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Orijinal araştırma (Original article)

Improved methodology for resistance screening in spring wheat against the root lesion nematode, *Pratylenchus thornei* (Sher et Allen) (Tylenchida: Pratylenchidae)

Buğdayda Kök yara nematodu, *Pratylenchus thornei* (Sher et Allen) (Tylenchida: Pratylenchidae)'ye karşı dayanıklılık çalışmalarında kullanılan yöntemlerin uygunluğunun araştırılması

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Summary

The root lesion nematode, *Pratylenchus thornei* (Sher et Allen) is a polyphagous and economically important nematode in wheat production systems, particularly in rainfed environments. Chemical management of this nematode is not economically or environmentally sound, leaving cultural practices like crop rotation as the most widely accepted option. Long-term control is best achieved in established wheat monoculture systems through genetic improvement, which provides both economic and environmental benefits to the growers. Intensive screening under controlled conditions can facilitate and accelerate the identification of resistance and its subsequent deployment in commercial wheat cultivars. In this study, a number of variables were assessed to optimize *P. thornei* screening, including initial nematode density, soil type, container size, reference cultivars, harvest time and watering regime with perlite. Growth room experiments showed clear separation between the resistant and susceptible cultivars, using sandy growth medium (70:29:1 sand, field soil and organic matter), small container (15 mm diameter x 100 mm in long), inoculation density with 400 individuals per plant, 9 week growing period and bottom perlite irrigation system.

Key words: Resistance, wheat, screening methods

Özet

Kök yara nematodları, *Pratylenchus thornei* (Sher et Allen) polifag bir zararlı olup, özellikle kurak alanlarda yapılan buğday üretiminde çok önemli ekonomik kayıplara neden olmaktadır. Buğday ıslah programlarında dayanıklı çeşit geliştirebilmek için ıslah materyalinin hızlı ve doğru şekilde kontrollü koşullarda test edilmesi gerekmektedir. Kök yara nematodlarına karşı dayanıklı çeşit elde etmek amacıyla laboratuvar koşullarında yürütülecek nematod bitki interaksiyonu çalışmalarında öncelikli olarak en uygun in-vitro koşulların belirlenmesi gerekmektedir. Bu amaçla 2 farklı toprak tipi, 2 hasat zamanı, 2 çeşit, 2 sulama sistemi, 3 saksı tipi, 2 inokulum miktarı araştırılmış ve 7 tekerrürlü olarak denemeye alınmıştır. Çalışmada *P. thornei*'ye karşı dayanıklılık denemelerinde en uygun parametrelerin; kumlu toprak tipi (70:29:1 kum:tarla toprağı:organik madde), küçük boy saksı (boru tipi, çap: 15 mm, yükseklik:100 mm), inokulum yoğunluğu 400 birey, 9 hafta hasat süresi, alttan perlitle yapılan sulama sistemi olduğu belirlenmiştir.

Anahtar sözcükler: Dayanıklılık, buğday, testleme yöntemleri

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Introduction

Plant-parasitic nematodes are important pathogens of many agricultural crops worldwide. The most common and damaging plant-parasitic nematodes are the root-knot (*Meloidogyne* spp.), cyst (*Globodera* and *Heterodera* spp.), burrowing (*Radopholus* spp.), rice root (*Hirschmanniella* spp.) and root-lesion (*Pratylenchus* spp.) groups. The last group of nematodes penetrates the plants root system where they cause typical elongated necrotized lesions. Root-lesion nematodes rank third after root-knot and cyst nematodes in terms of their worldwide impact on agricultural crops (Sasser & Freckman, 1987; Nicol, 2002). The genus *Pratylenchus* comprises 68 valid species (Castillo & Vovlas, 2007). Many of these species are of little or no economic importance but some are responsible for substantial damage and high yield losses in a wide range of crop varieties. *Pratylenchus thornei* (Sher et Allen) is one of the root-lesion nematode that are considered important plant pathogens. It has a worldwide distribution and has been reported in many countries, including Algeria, Australia, Canada, India, Israel, Italy, Mexico, Morocco, Pakistan, Syria, Turkey and Yugoslavia (Nicol et al., 2004).

One of the most preferred and accepted methods for controlling *P. thornei* is the use of resistance in wheat germplasm, which can reduce populations below economic thresholds. Pre-plant populations of *P. thornei* are often negatively correlated with wheat yield (Nicol et al., 1999; Hollaway, 2000). Economic damage thresholds for *P. thornei* on wheat vary greatly from region to region. For example, thresholds that ranged between 420 and 2,500 *P. thornei* per kg of soil in Mexico (Van Gundy et al., 1974; Nicol & Ortiz-Monasterio, 2000) and Queensland (Thompson et al., 1993). A threshold as high as 30,000 *P. thornei* per kg of soil was reported for atolerant variety in South Australia (Nicol et al.,1999). Populations as low as 4,000 *P. thornei* per kg of soil have caused severe damage to intolerant wheat at other locations in South Australia (Taylor et al., 1999). In Turkey, studies showed that yield losses caused by *Pratylenchus* spp. ranged between 20% and 32% (Gözel, 2001; Toktay, 2008).

A number of attempts have been made to screen wheat germplasm against the root lesion nematode (*P. thornei*) in order to find sources of resistance (Vanstone et al., 1998; Thompson et al., 1999; Nicol 2004; Sheedy et al., 2004; Toktay et al., 2006). Improved screening methods could further facilitate and enhance genetic engineering studies for incorporation of resistance genes against this nematode into wheat.

Researchers are using different techniques in their screening studies, which ultimately affect the results obtained. This is very important when an international seed exchange occurs to screen for the same species or different pathotype. Improving screening methodologies such as soil type, size of pot, watering system and inoculation density would improve nematode penetration and produce more reliable results. Comparing these methods to find the best one could result in adopting a standard method to be used in different research programs.

The main objectives of this study were therefore to improve screening methods for spring wheat varieties against *P. thornei* and to determine source(s) of resistance by evaluation of various inoculation methods, growth media, cultural methods, watering regimes and time of assessment.

Material and Methods

A full factorial experiment with 7 replicates was conducted to examine the factors listed in Table 1. Two growth media were used, combining river sand, field soil and organic matter (cow manure) at

different concentrations which were loamy soil 29:70:1 -sand (S),:field soil (F) organic matter (O)) and sandy soil (70:29:1- S:F:O).

Table 1. Factors used to determine the best combination for screening wheat varieties for resistance to *Pratylenchus thornei*

Factors	Treatments
Container size	Large (30 ϕ x 125 mm), medium (20 ϕ x 100 mm) and small (15 ϕ x 100 mm)
Growth medium	Loamy soil (29:70:1 – sand (S),field soil (F) organic matter (O)) and sandy soil (70:29:1- S:F:O)
Inoculum density	400 and 600 nematodes/plant
Irrigation system	Cloth and Perlite (Bottom)
nematode extraction	Root and Soil
Assessment time	9 and 13 weeks

The growth medium contained the field soil, (73% clay, 16.5%, silt and 10% sand), organic matter (cow manure) and sand (river sand). The growth medium was sterilized in an autoclave for 15 minutes at 121 ^{o}C. Seeds were surface sterilized with sodium hypochlorite (4.5%) and pre-germinated on wet filter paper in sterile petri dish. One sterilized and pre-germinated seed with approximately 3 equidistant, 1-cm long seminal roots was planted per screening container (Nicol, 1996).

Nematodes were grown *in vitro* on carrot cultures according to Moody et al. (1973). From carrot culture, the nematodes were extracted by placing the chopped carrot discs into a misting chamber for 2 - 4 days. The inoculation suspensions were prepared with sterile water and freshly extracted nematodes. *P. thornei* individuals were counted under a binocular microscope and suspended in a 50 ml of tap water in flask, then one week after sowing each plant was injected with 1 ml of water consisting of 400 and 600 individuals in 1 ml water. Plants were inoculated with nematodes one week after transplanting.

Two kinds of bottom irrigation system were used in order to irrigate plants homogeneously and to minimize labor. Water was added to perlite and thick cloth which are highest absorbing materials.

The experiment was arranged in a randomized complete block design and each treatment was replicated 7 times. Plants were grown at 20^{o}C with 16 hours of artificial lightning regime in a controlled growth chamber. All treatments were conducted at the same time. At nine weeks and thirteen weeks after nematode inoculation, plants were harvested and nematodes from both soil and roots were extracted and counted.

At harvest, shoots were removed and *P. thornei* vermiform nematodes were extracted from roots and soil using the modified Baermann funnel and mister extraction method (Southey, 1986). The total number of *P. thornei* nematodes both root and soil in the pot was account under microscope.

Data were analysed according to standard analysis of variance procedures using SPSS 17.0 program for Windows (SPSS Inc., Illinois, USA). Differences among treatments were tested using one way analysis of variance (ANOVA), followed by the Duncan Test for mean comparison, if the F-value was significant ($P < 0.05$).

Results and Discussion

Container Size

Three different cylindrical container sizes (Table 1) were evaluated. The statistical analysis showed that there were no significant differences in reproduction of nematodes when using small, middle and large tube sizes (1260 (a), 1287 (a) and 1305 (a), respectively). Therefore, the smallest tube size is recommended to optimize the use of space and reduce costs.

Soil type

P. thornei reproduced at a higher rate in clay soil than in sandy soil, in both susceptible (Warigal) and resistant (AUS 4930. 7.2) wheat varieties (Figure 1). However, nematode extraction was more difficult and the standard error was higher compared to sandy soil in screening experiments. Sandy soil showed more stable and accurate results in the replications and therefore sandy soil should be used for screening experiments.

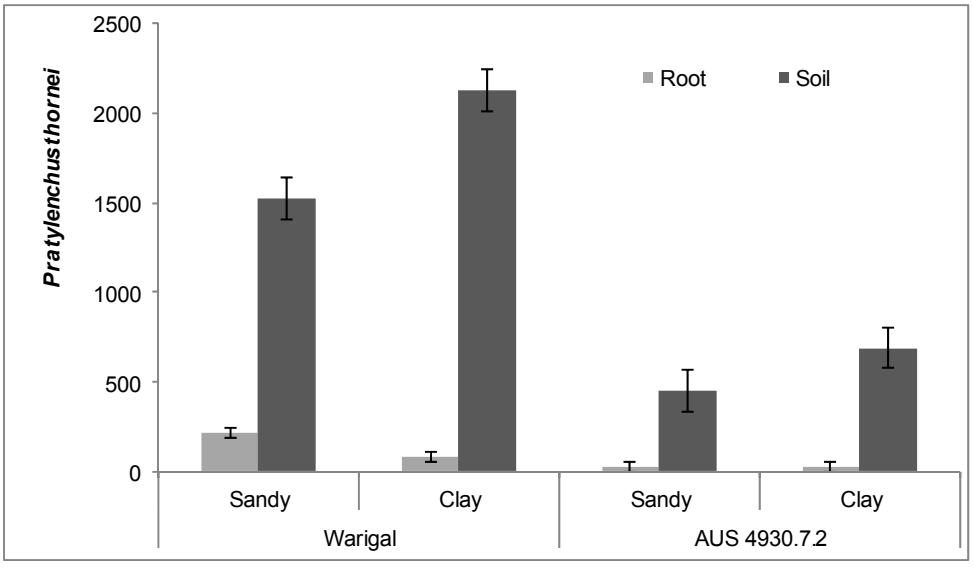

Figure 1. The effects of two different soil types on *Pratylenchus thornei* reproduction in two wheat germplasms AUS 4930 7.2 and Warigal.

Inoculum density

The multiplication rate MR (Pf/Pi) is commonly used in plant nematology as a quantitative value to determine resistance. After inoculation with 400 and 600 nematodes per plant, the MR was compared (Figure 2). The highest nematode reproduction factor was determined at an inoculum density (ID) of 400 (MR = 2.83). At 600 nematodes per plant inoculum density, the MR fell to 2.39. Therefore, inoculation with 400 nematodes is less-damaging, which means the plants are not destroyed after inoculation. As a result, an inoculum density of 400 nematodes/plant is recommended for screening purposes.

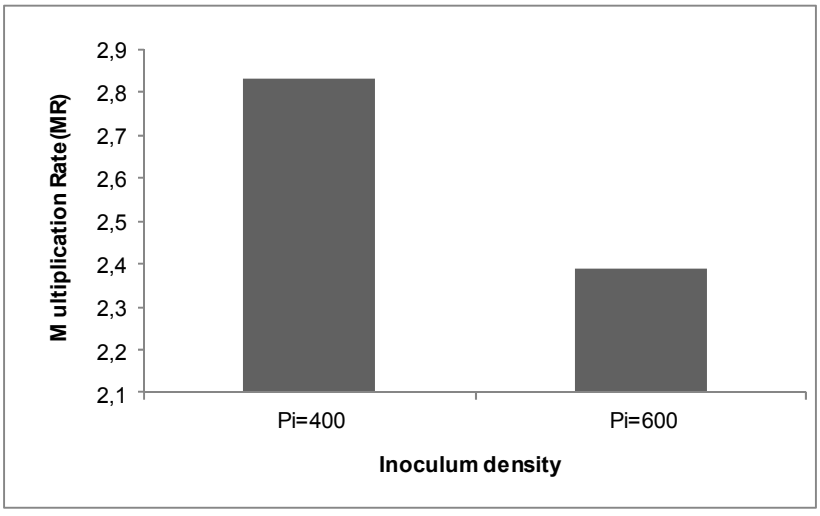

Figure 2. The effects on the multiplication rate (MR) of *Pratylenchus thornei* of two inoculum levels of *Pratylenchus thornei*.

Watering System

Watering systems with Perlite and cloth yielded 1821 and 574 nematodes per plant, respectively (Figure 3). The bottom watering system using perlite gave consistent water flow to the plants with minimum water loss and labor.

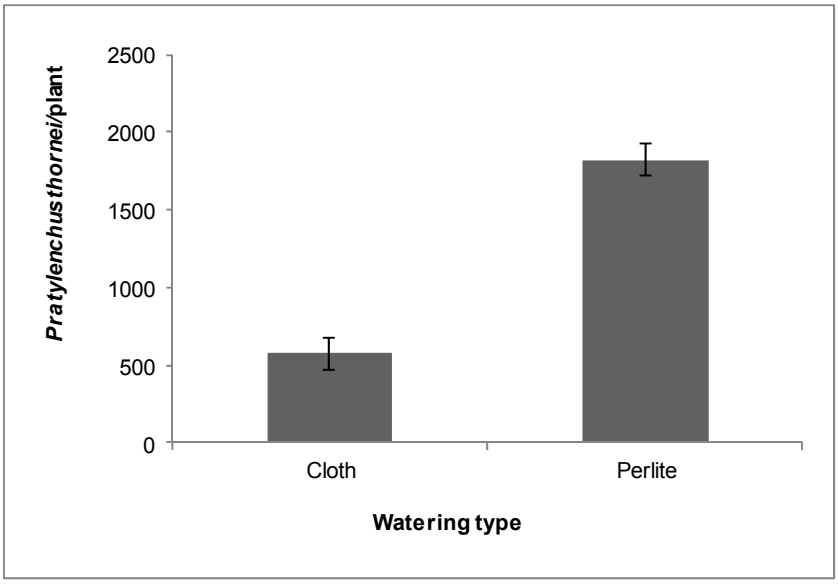

Figure 3. The effects of two different irrigation systems on *Pratylenchus thornei* number.

Nematode extraction source

Generally, total numbers of nematode on both root and soil were calculated in the screening experiments. Nematode final populations extracted from soil and root of the susceptible wheat variety “Warigal” were 3 times higher when compared to the resistant wheat varieties “AUS4930”, 1973 and 593respectively. More nematodes were extracted from soil than from roots. This study showed that nematode extraction from root systems is sufficient to screen susceptible and resistant varieties.

However, it is recommended that nematodes be extracted from both soil and roots since root lesion nematodes are migratory and extracting from only roots will be insufficient. Therefore, total nematode number per plant should be evaluated for screening experiments.

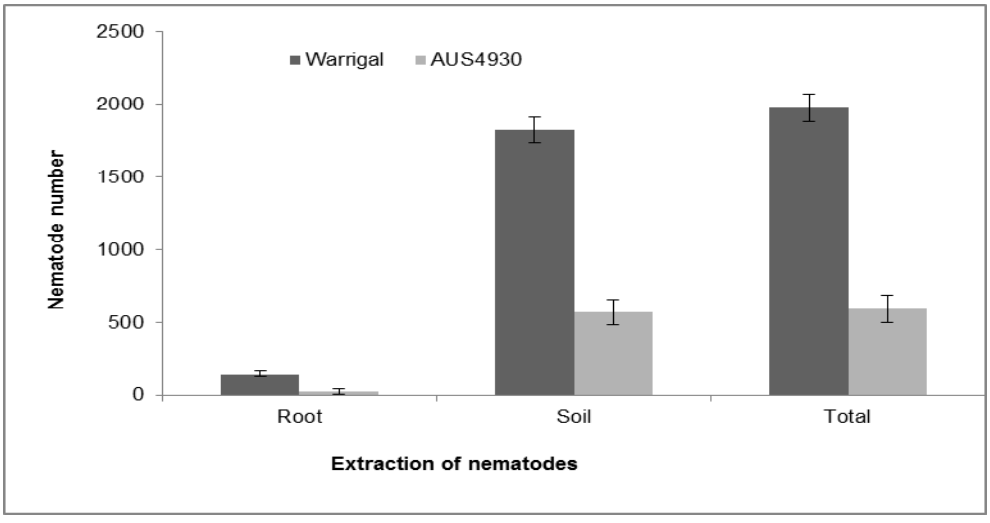

Figure 4. Number of nematodes from susceptible and resistant wheat varieties after extraction from roots and soil.

Harvest time

The susceptible (Warigal) and resistant (AUS4930 7.2) wheat germplasms were used to compare nematode number 9 and 13 weeks after harvest time. Nematode number was higher at 13 weeks growing time than 9 weeks in both the susceptible and resistant varieties, 2923 and 989, respectively. However, harvesting after 9 weeks showed a lower number of nematodes in resistant germplasm AUS4930 7.2 (199). Therefore, harvesting after 9 weeks is more appropriate than 13 weeks for screening experiments (Figure 5).

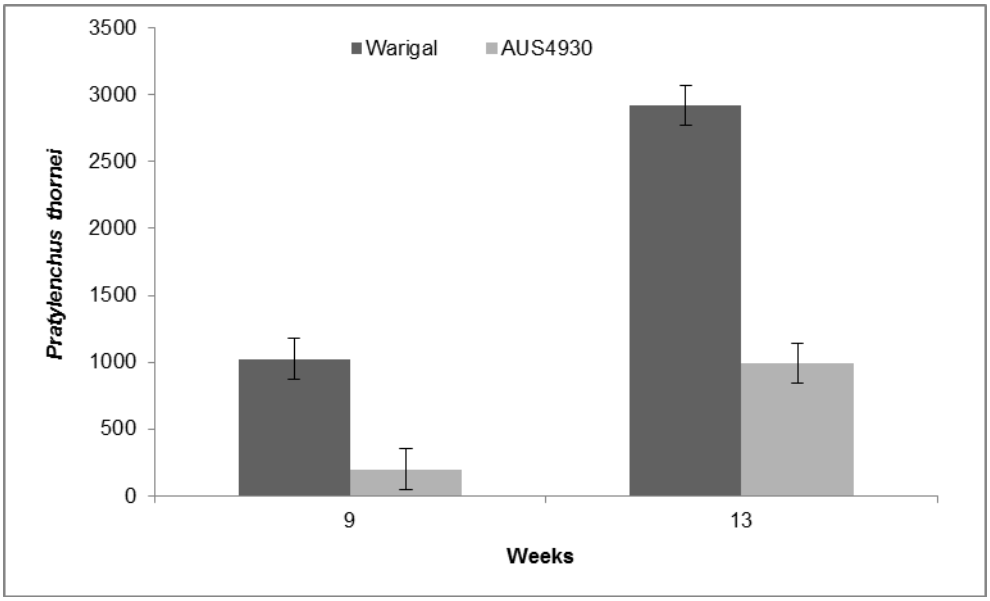

Figure 5. The effect of two different harvest time on *Pratylenchus thornei* numbers on wheat varieties AUS 4930 7.2 and Warigal.

In conclusion, this study demonstrated that inoculating each plant with 400 nematodes, use of sandy soil type (70:29:1 sand, field soil, organic matter), small tube size of 15 x 100 mm, 9 weeks harvest time and perlite watering system are the best choices for screening experiments.

Duration of the screening experiment is very important for research programs. Keil et al. (2009) reported that the best harvesting time in order to extract nematodes was less than 12 weeks. Also, they compared nematode inoculum densities but their results did not show a significant difference between 400 and 600 nematodes per plant.

An optimized screening method guarantees successful resistance screening. This study yielded results with the following advantages: (a) the system can be used for screening of wheat against migratory endoparasitic nematodes such as *P. thornei* and *P. neglectus* under controlled conditions (b) nematode differentiation between resistant and susceptible varieties is very clear with lower standard error, (c) plant tubes cover less space in the controlled unit, (d) irrigation is standardized for all plants; and (d) maintenance is less labor intensive. Furthermore, less space and time are needed for plant multiplication and maintenance. Also, the experiment time is reduced to 9 weeks. Harvest time is very important for screening experiments. The analysis (nematode extraction, nematode counting) is easier to handle since work is carried out on a smaller scale. A disadvantage, however, is the variable reproduction of the same nematode population between experiments. It is therefore very important to use a susceptible and resistance reference genotypes, like Warigal and AUS 4930, in all screening experiments.

Routine screenings of wheat accessions against members of the genus *Pratylenchus* are performed in the glasshouse. This enables the usage of a small space at minimal cost to establish appropriate growth conditions in a misting chamber. Although conditions in the glasshouse can be largely controlled, there are still environmental factors with a large impact on reliability and repeatability of the data. Especially the temperature can affect nematode reproduction (Kimpinski & Willis, 1981, Umesh & Ferris, 1994, Mizukubo & Adachi, 1997). To avoid such effects, this test can easily be implemented under more controlled conditions in a growth chamber where light and temperature can be controlled more precisely.

For wheat, an *in vitro* screening procedure has been successfully developed. It facilitates the identification of resistance against the root-lesion nematode, *P. thornei* at harvesting time. In addition, this system could be useful to the study of resistance mechanisms. However, it is important to note that resistance revealed under *in vitro* conditions must be confirmed in the field.

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