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Original article (Orijinal araştırma)

Host suitability of wheat cultivars to *Pratylenchus thornei* Sher & Allen, 1953 and *Pratylenchus neglectus* (Rensch, 1924) (Tylenchida: Pratylenchidae)

Buğday çeşitlerinin *Pratylenchus thornei* Sher & Allen, 1953 ve *Pratylenchus neglectus* (Rensch, 1924) (Tylenchida: Pratylenchidae)'a karşı konukçu uygunluğu

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Abstract

Root lesion nematodes (RLNs), *Pratylenchus thornei* Sher & Allen, 1953 and *Pratylenchus neglectus* (Rensch, 1924) (Tylenchida: Pratylenchidae) are important plant-parasitic nematodes that cause economic yield losses in wheat cropping systems worldwide. The use of resistant and tolerant cultivars is the most effective method to control these nematodes in wheat. There are currently no commercial wheat cultivars identified as completely resistant to the RLN species. The aim of this research was to evaluate 19 Turkish spring wheat cultivars for reaction to *P. thornei* and *P. neglectus* under *in-vitro* conditions over 16 weeks in 2019. In the result of the study, nine wheat cultivars (Adana99, Ata89, Bürküt, Cumhuriyet75, Gönen98, Marmara86, Meta2002, Troya and Uludağ) were found to have moderate resistance against *P. thornei*, whereas five wheat cultivars (Adana99, Alibey, Ata89, Ceyhan99 and Uludağ) were moderately resistant to *P. neglectus*. The study also showed that Adana99, Ata89 and Uludağ are resistant to both nematode species, and these cultivars, thus, are considered to be excellent sources of genes for further development RLN resistant commercial wheat cultivars.

Keywords: *Pratylenchus neglectus, Pratylenchus thornei*, resistance, root lesion nematode, wheat

Öz

Kök yara nematodları (RLN), *Pratylenchus thornei* Sher & Allen, 1953 ve *Pratylenchus neglectus* (Rensch, 1924) (Tylenchida: Pratylenchidae) dünya genelinde buğday yetiştiriciliğinde ekonomik ürün kayıplarına neden olan önemli bitki paraziti nematodlarıdır. Dayanıklı ve tolerant çeşit kullanımı bu nematodlar ile mücadelede en etkin metot olarak bilinmektedir. Günümüzde, RLN türlerine karşı tamamen dayanıklı ticari herhangi bir çeşit yoktur. Bu çalışmada 19 adet yazlık buğday çeşidinin kontrollü koşullarda 16 hafta süreyle *P. thornei* ve *P. neglectus*'a karşı reaksiyonları 2019 yılında değerlendirilmiştir. Çalışma sonucunda Adana99, Ata89, Bürküt, Cumhuriyet75, Gönen98, Marmara86, Meta2002, Troya ve Uludağ'ın içinde olduğu dokuz buğday çeşidi *P. thornei*'ye karşı orta dayanıklı, buna karşın Adana99, Alibey, Ata89, Ceyhan99 ve Uludağ'ın içinde olduğu beş buğday çeşidi *P. neglectus*'a karşı orta dayanıklı bulunmuştur. Ayrıca çalışmada Adana99, Ata89 ve Uludağ'ın her iki nematod türüne dayanıklılık gösterdiği ve bu çeşitlerin RLN'e karşı dayanıklı ticari buğday çeşitleri geliştirmek için oldukça iyi dayanıklılık gen kaynakları olduğu düşünülmektedir.

Anahtar sözcükler: *Pratylenchus neglectus, Pratylenchus thornei*, dayanıklılık, kök yara nematodu, buğday

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Introduction

Wheat is the most important food crop and accounts for almost one-third of the world's edible grain production (FAOSTAT, 2020). In Turkey, wheat is cultivated mostly in arid and semiarid areas with an average size of 9.83 Mha (TUIK, 2019). There is variability caused by abiotic and biotic stress factors in the amount of production; however, total wheat grain production reached 19 Mt in 2019 (FAOSTAT, 2020). Unfortunately, the production is still inadequate to meet nutritional requirements by a growing population (Sirat & Sezer, 2014). Also, wheat production is often subjected to various constraints, such as lack of fertilizer and irrigation water, and soilborne pathogens including several plant-parasitic nematodes (Shroyer et al., 1990; Dababat & Fourie, 2018).

Root lesion nematodes (RLNs), *Pratylenchus*, are migratory plant-parasitic nematodes that are economically important in many crops, including cereals, and have spread to agricultural soils around the world (Nicol & Ortiz-Monasterio, 2004; Thompson, 2008; Moens & Perry, 2009; Mokrini et al., 2016; Thompson et al., 2017). Wheat is severely affected by several RLNs species of which *Pratylenchus thornei* Sher & Allen, 1953 and *Pratylenchus neglectus* (Rensch, 1924) (Tylenchida: Pratylenchidae) are the most destructive (Yu et al., 2012; Dababat et al., 2016). *Pratylenchus thornei* and *P. neglectus* have a broad host ranges including cereals and legumes (O'Brien, 1982; Vanstone & Russ, 2001a, b; Owen et al., 2014). Yield losses caused by *P. neglectus* in Europe, North America and Australia have not been fully investigated; nevertheless, 16-23% yield loss has been recorded in southern Australia (Taylor et al., 1999). Also, the spring wheat yield losses associated with *P. neglectus* populations in Oregon, USA were 36-85% (Smiley et al., 2005; Smiley, 2010). In contrast, 38-85% wheat yield loss due to *P. thornei* is reported in Australia, 50% in Oregon, USA, 12-37% in Mexico and 70% in Israel (Nicol et al., 2004), as well as 19-32% in Turkey (Toktay, 2008).

Management of root lesion nematodes is difficult for several reasons. Adult female RLNs are highly fecund and can survive/feed in numerous places: in the soil, in the root fragments or feed on weeds for extended periods. Growing resistant cultivars, applying chemicals, cultural practices and biological control are the main methods that have been used to control root lesion nematodes (Urwin et al., 1997). The effectiveness of these methods to control plant-parasitic nematodes increases when used in combination. The ideal control approach relies upon the availability of resistant cultivars and the value of the crop (Riggs & Schuster, 1997). Plants, where nematodes cannot multiply are considered completely resistant whereas plants, where nematodes can freely multiply, are defined as non-resistant or susceptible (Cook & Evans, 1987). Tolerant plants are described as plants that are poorly damaged by severe nematode infection and intolerant plants are described as plants that are severely damaged (Cook & Evans, 1987).

Although, plant resistance does not exist in many crops plants and efficiency is frequently limited to a few races of a nematode species. This situation may result in cultivars becoming susceptible to virulent nematode biotypes or related species in field populations (Gheysen et al., 1996; Whitehead, 1998). Using more widely-based resistance would lessen this problem for resistant cultivars. A few reports indicate that *P. thornei* and *P. neglectus* often occur together in the major wheat cultivating areas of Turkey (Sahin et al., 2008; İmren et al., 2017; Dababat et al., 2018). The major objective of this study was to find novel sources of resistance to *P. thornei* and *P. neglectus* among a core set of spring wheat cultivars for further pyramiding into elite cultivars. The current research aimed to (1) examine the host suitability of wheat cultivars to *P. thornei* and *P. neglectus* and (2) provide a common wheat pool with RLN resistance information to producers.

Materials and Methods

Plant material

The 19 spring wheat cultivars obtained from institutes of the General Directorate of Agricultural Research and Policies (Eastern Aegean Agricultural Research Institute, Field Crops Central Research Institute, Mediterranean Agricultural Research Institute and GAP Agricultural Research Institute) were evaluated for their suitability to *P. thornei* and *P. neglectus* (Table 1). Durum wheat cv. Gatcher and GS50a were used as susceptible and resistant check lines, respectively (Thompson et al., 2008). Two independent experiments were performed for phenotyping cultivars against *P. thornei* (Experiment 1) and *P. neglectus* (Experiment 2) under *in-vitro* conditions in a growth room at 21 ± 3°C with a 16:8 h L:D photoperiod for 16 weeks. Seeds of each cultivar were surface-disinfested with 70% ethanol for 1 min, washed in twice sterilized water, air dried, transferred to Petri dishes containing moistened filter paper and maintained for 3-4 days at 23-25°C to promote germination. A germinated seed of each cultivar with three seminal roots were transplanted to plastic tubes (1.5 x 12 cm) filled with a pasteurized mix of field soil and sand (1:3 v/v). Seven replicates of each cultivar were arranged in a randomized block design.

Nematode inoculum

Pathogenicity experiments were conducted with populations of *P. thornei* (PT18) and *P. neglectus* (PN2) obtained from the provincial center and Gerede District, Bolu Province in 2019, respectively (Dababat et al., 2019). The carrot discs were used to culture and maintain nematode populations *in-vitro* according to Moody et al. (1973). Nematode growth on the carrot discs was monitored and when the desired density was reached, the Baermann-funnel method was use to collect nematodes from chopped carrot discs in a moist chamber for 3 days (OEPP/EPPO, 2013). The nematode suspension was used to inoculate seedlings 1 week after planting, at a density of 400 individuals/ml of water (Keil et al., 2009; Toktay et al., 2012). The suspension of each nematode was pipetted into three 2-cm deep holes at a distance of 0.5 cm from the seedling. The plants were kept in the growth chamber and watered daily. The plants were fertilized with liquid fertilizer [NPK(Mg), 15-8-15-(2), Spiess-Urania Chemicals GmbH, Hamburg, Germany] at 3 and 6 weeks after planting.

Assessment of resistance

The experiment was terminated 16 weeks after the inoculation, and a modified Baermann funnel method was used to extract motile nematodes (juveniles and adults) from 80 g of soil and roots from each tube (Hooper, 1986). To the nematode density of the suspension was determined for three subsamples from each tube under a stereomicroscope (Zeiss Stemi 305, Carl Zeiss, Jena, Germany) at 64x magnification. For the evaluation of host suitability to *P. thornei* and *P. neglectus*, the reproduction factor (*Rf*) was calculated as $Rf = Pf/Pi$, where *Pf* is the final population and *Pi* is the initial population in the tube (400 in these experiments). If no nematodes were extracted from the soil and plant roots, the cultivar were considered resistant. An *Rf* of less than 1 was considered to be moderately resistance and 1 or more as susceptible (Thompson et al., 2008; Keil et al., 2009; Toktay et al., 2012). The experimental data were analyzed using a one-way analysis of variance. The mean number of nematodes was separated using Tukey's test at a significance level of $P < 0.05$ using the SPSS statistics package (SPSS version 20.0 for Windows; IBM, Armonk, NY, USA), the principal component analysis performed using XLSTAT 2016.02.28451 software (Addinsoft, Paris, France).

Results and Discussion

Pratylenchus thornei and *P. neglectus* survived and/or increased on all wheat cultivars, including the check lines. All wheat cultivars tested for resistance to *P. thornei* and *P. neglectus* were moderately resistant or susceptible to both nematode populations, but none of the cultivars were resistant to these nematodes (Table 1). The number of individuals *P. thornei* and *P. neglectus* calculated for each cultivar ranged from 106 to 1,980 and from 100 to 3,495, respectively. There was also a significant difference ($P < 0.001$) in the host suitability of wheat cultivars to *P. thornei* and *P. neglectus*. The *Rf* ranged from 0.43 to 6.06 for across both species. In the susceptible check, Gatcher, the average final numbers of *P. thornei* and *P. neglectus* per plant were 1,116 and 1,826, respectively, whereas in the moderately resistant check (GS50a) they were 194 and 286, respectively.

Table 1. Host suitability of tested wheat cultivars to root lesion nematodes

Cultivar	*Pratylenchus thornei*				*Pratylenchus neglectus*			
	Nematodes*		*Rf*	Phenotype	Nematodes*		*Rf*	Phenotype
Adana99	200 ± 33.5 (140-216)	fg	0.50	MR	172 ± 67.6 (110-280)	d	0.43	MR
Alibey	1032 ± 113.2 (684-1273)	bc	2.58	S	240 ±102.2 (120-400)	d	0.60	MR
Altınbaşak	1622 ± 371.7 (1000-1980)	a	3.83	S	464 ± 35.7 (320-860)	d	1.3	S
Ata89	332 ± 23.9 (298-354)	dg	0.83	MR	232 ± 107.9 (160-420)	d	0.58	MR
Basribey95	592 ± 23.0 (580-628)	dg	1.48	S	1488 ± 626.0 (1110-2600)	bc	3.72	S
Bürküt	340 ± 119.1 (144-427)	dg	0.85	MR	832 ± 486.1 (400-1600)	cd	2.08	S
Ceyhan99	516 ± 58.9 (440-580)	dg	1.29	S	296 ± 101.1 (140-420)	d	0.94	MR
Cumhuriyet75	276 ± 44.9 (204-324)	eg	0.69	MR	552 ± 180.1 (230-640)	d	1.38	S
Gökkan	708 ± 52.8 (653-764)	cd	1.77	S	2152 ± 430.4 (1800-2880)	ab	5.38	S
Gönen98	324 ± 80.6 (285-468)	dg	0.81	MR	674 ± 282.0 (360-1110)	cd	1.68	S
İzmir85	1404 ± 303.9 (869-1621)	ab	3.51	S	560 ± 448.3 (320-1360)	d	1.40	S
Kakliç88	1612 ± 482.8 (756-1928)	a	4.03	S	416 ± 270.3 (140-860)	d	1.64	S
Kaşifbey95	644 ± 80.2 (542-765)	dg	1.61	S	2316 ± 635.4 (1600-3290)	ab	5.79	S
Marmara86	180 ± 46.9 (120-228)	g	0.45	MR	472 ± 238.1 (120-700)	d	1.18	S
Menemen	756 ± 12.5 (564-894)	cd	1.89	S	504 ± 225.3 (330-870)	d	1.26	S
Meta2002	392 ± 26.7 (362-426)	dg	0.98	MR	548 ± 190.1 (410-860)	d	1.37	S
Troya	372 ± 108.3 (258-524)	dg	0.93	MR	821 ± 249.0 (512-1134)	cd	2.94	S
Uludağ	186 ± 61.2 (106-276)	g	0.46	MR	198 ± 87.7 (100-340)	d	0.49	MR
Ziyabey98	1344 ± 116.3 (1200-1463)	ab	3.36	S	2424 ± 794.1 (1600-3495)	a	6.06	S
GS50a (R control)	194 ± 42.2 (160-265)	fg	0.58	MR	286 ± 70.6 (160-320)	d	0.74	MR
Gatcher (S control)	1116 ± 419.9 (423-1421)	bc	2.79	S	1826 ± 33.8 (624-2326)	ab	3.02	S

* Mean ± SD (range). Means followed by the same letter within columns are not significantly different ($P < 0.05$, Tukey's test), *Rf,* reproduction factor*;* MR, moderately resistant; S, susceptible.

Cvs Marmara86 and Uludağ had the lowest average number of *P. thornei* at 180 and 186, respectively and cvs Altınbaşak and İzmir85 had the highest average at 1,622 and 1,612, respectively (Table 1). The *Rf* of *P. thornei* on the 19 cultivars ranged from 0.45 (Marmara86) to 4.03 (Kakliç88). Nine cultivars (Adana99, Ata89, Bürküt, Cumhuriyet75, Gönen98, Marmara86, Meta2002, Troya and Uludağ) had *Rf* of <1, with Marmara86 having the lowest value.

Three groups were apparent among the 21 cultivars assessed (including the two check lines) based on the resistance to *P. thornei* (Figure 1). The first group (moderately resistant) included GS50a and nine moderately resistant cultivars: Adana99, Ata89, Bürküt Cumhuriyet75, Gönen98, Marmara86, Meta2002, Troya and Uludağ. The second group (S-I) included Gatcher and six susceptible cultivars: Gökkan, Basribey95, Kaşifbey95, Ceyhan99, Menemen and Alibey. The *Rf* of S-I was >1 but, lower than the *Rf* of Gatcher (2.79). The final group (S-II) included four susceptible cultivars: Altinbaşak, İzmir85, Kakliç88, and Ziyabey98. The *Rf* of S-II was higher than the *Rf* of Gatcher (Figure 1).

Cvs Adana99, Marmara86 and Uludağ had lower numbers of *P. thornei* than GS50a (Figure 1) (Table 1). Cvs Ata89, Bürküt, Cumhuriyet75, Gönen98, Meta2002 and Troya had significantly lower *Rf* (<1) and higher numbers than GS50a (*Rf* = 0.58). Cvs Basribey, Ceyhan99, Gökkan, Kaşifbey95, Menemen, grouped in S-I for *P. thornei*, had *Rf of* >1 and lower nematodes than Gatcher. Cvs Ziyabey98. Altinbaşak, İzmir85 and Kakliç88 had a higher number of nematodes than Gatcher and were grouped in S-II for *P. thornei.*

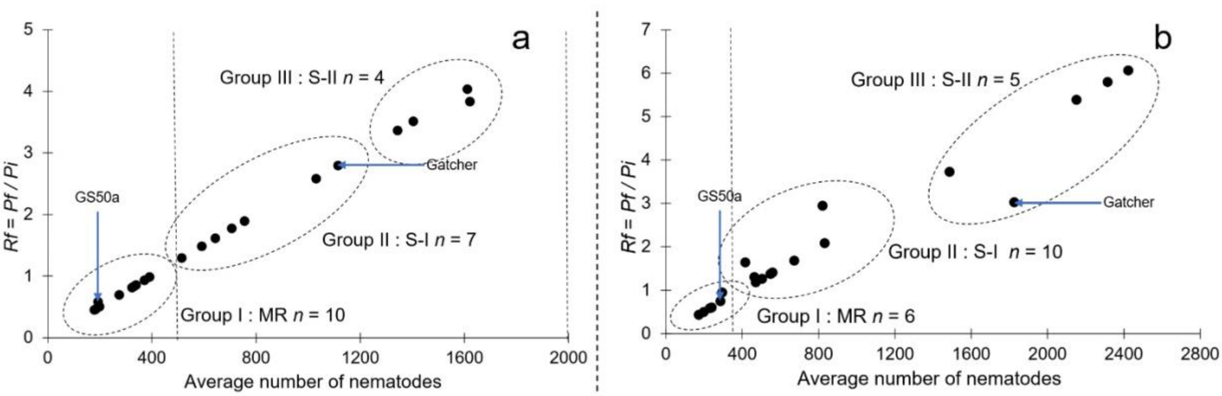

Figure 1. Principal component analysis (Kendall type) showing the plant population structure for a set of GS50a and Gatcher replicates based on their resistance reaction to a) *Pratylenchus thornei* and b) *Pratylenchus neglectus*.

For *P. neglectus,* the lowest numbers extracted where for cvs Adana99 and Uludağ were 172 and 198, respectively (Table 1) and the highest at 2,424 and 2,316 for cvs Ziyabey98 and Kaşifbey95, respectively. The *Rf* for *P. neglectus* ranged from 0.43 (Adana99) to 6.06 (Ziyabey98). The *Rf* values of the five cultivars were <1 and was the lowest for cv. Adana 99 (*Rf* = 0.43).

The phenotyping of *P. neglectus* revealed three groups of cultivars (Figure 1). The first group included GS50a and five moderately resistant cultivars: Ata89, Alibey, Adana99, Ceyhan99 and Uludağ. The second group (S-I) included 9 susceptible cultivars: Altınbaşak, Bürküt, Cumhuriyet75, Gönen98, İzmir85, Kakliç88, Marmara86, Menemen, Meta2002 and Troya. The *Rf* of these was >1 but, lower than the *Rf* value for Gatcher (3.02). The final group (S-II) included Gatcher and four susceptible cultivars: Basribey95, Gökkan, Kaşifbey95 and Ziyabey98. The *Rf* of the S-II group was higher than the *Rf* of Gatcher.

Cvs Ata89, Adana99, Alibey, and Uludağ had lower numbers of *P. neglectus* than GS50a (Table 1). Ceyhan99 had a significantly lower *Rf* (<1) and higher number of nematodes than GS50a. Cvs Altinbaşak, Bürküt, Cumhuriyet75, Gönen98, İzmir85, Kakliç88, Marmara86, Menemen, Meta2002 and Troya had significantly higher *Rf* (>1) and less nematodes than Gatcher. Cvs Basribey, Gökkan, Kaşifbey95 and Ziyabey98 had more nematodes than Gatcher and were grouped as S-II for *P. neglectus*.

The present study determined the host responses of 19 spring wheat cultivars to the two RLNs as moderately resistant and susceptible. RLN resistance in wheat has been studied in either the field or in pots (greenhouse and growth chamber) (Thompson et al., 2015; Dababat et al., 2016). A certain degree of resistance to *P. thornei* has been identified in India (Kranti & Kanwar, 2012), Australia (Thompson & Seymour, 2011) and Turkey (Toktay et al., 2012). For example, several lines were found to be resistant to the Indian population of *P. thornei* among 20 wheat lines (Kranti & Kanwar, 2012), and consistent with the present studies, some wheat lines were moderately resistant to both *P. thornei* and *P. neglectus*. In particular, three cultivars (Ata89, Adana99 and Uludağ) had moderately resistant reactions both of RLN species. This is useful as these species often occur together in wheat fields (Thompson et al., 2010). Cvs Adana99 and Ceyhan99 showed useful resistance to the tested nematodes, which confirms the result of the studies of Toktay et al. (2012), who found resistance in these cultivars. This information allows growers to choose tolerant/resistant crops when both RLN species are present.

As the result of the current study, three wheat cultivars (Marmara86, Adana99 and Uludağ) reduced *P. thornei* densities below that with GS50a showing moderate resistance to *P. thornei.* Also, four wheat cultivars (Ata89, Adana99, Alibey and Uludağ) reduced *P. neglectus* densities below that with GS50a, which is moderately resistant to *P. neglectus.* Numerous sources of resistance to *P. thornei* and *P. neglectus* have been described in wheat germplasm (Thompson & Haak, 1997; Vanstone et al., 1998; Thompson et al., 1999, 2009; Taylor et al., 2000; Toktay et al., 2012). For example, GS50a was reported to be the first source of resistance to *P. thornei* in Australia, which was primarily selected from the cultivar of Gatcher (Thompson & Clewett, 1986). Ten times less RLN reproduction was found in GS50a in comparison to local control lines (Thompson et al., 1999). A reasonable number of Iranian landraces of wheat were assessed for resistance to RLN and 25 of those accessions showed more resistance than GS50a (Sheedy & Thompson, 2009). Similarly, Thompson et al. (2009) performed the screening experiment with wheat accessions from North Africa and West Asian regions and found some additional sources resistance to *P. thornei*.

Mapping of QTLs and phenotypic identification of resistance sources have been used to identify resistance sources to RLNs. The QTLs linked to resistance to *P. thornei* resistance are mapped on different chromosomes of bread wheat (Schmidt et al., 2005; Zwart et al., 2005). The *Rlnn1* locus which is located on the 7A chromosome offers substantial resistance to *P. neglectus* at the seedling stage (Williams et al., 2002). According to Williams et al. (2002), *Rlnn1* originated from an Australian wheat cv. Excalibur, which has been validated for its better resistance to *P. neglectus*. Similarly, another locus conferring resistance to *P. neglectus* has been characterized and identified on the 4D chromosome (Zwart et al., 2005). The relationships between resistance reactions and markers were adequately constant to demonstrate the value of using the marker selection to increase *Pratylenchus* resistance in wheat. The *Rlnn1* marker has been successfully used in this way and is actively implemented as part of international wheat breeding programs in CIMMYT at a global level and in Australia (Williams et al., 2002).

Wheat breeding routinely aims to increase the level of durable resistance of wheat to gain a reasonable yield even in soils with high nematode population densities. To date, several genotypes having RLN-resistance have been identified from the International Winter Wheat Improvement Program (www.iwwip.org) sources, but the genetic basis of resistance is still unknown. Thus, it is important to understand the novelty of the resistance of these cultivars and to use this resistance in different genetic backgrounds by crosses and pyramids to achieve new resistance and high yields that increase grain yield for food security. This study has determined the resistance of some Turkish commercial wheat cultivars, with superior agronomic properties have already, to two RLNs. Therefore, it is suggested that the potentially useful resistance sources determined in the study should be included in breeding studies.

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