result and Discussion

One of the major obstacles and challenges to use genetic host resistance is the understanding of the CCN species and pathotypes in different regions of the world where the nematode is considered to be economically important. This restricted study clearly indicated that the known published *Cre* genes found in bread wheat backgrounds have range of reactions in the regions where they were tested, both regionally and within region in some cases. This work demonstrates the importance of collecting representative populations of CCN and sharing known resistant germplasm to determine the effectiveness of such resistance in differential pathotypes virulence and utilization of resistance sources to control CCN. Current resistance genes might be overcome by emerging virulent pathotypes which is an essential point to research for new resistance specificities. Therefore it is crucially important to know Turkish *Heterodera* species with different pathotypes population reaction against resistance sources. In this study reaction of resistance genes, *Cre*R, *Cre*1, *Cre*2, *Cre*3, *Cre*7 and *Cre*8, against the cereal cyst nematodes, *H. avenae*, *H. filipjevi* and *H. latipons* were given in Table 2.

Table 2: The reactions of some of the carrying resistance genes found in the wheat lines, tweny-five cereal lines, against to *H. avenae, H. filipjevi* and *H. latipons*

No	Line name	Gene	Results							
			Heterodera avenae			*Heterodera filipjevi*			*Heterodera latipons*	
			Beşaslan	İmece	Karlık	Afşin	Elbistan	Yozgat	Elbeyli	Karkamış
1	6R(6 D)	*Cre* R	S	S	HS	MR	MR	MR	MR	MR
2	FRAME	*Cre* 8	S	MS	S	MR	MR	MR	MS	MR
3	SILVERSTAR	*Cre* 1	R	R	MR	MR	R	MR	R	R
4	VP5053 (WA#Fm/201/23*2/GS50A)	*Cre* 8	S	MS	S	S	MS	S	MS	MS
5	T-2003	*Cre* 7	R	R	MR	S	MS	S	R	R
6	RAJ 1		MR	MR	MR	S	MR	S	MR	MR
7	ID-2150	*Cre* 2	S	MS	S	MS	MS	MS	S	S
8	MILAN		MS	MS	MS	MS	MR	S	R	MR
9	AUS 4930 .7/2 *PASTOR		MS	MR	MS	MS	MS	S	MR	MR
10	AUS GS50AT34/ SUNCO//CUNNINGHAM		MR	R	MR	MR	R	MS	R	R
11	VL 411R		S	MR	S	MR	R	MR	S	MS
12	CROC 1/AE.SQUARROSA (224)//OPATA		MR	R	MS	MR	R	MS	MR	R
13	CROC 1/AE. SQUARROSA (224)//OPATA		S	R	MR	S	S	S	MR	MR
14	VP 1620 (VF304/TTAU. 69.5-3//YANAC)	*Cre* 3	MR	MR	MR	S	R	HS	MR	MR
15	F 130L 1. 12 /ATTILA		MS	MR	MS	S	MR	MR	MR	MR
16	SONMEZ 2001		S	MS	S	MS	MS	S	MR	MS
17	CPI 133859		MS	S	S	MR	MR	MR	S	S
18	CPI 133872		S	S	S	MS	MS	MS	S	MS
19	KATE A-1		MS	S	S	MS	MS	MS	MS	MS
20	PRINS		MS	S	HS	MS	MS	MS	MR	MR
21	MIRZABEY2000		S	MS	MS	MR	R	MR	S	MS
22	AU/CO652337//2*CA8-155/3/F474S1-.1		MR	R	MS	MR	R	MR	MR	MR
23	F372		S	MR	S	S	MS	MS	S	MS
24	TAIKONG		S	MS	S	MS	MS	MR	MS	S
25	ZHONGYU		S	MR	S	MS	MS	MR	MR	MR

The obtained results showed that *Cre*1 gene was only found as completely resistant to all (*H.avenae*, *H. filipjevi* and *H. latipons*) nematode species extracted from the Eastern Mediterranean Region of Turkey. *Cre*3 and *Cre*7 were found resistant to *H. avenae* and *H. latipons*. *Cre* R was also determined as resistant to *H. filipjevi* and *H. latipons* populations but *Cre*8 was only found resistant against to *H. filipjevi* population. Any resistance was not found in Cre 2 gene against to all nematode populations. Additionally, 2 resistance gene-free variety and lines were found resistant to *H. avenae*; 3 wheat lines to *H. filipjevi* and 11 wheat genotypes were found moderately resistant to *H. latipons*.

Wheat lines having *Cre*1, *Cre*3 and *Cre*7 gene showed resistance response against three populations of *H. avenae*, while wheat lines having resistant genes (*Cre*R, *Cre*8 and *Cre*2) reacted as susceptible. However, wheat lines, RAJ1 and AUSGS50AT34/SUNCO//CUNNINGHAM do not have resistance genes were determined as resistant against all studied *H. avena*e populations. Wheat lines caring *Cre*1, *Cre*8 and *Cre*R genes gave resistant reaction to the three populations of *H. filipjevi*, but *Cre*2, *Cre*3 and *Cre7* genes showed susceptible reaction. Interestingly, wheat lines, CPI 133859, MIRZABEY 2000 and AU/CO652337//2*CA8-155/3/F474S1-1, which do not have resistance genes were recorded to have resistant reaction against all *H. filipjevi* populations which indicates other unknown

source/s of resistance are exist. Resistance genes of *Cre*1, *Cre*3 *Cre*7 and *Cre*R gave resistant response to two populations of *H. latipon*s, but *Cre*2 and *Cre*8 genes were not resistant against same populations. Wheat lines, RAJ1, MILAN, AUS 4930 .7/2 *PASTOR, AUSGS50AT34/SUNCO//CUNNINGHAM, CROC 1/AE.SQUARROSA (224)//OPATA, CROC 1/AE. SQUARROSA (224)//OPATA, F 130L 1. 12 /ATTILA, CPI 133872, PRINS, AU/CO652337//2*CA8-155/3/F474S1-.1 and ZHONGYUwhich do not have resistance gene, gave resistant reaction against *H. latipons* populations.

Using host-plant resistance is one of the most effective methods to control cereal nematodes. Resistance is defined as the ability of the host to inhibit nematode multiplication (Cook & Evans, 1987). Preferably resistance should be combined with tolerance, which is the ability of the host plant to maintain yield potential in the presence of the nematode (Cook & Evans, 1987). The use of cultivars that are both resistant and tolerant offers the best control option, as well as being environmentally sustainable and requiring no additional cost. However, using resistance needs a sound knowledge of the virulence range from the targeted species and pathotypes. Wheat cultivars are able to show diverse reaction in different regions. Moreover, some wheat cultivars resistant to *Heterodera* species populations in one region may be fully susceptible to populations in other regions.

These results of this study were similar to that one reported by Rivoal et al. (2001) which indicated that *Cre*3 genes did not give exact resistance on the 14 populations of *H. avenae*, *H. filipjevi* and *H. latipons* populations. Additionally, Nicol et al. (2009) indicated that *Cre*1, *Cre*5 and *Cre*R, genes provide moderate resistance to Haymana populations of *H. filipjevi* but *Cre*3 and *Cre*8 genes were found to be susceptible in their reaction. Özarslandan et al. (2008) and Toktay et al. (2012) found that *Cre*1 gene was not resistant to different Yozgat populations of *H. filipjevi*. As it is understand in previous studies that the different populations of *H. filipjevi* can give diverse reaction in same genes.

According to our results, Silverstar which has *Cre*1 gene showed resistance reactions to all studied populations of three nematode species. However, some lines were found resistance to only 1 or 2 species. For example; *Cre*3, and *Cre*7 genes were found resistant to both *H. avenae* and *H. latipons* populations and also *Cre*R gene was found resistant to both *H. filipjevi* and *H. latipons* populations. In breeding programs, final target is aimed to obtain multiple resistances against to nematodes species damaged on wheat. In this regard, *Cre* 1 gene can be used in breeding programs.

In conclusion, the results obtained from this study show that the known resistance gene/s (*Cre* genes) against the cereal cyst nematodes cannot control all *Heterodera* populations except Cre 1 gene. However, other source of resistance is seemed to be exited in wheat lines which do not have *Cre* genes. Finding new sources of resistance would be very much demanded to control the different populations especially in areas where a mixture of *Heterodera* species occurs. Therefore, wheat origin countries including Turkey, Iran, Iraq and Syrian obtained wheat wild relatives should be screened to *Heterodera* species. Taking advantage of these sources of resistance, it is necessary to know the reaction of genotypes against to nematode species in Turkey.

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