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Screening of common bean (*Phaseolus vulgaris* L.) cultivars against root-lesion nematode species*

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Abstract: In this study, screening of 15 *Phaseolus vulgaris* cultivars and genotypes (Zülbiye, Kınalı, Perla, Kwintus, Özayşe, Tokat Sırık, Musica, Şelale, Nadide, Gina, Serra, Karabağ, Funda, and Lepus) and 1 *Pisum sativum* cultivar (Araka) for host suitability to 4 root-lesion nematodes, *Pratylenchus thornei*, *P. crenatus*, *P. neglectus*, and *P. penetrans*, was carried out in 2010 and 2011 under controlled conditions. The experiment was set up in a completely randomized block design with 7 replicates. Approximately 1000 adults + juveniles + eggs of root-lesion nematodes in 10 mL of distilled water were inoculated around the base of each bean seedling. The experiments were harvested after a 10-week duration. Nematodes were extracted from soil and roots by modified Baermann funnel technique and counted under a light microscope. Common bean cultivars and genotypes showed differences in host suitability in the 4 root-lesion nematodes. *Pratylenchus penetrans* reproduced on Zülbiye, Kınalı, Nadide, Gold Nectar, Karabağ, and Funda. Additionally, *P. penetrans* had a reproduction index value of slightly higher than 1 on Tokat Sırık, Şelale, and Serra. However, Gina, Musica, Perla, Kwintus, and Özayşe showed poor host reaction to *P. penetrans*, and *P. penetrans* did not develop on pea cultivar Araka. *Pratylenchus thornei* multiplied on Zülbiye, Şelale, Kınalı, Lepus, Perla, Nadide, and Araka. The value of the reproduction index was in the range of 1.8–3.1, and there were no significant statistical differences among these cultivars and genotypes. Özayşe, Tokat Sırık, Gold Nectar, Kwintus, Serra, Gina, Funda, and Musica significantly reduced *P. thornei* populations ($P < 0.05$). Reproduction indices of *P. neglectus* and *P. crenatus* were extremely low in all bean cultivars.

Key words: Common bean, *Phaseolus vulgaris*, *Pratylenchus* spp., root lesion nematodes

1. Introduction

Root-lesion nematodes are common migratory endoparasites associated with field crops in Turkey (Yıldız, 2007; Şahin, 2009). Several root-lesion nematode species can be found in mixed populations in the field crops and temperate fruit orchards (Smiley et al., 2004; Şahin et al., 2008; Söğüt and Devran, 2011). Surveys of parasitic nematodes in legume fields were conducted on the most common species: *Pratylenchus thornei* Sher & Allen, 1953; *P. penetrans* Filipjev & Schuurmans Stekhoven, 1941; and *P. mediterraneus* Corbett, 1983 (Di Vito et al., 1994b; Kepenekçi and Ökten, 1999). *Pratylenchus thornei* is among the most important parasites of cereal and legumes and this nematode is distributed in 72% of chickpea fields in Syria, 61% in Turkey, 92% Spain, and 28%–61% in North Africa (Greco et al., 1992; Di Vito et al., 1994a, 1994b; Castillo et al., 1996). In addition to these nematodes, *P. neglectus* Filipjev & Schuurmans Stekhoven, 1941 and *P. crenatus* Loof, 1960 were also identified as parasites of cereal crops. Root-lesion nematodes feed on young roots and cause root reduction or inhibition associated with the formation of local lesions on the young roots. Roots invaded by nematodes are susceptible to root-rotting pathogens (Back et al., 2002). *Pratylenchus penetrans* and *P. thornei* are usually found in temperate areas, have wide host ranges, and are common parasites of legume species (Vovlas and Troccoli, 1990; Castillo et al., 1998; Di Vito et al., 2000).

Turkey is the second-largest bean-producing country (*Phaseolus vulgaris* L.) in the world at approximately 1,000,000 t (TÜİK, 2012). Fresh beans and dry beans are commonly produced in the Central Anatolian provinces in Turkey, and root-lesion nematodes are the most commonly encountered parasitic species in bean fields (Di Vito et al., 1994b; Kepenekçi and Ökten, 1999). Söğüt et al. (2010) reported that *P. thornei* was common in fresh

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bean cultivation areas of Bilecik and it reproduced well on fresh, local bean cultivars Ayşekadın, Siyah Boncuk, and Osmanlı. The highest reproduction indices were found to be 84.6%, 12.0%, and 15.4% on Ayşekadın, Siyah Boncuk, and Osmanlı, respectively. The highest yield loss was 14.2% on cultivar Ayşekadın.

Fumigant pesticides and nematicides such as metam-sodium, dazomet, and 1,3-dichloropropene are the main approaches for nematode control, but these are nonprofitable in field crops and cause environmental pollution (Broun and Supkoff, 1994). Therefore, exploitation of host plant resistance would be the most practical and cost-effective management strategy. Nematode multiplication is used as a basic measurement of resistance and/or susceptible in nematology. It is defined as the ability of a host plant to prevent multiplication of the nematode (Cook and Evans, 1987; Trudgill, 1991). Singh and Schwartz (2011) reported that there was limited information on the reaction of common bean cultivars and germplasm to root-lesion nematodes; additionally, there were no records of genetic and breeding for resistance to root-lesion nematodes.

The objective of this study was to evaluate the host status of some common bean cultivars used in Turkey against common root-lesion nematode species *P. penetrans*, *P. thornei*, *P. neglectus*, and *P. crenatus*.

2. Materials and methods

Fifteen bean cultivars and 1 pea cultivar obtained from private sources in Turkey were screened for host reaction to root-lesion nematodes under controlled conditions. Experiments were conducted with Zülbiye, Kınalı, Perla, Kwintus, Gold Nectar, Özayşe, Tokat Sırık, Musica, Funda, Şelale, Lepus, Nadide, Karabağ, Gina, and Serra bean cultivars and Araka pea cultivar in 2010 and 2011 (Table 1).

2.1. Nematode multiplication

Four root-lesion nematode species, *P. penetrans*, *P. thornei*, *P. neglectus*, and *P. crenatus*, were used from survey studies conducted by Söğüt and Devran (2011). The nematodes were multiplied from pure culture, using the carrot disk culture technique in an incubator at 22 ± 1 °C. Mass culture production was established according to the protocol described by Verdejo-Lucas and Pinochet (1992), Castillo et al. (1995), and Tülek et al. (2009). Ten to 15 individuals from each population were placed on surface-sterilized and peeled carrot disks to establish pure nematode culture in the incubator for multiplication. Approximately 4 months later, the nematodes were extracted from carrot disks by passing the water solution through a 20-µm sieve. All vermiform and egg stages of root-lesion nematodes were collected in a centrifuge tube and stored at 10 °C in the refrigerator until inoculation. Root-lesion nematodes in various stages were then prepared by counting under a light microscope for inoculation of the germinated bean seedlings.

2.2. Plant preparation

In the first experiment, 6 bean cultivars (Zülbiye, Şelale, Kınalı, Lepus, Perla, and Nadide) and 1 pea cultivar (Araka) were screened. In the second experiment, 15 bean cultivars (Zülbiye, Kınalı, Perla, Kwintus, Gold Nectar, Özayşe, Tokat Sırık, Musica, Funda, Şelale, Lepus, Nadide, Karabağ, Gina, and Serra) were used to determine nematode colonization and population formation. Seeds of all cultivars were germinated on a moistened filter paper in petri dishes and transplanted 1 cm deep in 250-cm^3 pots (6 cm in diam. with 1 germinated seed per pot) containing autoclaved sandy loam soil (ca. 68.3% sand, 18.4% silt, and 13.3% clay). Plants were watered daily with 50 mL of water and fertilized twice with 100 mL of nutrient solution (Crop-TEC, Science Products Company, USA).

2.3. Nematode inoculation and accounting

The host status of the 4 root-lesion nematode species was tested using 6 bean and pea cultivars in the first experiment and 15 bean cultivars in the second experiment. The experiments were conducted twice under controlled

Table 1. Common bean (*Phaseolus vulgaris* L.) and pea (*Pisum sativum* L.) cultivars and genotypes used in the experiments.

French bean	Dry bean	Kidney bean	Pea bean
Gina cv.	Zülbiye cv.	Kınalı cv.	Araka cv.
Özayşe cv.	Kwintus cv.	Serra cv.	
Nadide cv.	Gold Nectar cv.	Şelale cv.	
Perla cv.		Lepus cv.	
Musica cv.			
Funda domestic genotype			
Tokat Sırık domestic genotype			
Karabağ domestic genotype			

conditions of 25 ± 1 °C constant temperature, 60 ± 5% relative humidity, and 16-h light/8-h dark photoperiod. The experiments were conducted as a completely randomized block design with 7 replications. One week later, bean seedling with 4 true leaves were inoculated with a mixture of adult, juveniles, and eggs of *P. penetrans* (ca. 700 in experiment 1 and ca. 1000 in experiment 2), *P. thornei* (ca. 860 in experiment 1 and ca. 1020 in experiment 2), *P. neglectus* (ca. 520 in experiment 1 and ca. 940 in experiment 2), and *P. crenatus* (ca. 860 in experiment 1 and ca. 1120 in experiment 2) in 10 mL of distilled water for experiments 1 and 2, separately. Control plants were treated with 10 mL of distilled water. The experiments were terminated 10 weeks after inoculation. The entire root system was removed and washed under tap water. Root-lesion nematodes were extracted from the 100 g of soil and from roots with the modified Baermann funnel technique (Hooper, 1986). Nematodes were quantified by counting under a light microscope and expressed as number per 100 g soil and 0.1 g dry root weight.

2.4. Statistical analysis

For each cultivar, the reproduction index, RI = Pf / Pi, was calculated, where Pf = total number of root-lesion nematodes extracted from the soil and entire root system and Pi = initial population of nematodes inoculated per pot (Ferris and Noling, 1987). The number of root-lesion nematodes per 100 g soil and per 0.1 g dry root and the RI values were used in statistical analysis. Before statistical analysis, nematode counts were transformed using the log10 (x + 1) transformation to homogenize variance. Data were analyzed by ANOVA and means were separated using Duncan's multiple range test at the P < 0.05 significance level. All statistical analyses were performed with SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

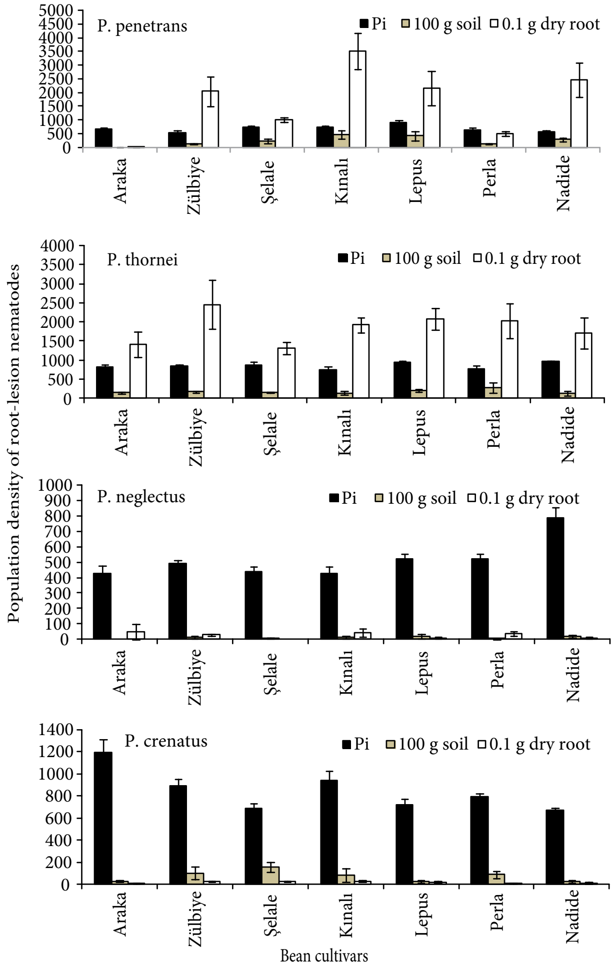

Figure 1. Population densities of root-lesion nematodes on the common bean cultivars in experiment 1 [mean ± SE of adult + juveniles of root-lesion nematodes/100 g soil, mean ± SE adult + juveniles of root-lesion nematodes/0.1 g dry root, and mean ± SE of initial population (Pi)].

3. Results

Host reactions of 15 bean cultivars and genotypes and 1 pea cultivar were determined for *P. penetrans*, *P. thornei*, *P. neglectus*, and *P. crenatus* in this study. *Pratylenchus penetrans* and *P. thornei* were extracted from the roots and soil of all bean cultivars. However, *P. neglectus* and *P. crenatus* populations were not able to reproduce in both experiments.

Pratylenchus penetrans had extremely high root and soil population densities in Zülbiye, Kınalı, Lepus, and Nadide in experiment 1 (Figure 1) and in Gold Nectar, Karabağ, Funda, Nadide, Zülbiye, and Kınalı in experiment 2 (Figure 2). On the other hand, population densities of *P. penetrans* on Gina, Musica, Perla, Kwintus, and Özayşe were lower than the initial inoculum level (Pi). The *Pratylenchus penetrans* population was dramatically reduced in the Araka pea cultivar in experiment 1. The highest RI value of *P. penetrans* was observed on Kınalı and Nadide, whereas the lowest RI was observed on Perla in experiment 1 (P < 0.05; Table 2). In experiment 2, Karabağ, Funda, and Gold Nectar had extremely high RI values for *P. penetrans*. However, Gina, Kwintus, Özayşe, Perla, and Musica had reduced RI values of *P. penetrans* (P < 0.05; Table 3). *Pratylenchus penetrans* had a RI value of slightly higher than 1 on Tokat Sırık, Şelale, and Serra and statistically significant differences were not observed among these cultivars (P < 0.05) (Table 3).

Pratylenchus thornei had a lower population density and RI value than *P. penetrans* in both experiments. Bean and pea cultivars also differed significantly for their host reaction to *P. thornei*. Zülbiye, Şelale, Kınalı, Lepus, Perla, Nadide, and Araka showed moderately susceptible host reactions to *P. thornei* in experiments 1 and 2 (Figures 1 and 2). The value of the RI was in the range of 1.8–3.1 and

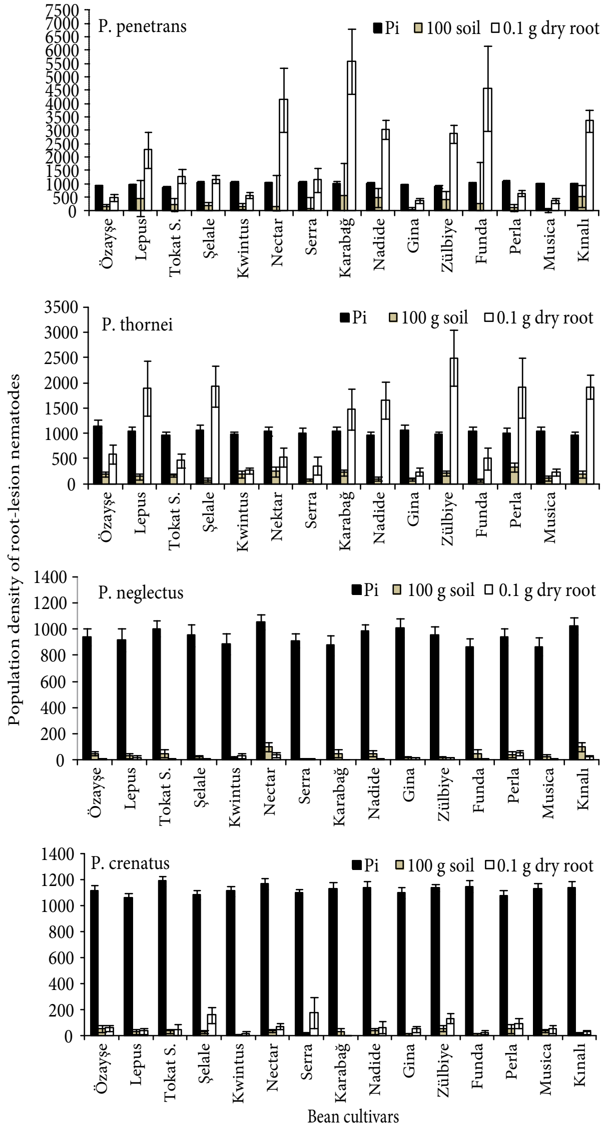

Figure 2. Population density of root-lesion nematodes on the common bean cultivars in experiment 2 [mean ± SE of adult + juveniles of root-lesion nematodes/100 g soil, mean ± SE adult + juveniles of root-lesion nematodes/0.1 g dry root, and mean ± SE of initial population (Pi)].

there were no significant statistical differences between these cultivars (Tables 2 and 3). However, Özayşe, Tokat Sırık, Gold Nectar, Kwintus, Serra, Gina, Funda, and Musica significantly reduced *P. thornei* populations and showed poor host reactions in the second experiment (Figure 2). The lowest populations of *P. thornei* were observed on Kwintus, Serra, Gina, and Musica (Figure 2). The RI values of *P. thornei* on these cultivars were also the lowest in experiment 2 (P < 0.05) (Table 3). The Karabağ genotype showed a moderately susceptible host reaction to *P. thornei* and the RI value was 1.67.

Pratylenchus crenatus and *P. neglectus* did not develop well in all tested cultivars (Figures 1 and 2). The RIs of these root-lesion nematodes were generally much lower than those of *P. penetrans* and *P. thornei* (Tables 2 and 3). All bean cultivars showed resistant host reactions to *P. neglectus* and *P. crenatus*.

4. Discussion

Pratylenchus penetrans, *P. thornei*, *P. neglectus*, *P. scribneri*, and *P. alleni* feed on bean cultivars (Sikora et al., 2005). Elliot and Bird (1985) reported a 0.5 *P. penetrans*/cm^3 soil economic threshold level for 3 dry bean cultivars. Thomason et al. (1976) showed that *P. scribneri* caused extensive root necrosis on a lima bean and suppressed plant top growth on a snap bean cultivar. However, the impact of the other cosmopolitan species, *P. thornei*, *P. neglectus*, and *P. crenatus*, to common bean cultivars has not been determined. Our results indicated that *P. penetrans* and *P. thornei* were pathogenic species on common bean. However, *P. neglectus* and *P. crenatus* could not reproduce on any of the cultivars that we tested.

The common bean cultivars used in our experiments showed various host reactions to the 4 root-lesion nematode species. The Araka pea cultivar was found to be a poor host for *P. penetrans*, whereas Oyekan et al. (1972) reported that a number of *P. penetrans* infected and reproduced in the cortex of pea roots. Subsequent nematode activities resulted in extensive breakdown of the cortex. In the present study, *P. thornei* developed and multiplied on the Araka pea cultivar. In contrast, Hollaway et al. (2000) recorded that 13 pea cultivars showed resistant host reactions to *P. thornei*.

There are several studies related to host suitability of *Phaseolus vulgaris* to root-lesion nematodes. Elliot and Bird (1985) reported that *P. penetrans* infection varied among 6 dry bean cultivars. The relative growth rate was not affected by *P. penetrans* infection in Saginaw, Gratiot, and Kentwood beans, indicating that they are tolerant. In contrast, the relative growth rate of Sanilac, Seafarer, and Tuscola was decreased, and these cultivars were highly susceptible to infection by *P. penetrans*. In our experiment, Kwintus, Özayşe, Gina, and Musica bean cultivars showed poor host reactions to both *P. penetrans* and *P. thornei*. Perla was a poor host to *P. penetrans*, whereas *P. thornei* reproduced on this cultivar in experiment 1. Karabağ, Funda, Gold Nectar, and Kınalı were susceptible to *P. penetrans*. However, Funda, Gold Nectar, and Tokat Sırık showed moderately resistant host reactions to only *P. thornei*. Söğüt et al. (2010) reported that *P. thornei* had different RI values on 3 bean cultivars in open-field experiments in Bilecik Province of Turkey. In that study, the highest RI value was observed on the Ayşekadın cultivar. Other legume plants, chickpea (*Cicer arietinum*)

Table 2. Reproduction index of root-lesion nematodes on common bean and pea cultivars in experiment 1 (mean of reproduction index ± SE).

Cultivars	*P. penetrans*	*P. thornei*	*P. neglectus*	*P. crenatus*
Araka (pea cv.)	0.01 ± 0.01 A*	1.88 ± 0.51 A	0.12 ± 0.12 A	0.03 ± 0.01 A
Zülbiye	4.04 ± 1.19 CD	3.13 ± 0.79 A	0.09 ± 0.01 A	0.15 ± 0.05 AB
Şelale	1.67 ± 0.29 ABC	1.66 ± 0.20 A	0.02 ± 0.01 A	0.27 ± 0.08 B
Kınalı	5.27 ± 1.11 D	2.76 ± 0.27 A	0.13 ± 0.07 A	0.12 ± 0.05 AB
Lepus	2.76 ± 0.68 BCD	2.41 ± 0.36 A	0.06 ± 0.01 A	0.06 ± 0.03 A
Perla	1.04 ± 0.20 AB	2.88 ± 0.50 A	0.08 ± 0.03 A	0.13 ± 0.05 AB
Nadide	4.83 ± 1.42 D	1.93 ± 0.38 A	0.04 ± 0.02 A	0.06 ± 0.03 A

*: Means of reproduction index in each column followed by the same letter are not significantly different at P < 0.05 according to Duncan's multiple range test.
Reproduction index < 1: resistant reaction, reproduction index > 1: susceptible reaction.

Table 3. Reproduction index of root-lesion nematodes on common bean cultivars in experiment 2 (mean of reproduction index ± SE).

Cultivars	*P. penetrans*	*P. thornei*	*P. neglectus*	*P. crenatus*
Özayşe	0.67 ± 0.14 A*	0.79 ± 0.26 A–C	0.05 ± 0.02 AB	0.10 ± 0.04 AB
Lepus	2.79 ± 0.83 A–D	2.08 ± 0.56 D	0.05 ± 0.02 AB	0.07 ± 0.04 AB
Tokat Sırık	1.72 ± 0.37 A–C	0.65 ± 0.15 A–C	0.05 ± 0.03 AB	0.07 ± 0.04 AB
Şelale	1.27 ± 0.15 AB	2.04 ± 0.54 D	0.03 ± 0.01 AB	0.18 ± 0.06 B
Kwintus	0.71 ± 0.10 A	0.47 ± 0.10 AB	0.05 ± 0.02 AB	0.02 ± 0.02 A
Gold Nectar	4.10 ± 1.12 DE	0.82 ± 0.23 A–C	0.12 ± 0.04 B	0.09 ± 0.02 AB
Serra	1.30 ± 0.54 AB	0.46 ± 0.16 AB	0.01 ± 0.00 A	0.18 ± 0.11 B
Karabağ	6.22 ± 1.55 E	1.67 ± 0.47 B–D	0.06 ± 0.04 AB	0.03 ± 0.02 AB
Nadide	3.60 ± 0.54 B–D	1.78 ± 0.33 CD	0.03 ± 0.02 AB	0.09 ± 0.06 AB
Gina	0.43 ± 0.11 A	0.33 ± 0.09 A	0.03 ± 0.02 AB	0.06 ± 0.02 AB
Zülbiye	3.57 ± 0.24 B–D	2.82 ± 0.66 D	0.03 ± 0.01 AB	0.17 ± 0.04 AB
Funda	4.77 ± 1.55 DE	0.61 ± 0.28 A–C	0.06 ± 0.04 AB	0.03 ± 0.01 AB
Perla	0.75 ± 0.16 A	2.21 ± 0.59 D	0.11 ± 0.03 AB	0.08 ± 0.02 AB
Musica	0.41 ± 0.09 A	0.32 ± 0.08 A	0.03 ± 0.02 AB	0.05 ± 0.01 AB
Kınalı	3.89 ± 0.56 CD	2.28 ± 0.38 D	0.12 ± 0.05 B	0.04 ± 0.02 AB

*: Means of reproduction index in each column followed by the same letter are not significantly different at P < 0.05 according to Duncan's multiple range test.
Reproduction index < 1: resistant reaction, reproduction index > 1: susceptible reaction.

and broad bean (*Vicia faba*), were good hosts for *P. thornei* (Di Vito et al., 1994a, 2000).

Pratylenchus neglectus and *P. crenatus* were reported on chickpea, field pea, and faba bean cultivars (Greco et al., 1992; Di Vito et al., 1994a, 1994b). Di Vito et al. (2000) indicated that the tolerance limit of faba bean to *P. neglectus* was 2 nematodes/cm^3 soil. Taylor et al. (2000) reported that chickpea was a good host, whereas field pea and faba bean were poor hosts for *P. neglectus*. Assessment of the impact of *P. crenatus* on legume cultivars has not been conducted in detail yet.

Legume plants are grown generally on the Anatolian Plateau, and these cultivars were rotated with winter cereals. Legume and cereal plants are good hosts for these cosmopolitan root-lesion nematodes. Therefore, the population densities of these species may remain high in the soil throughout all seasons. As a result, yield losses and plant damages of legume species could occur and plant growth could be further reduced by root damage caused by interaction with soil-borne pathogens. It is difficult to control root-lesion nematodes in legume plants because there is no satisfactory specific management method. Moreover, there are no resistant bean cultivars available commercially in the world. For the satisfactory control of root-lesion nematodes, it is important to conduct screening programs to identify resistant sources, monitoring the aggressiveness of nematode populations and development of resistant cultivars. Ferriera et al. (2010) and Dos Santos et al. (2012) reported that there is genetic variability for resistance against root-knot nematodes among the *Phaseolus vulgaris* cultivars of both dry and fresh beans. Furthermore, the resistance in some genotypes could involve different host reactions when challenged with different nematode species and races. We obtained significant results about the host suitability of 4 root-lesion nematodes on bean cultivars in our experiments. Some of the cultivars that we used significantly reduced root-lesion nematode populations. However, the field performance of these cultivars against root-lesion nematode attacks should be evaluated. Furthermore, detailed screening studies should be carried out for successful resistance breeding programs.

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