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Original article (Orijinal araştırma)

Histopathological changes of some resistant and susceptible wheat cultivars caused by plant parasitic nematodes[1]

Bitki paraziti nematodlarının neden olduğu bazı dayanıklı ve hassas buğday çeşitlerinin histopatolojik değişiklikleri

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Abstract

Root lesion nematodes, *Pratylenchus thornei* Sher & Allen, 1953 and *Pratylenchus neglectus* (Rensch, 1924) (Tylenchida: Pratylenchidae), and cereal cyst nematode, *Heterodera avenae* Wollenweber, 1924 (Tylenchida: Heteroderidae) are economically important migratory and sedentary endoparasites on host plant roots. In this study, cellular variations in roots of resistant and susceptible wheat cultivars infected by *P. thornei*, *P. neglectus* and *H. avenae* were investigated between 2017 and 2018. Wheat cultivars were infected with *P. neglectus, P. thornei,* and *H. avenae,* separately and *H. avenae* and *P. thornei* together. Twelve weeks after inoculation, roots were washed, embedded in paraffin, cut by microtome. The root cell slides were examined under light microscope for histopathological changes in the root cells. The root lesion nematodes moved from epidermal to cortical cells by damaging the cells along the feeding path. Due to the nematode infection, cortical cells of most wheat cultivars were destroyed by the development of many cavities in the root tissue. Also, it was observed that many root lesion nematodes fed collectively on the same site either stretched out or coiled in different cell layers. *Heterodera avenae* fed on the cortex cells as did root lesion nematode.

Keywords: Cereal cyst nematodes, *Heterodera avenae,* histopathology, root lesion nematodes, wheat

Öz

Kök lezyon nematodları, *Pratylenchus thornei* Sher & Allen, 1953 ve *Pratylenchus neglectus* (Rensch, 1924) (Tylenchida: Pratylenchidae) ve Tahıl kist nematodları *Heterodera avenae* Wollenweber, 1924 (Tylenchida: Heteroderidae) konukçu bitki köklerinde beslenen ve ekonomik olarak önemli hareketli ve hareketsiz endoparazitlerdir. Bu çalışmada, *P. thornei, P. neglectus* ve *H. avenae* ile bulaştırma yapılan dayanıklı ve hassas buğday çeşitlerindeki hücresel değişimler 2017-2018 yılları arasında incelenmiştir. Buğday çeşitleri, *P. neglectus, P. thornei* ve *H. avenae* türleri ile ayrı ayrı olarak ve *H. avenae* ile *P. thornei* birlikte inokule edilmiştir. İnokulasyondan on iki hafta sonra, buğday çeşitlerinin kökleri yıkanıp parafin içerisine sabitlenmiş, mikrotom ile kesilip kök hücre preparatları hazırlanmış ve kök hücrelerindeki histopatolojik değişimlerin ışık mikroskobu altında incelenmiştir. Kök lezyon nematodlarının, epidermal hücrelerden kortikal hücrelere doğru beslenme yolu boyunca hücrelere zarar vererek hareket ettiği gözlenmiştir. Nematod infeksiyonundan dolayı çoğu buğday çeşidinin, korteks hücrelerinde birçok oyuğun oluşumuyla kök dokusu tahrip olmuştur. Ayrıca, farklı hücrelerde düz ya da kıvrılmış şekilde, aynı bölgede toplu bir şekilde beslenen birçok kök lezyon nematodları gözlenmiştir. *Heterodera avenae* bireyleri de kök lezyon nematodları gibi korteks hücrelerinden beslenmiştir.

Anahtar sözcükler: Tahıl kist nematodları, *Heterodera avenae,* histopatoloji, kök lezyon nematodları, buğday

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Introduction

Wheat is one of the most important grains in the world. Global wheat consumption in 2019/20 was forecasted to reach around 757 Mt. The 2019 cereal harvest is forecast at 34.2 Mt. in Turkey (FAO, 2019 a, b). The production of wheat is impacted by many plant parasitic nematodes worldwide.

The genus *Pratylenchus* Thorne, 1949 (Tylenchida: Pratylenchidae) may include the broadest host range of plant parasitic nematodes. *Pratylenchus* spp. distributed worldwide and while most species are of little or no economic importance, some species are responsible for substantial yield losses in plants (Duncan & Moens, 2006). Plant parasitic nematodes are categorized into groups based on feeding behavior. Root lesion nematodes feed on roots as migratory endoparasitic nematodes. Migratory endoparasitic nematodes cause mechanical damage to the root system by moving through and destroying the cells on which they feed. Once they reach suitable host cells, they start extract water and nutrients. These nematodes move intercellular causing extensive rupturing of cell walls or necrosis (Vovlas & Troccoli, 1990).

Pratylenchus thornei Sher & Allen, 1953 and *Pratylenchus neglectus* (Rensch, 1924) (Tylenchida: Pratylenchidae) are commonly found in wheat fields in Turkey (Elekcioğlu, 1992; Mennan & Handoo, 2006; İmren et al., 2017). Rotation and resistant cultivars are more practical and cheaper ways of controlling these nematodes than other methods. Information on these subjects is of importance for the interaction and use of resistant cultivars as management strategies. Management of root lesion nematodes requires adequate information. Nowadays, most information in all areas of plant parasitic nematodes is related to the development of host-parasite interaction. Many studies have been published about resistance of lesion, cyst and root knot nematodes, yield loss and control of plant parasitic nematodes (Pariyar et al., 2016; Fard et al., 2018; Özdemir & Gözel, 2018; Dababat et al., 2019).

Since host-parasite relationships are important for control of plant parasitic nematodes, so understanding host-parasite relationships is valuable. The histopathology of infection by *Pratylenchus penetrans* (Cobb, 1917) (Tylenchida: Pratylenchidae) and structural change of *Heterodera glycines* Ichinohe, 1952, *Heterodera schactii* Schmidt, 1871 and *Heterodera avenae* Wollenweber, 1924 (Tylenchida: Heteroderidae) have been studied on several crops (Oyekan et al., 1972; Townshend & Stobbs, 1981; Kim et al., 1987, 2010; William & Fisher, 1993; Holtmann et al., 2000). There are few studies on the histopathology and structure of *P. thornei*, *P. neglectus* and *H. avenae* in wheat. The objective of this study was to investigate the migration, feeding, reproduction of cereal cyst nematode and root lesion nematodes in roots of resistant and susceptible wheat cultivars. Susceptible cv. Seri-82 and resistant cvs Adana-99, Ceyhan-99, Porsuk-2000, Atlı-2002 and Silverstar, which have *Cre1* and *Cre3* genes providing resistance to cereal cyst nematode, were selected (Kasapoğlu et al., 2016). This information would be important for determining the mechanisms of nematode damage in these cultivars. Given the importance for control of plant parasitic nematodes, host-parasite relationships ought to be understood thoroughly in nematology.

Materials and Methods

Nematode inoculum

Heterodera avenae was collected from infested soil in Sarıçam/Adana (Elekcioğlu, 1992; İmren, 2013). Cyst nematodes were separated from the soil using a court device, a modified form of the Fenwick (1940) method. The cysts were kept at 4°C for hatching of juveniles to get enough juvenile population (İmren, 2013). The root lesion nematodes were grown on carrot and the inoculum was obtained for the experiments according to Moody et al. (1973). To obtain inoculum, the infected carrot discs were extracted on a modified Baermann funnel method (Hooper, 1986). After extracting the nematodes, they were surface sterilized for 30 min in 0.01% streptomycin sulfate and rinsed several times in sterilized water. Each plant was inoculated with 175 *H. avenae* (juveniles), *P. thornei* and *P. neglectus* (mixed stages).

Wheat cvs Adana-99, Ceyhan-99, Porsuk-2000, Atlı-2002, Silverstar and Seri-82 were used in this study (Table 1). Wheat seeds were surface-disinfested (70% alcohol 30 s, 0.05% sodium hypochlorite 1 min) before germination. These seeds were placed on surface of wet filter paper at 21°C for 3 d in sterile Petri dishes then the germinated seeds were planted in small tubes (13 cm high and 3 cm diameter, 70 g soil) that contained autoclaved soil, (29% clay, 70% river sand and 1% organic matter) with 5 replicates in a climate chamber. Plants were grown in the climate chamber for 12 weeks at 21°C after inoculation, all plant roots were harvested, washed and fixed by hand.

Table 1. List of reactions of wheat cultivars used in experiments with *Pratylenchus thornei, P. neglectus* and *Heterodera avenae*

Cultivar	Wheat Type	Resistance	Reactions*	Reference	Resistance	Reactions*	Genes	Reference
Adana-99	Bread	*P. thornei,* *H. avenae*	R R	Toktay (2008) İmren (2013)	*H. avenae*** *P. thornei*	R	*Cre 1*	Kasapoğlu Uludamar (2018)
Ceyhan-99	Bread	*P. thornei,* *P. neglectus,* *H. avenae*	R S S	Toktay et al. (2015) İmren (2013)	*P. neglectus*	R	*Cre 1*	Kasapoğlu Uludamar (2018)
Porsuk-2000	Bread	*P. neglectus*	R	İmren (2015)	*H. avenae*** *P. thornei*	R	*Cre 1*	Kasapoğlu Uludamar (2018)
Atlı-2002	Bread	*P. thornei,* *P. neglectus*	R	İmren (2015)	*H. avenae*** *P. thornei*	R	*Cre 1* *Cre 3*	Kasapoğlu Uludamar (2018)
Silverstar	Bread	*H. avenae,* *H. latipons*	R	İmren (2013, 2014)	*H. avenae*	R	*Cre 1* *Cre 3*	Kasapoğlu Uludamar (2018)
Seri-82	Bread	*P. thornei,* *P. neglectus,* *H. avenae*	S	Toktay et al. (2015) İmren (2013)	*H. latipons* *H. avenae*	S	*Cre 1*	Kasapoğlu Uludamar (2018)

* R: Resistant, S: Susceptible;

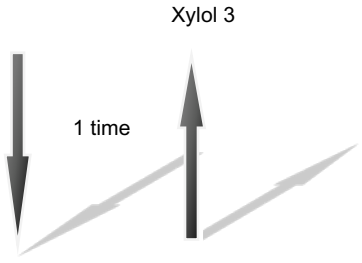
** Mixed inoculation of nematodes.

Histological studies

Root samples were kept in FPA 70 (formaldehyde-propionic acid-alcohol) solution for fixing the structure of the roots. Afterwards, they were dehydrated using tertiary butyl and ethyl alcohol series (70, 85, 95 and 100%) for 4 h at each step, air was evacuated for each sample via desiccator. The samples were kept in pure tertiary butyl alcohol overnight and then embedded in paraffin in 65°C. After 2 d, the samples were put on ice for hardening and placed on wood blocks for cutting process (Eti, 1987). The prepared samples were cut 10 µm thick with LEICA RM 2245 rotary microtome. Sections were emplaced on slides with 1:1 glycerine and egg white mixture, and left on a hot plate to dry and later transferred into an incubator (30°C) for 2 d. Then the slides with root samples were stained with 0.125% hematoxylin as shown in Table 2 and mounted in Entellan (Karabıyık et al., 2018). Slides were examined for infection sites under a LEICA DM 4000B light microscope equipped with LEICA DMC 4500 camera.

Table 2. Staining period of root cell preparations and time schedule

Solutions	Time (Min)
Xylol-1	10
Xylol-2	10
Isopropyl alcohol-1	5
Isopropyl alcohol-2	5
96% ethyl alcohol	3
70% ethyl alcohol	3
40% ethyl alcohol	3
20'% ethyl alcohol	3
Distilled water	15
Hematoxylin	30



Results

The objective of this study was to provide information on the histopathological changes of *P. thornei*, *P. neglectus* and *H. avenae* including penetration, migration to a feeding site, feeding, egg laying, and molting in resistant and susceptible wheat cultivars by using light microscopy to examine living nematodes in the roots. Histological observations indicated that many *P. thornei* larvae had reached the endodermis and cortex where they fed and reproduced (Figure 1a, b). Cavity of cortical layer follows the path of root lesion nematodes, also small nuclei, necrosis and cell wall thinning was observed in the adjacent cells. Most of the *P. thornei* moved together intracellularly. So, pictures were taken of the root damage caused by collective nematodes as shown in Figure 1c, d. Since *H. avenae* did not develop in cv. Atlı-2002 with *Cre1* and *Cre3* genes (Table 1), related pictures were not be able to taken (Figure 1a). Large cavities, necrosis and thickened walls occurred both on vertical and transverse sections. Necrosis were evident of nematode feeding. No evidence was found of nematodes feeding in endodermis.

Mixed inoculation of *H. avenae* and *P. thornei* showed low reproduction in Porsuk-2800. In the experiment, a few plant parasitic nematodes were found in vertical and transverse sections. So, some of cells did not show damage from *P. thornei* and *H. avenae* (Figure 2a, d) but the damage was seen in other sections (Figure 2b, c, e). *Heterodera avenae* and *P. thornei* did not feed in the xylem and phloem. Occasional damage and hyperthyroid nucleus were formed in the cortex (Figure 2d). The movement of *H. avenae* and *P. thornei* was limited within the cortex, resulting in undisturbed cells. Nevertheless, it allowed identification of eggs of nematodes and cytoplasm contained in intensely osmophilic grains and egg laid in cortical cells (Figure 2d). After hatching, females and juveniles moved on parenchymatous cells and caused enlargement of the lesion. Cortical cells adjacent to infected sites showed dense granular cytoplasm with large cavities and damage.

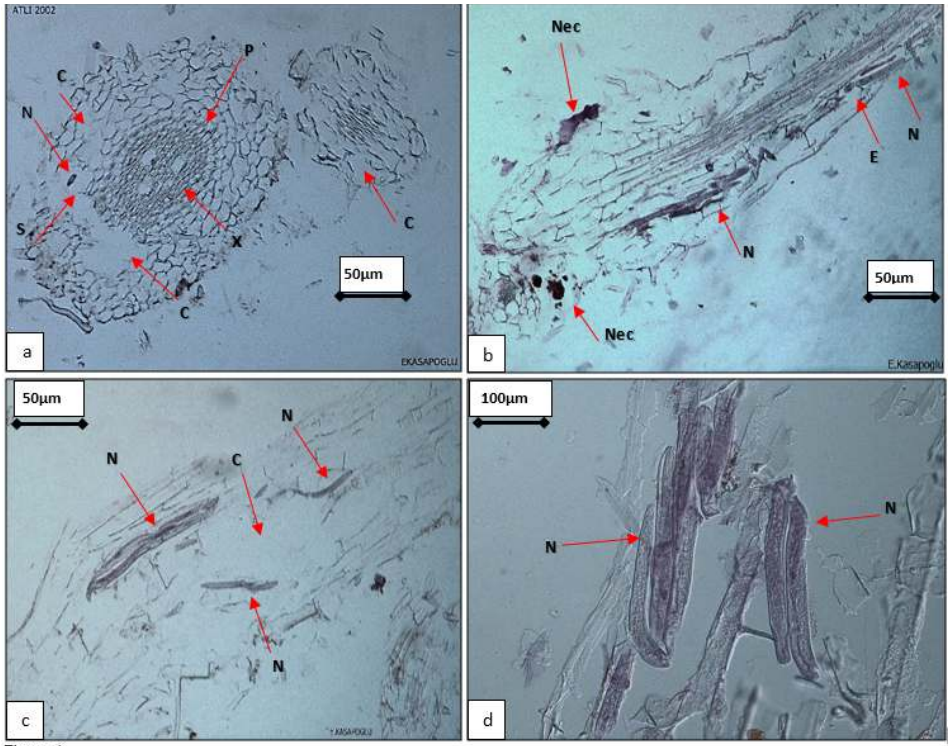


Figure 1. Structure of syncytia induced in the roots of Atlı-2002 by *Heterodera avenae* and *Pratylenchus thornei*: a) extensive cell; b & c) extensive damage of the cortex. Thickened and disrupted cell wall: d) root cell feeding of nematodes.
E: nematode egg, N: Nematode, Nec: Necrosis, S: Syncytium C: Cavity, X: Xylem, P: Phloem, H: Hypertrophied nuclei.

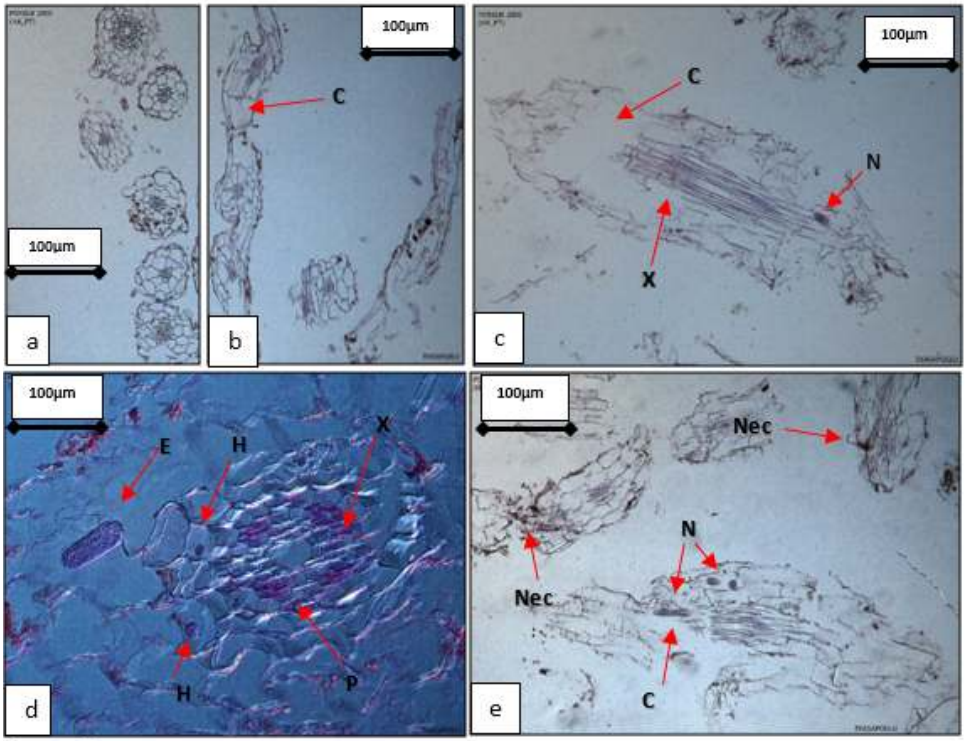


Figure 2. a) Healthy root cell image of cv. Porsuk 2800; b & c) extensive damage of the cell walls in the cortex of cv. Porsuk-2800 by *Heterodera avenae* and *Pratylenchus thornei*, showing hypertrophied cells, cell wall breakdown on transverse section and large cavity in old infections; d & e) transverse section of roots showing damaged endodermis, epidermis and cortex.
E: Nematode egg, N: Nematode, Nec: Necrosis, C: Cavity, X: Xylem, P: Phloem, H: Hypertrophied nuclei.

Figure 3 shows that root cells responded to *P. neglectus* individuals broken through in the susceptible cv. Ceyhan 99. Usually, nematodes affected three or four cells in each region like tunnels (Figure 3b, e, f, g). Feeding of *P. thornei* and *P. neglectus* caused large cavities in the cortex with their walls. These histological observations demonstrated feeding of root lesion nematodes. Seri-82 was susceptible to *P. thornei* and *P. neglectus* (Table 1). So, there were several straight and coiled nematodes in cell tissue. Especially, several nematodes fed together in damaged cells. It was observed that *P. neglectus* moved in the endodermal cells whereas *P. thornei* were found frequently in the cortex (Figure 3d, g). There were large vacuoles; nucleus. The cortex had very extensive cavities and cell walls were thickened.

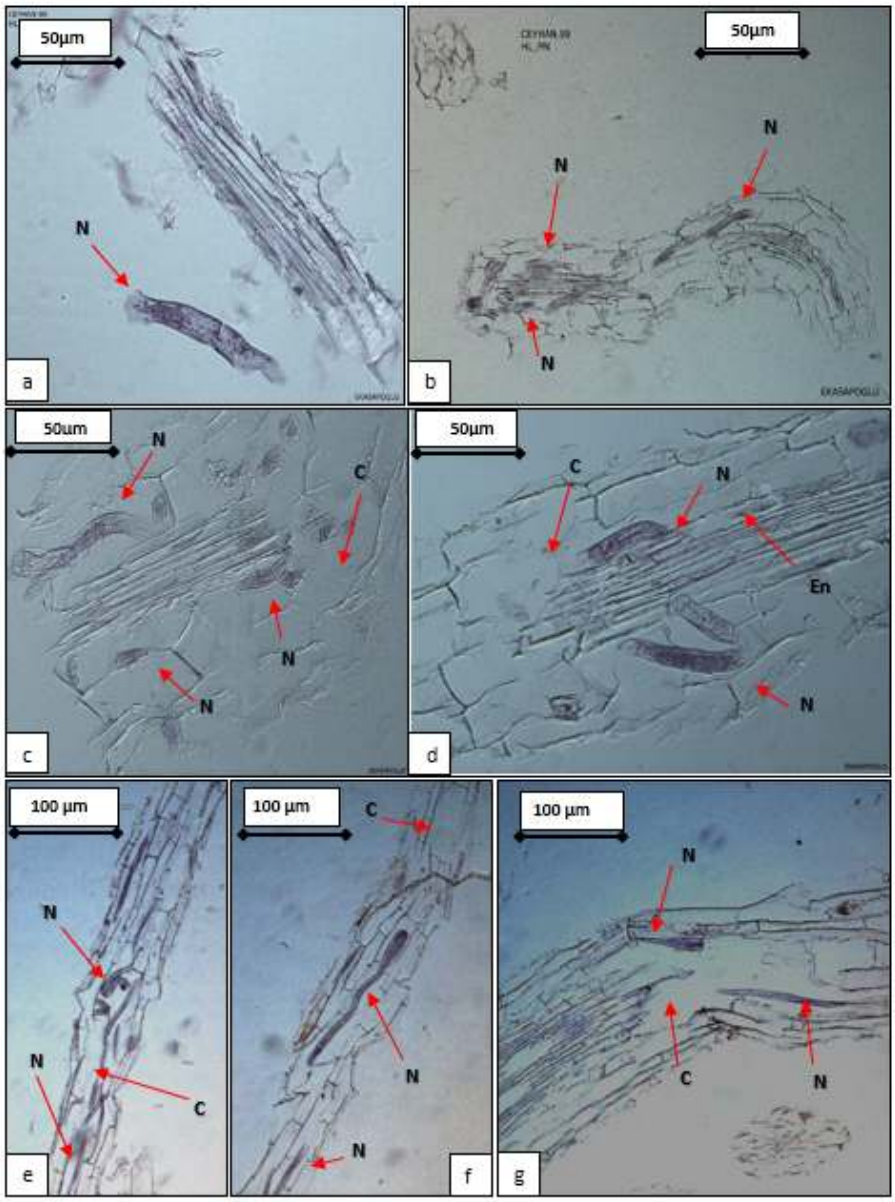


Figure 3. Histopathological changes induced by migration and feeding of the root lesion nematode *Pratylenchus neglectus* in root cell of Ceyhan-99: a) vulva region of *Pratylenchus neglectus* in Ceyhan 99; b,c & d) head, body, tail of *Pratylenchus neglectus* in root cell, feeding of nematodes in the cortex layer in Ceyhan 99; e & f) coiled *Pratylenchus thornei* occupying on different cell in Seri- 82; g) extensive damage (large cavity) in cortical layer in Seri-82.
En: Endodermis, N: Nematode, C: Cavity.

Wheat cv. Adana-99 is resistant to *H. avenae*. Syncytia had large vacuoles and hyperthyroid nuclei (Figure 4b, d). Syncytia developed longitudinally along root axis. Membrane thinning and degeneration was observed. Also, juveniles invaded the endodermal and cortical layers in Adana-99 (Figure 4a, b). Epidermis, exodermis and endodermis were damaged by the nematodes. Cell integrity deteriorated in many layers (Figure 4a, c) of root tissue. According to feeding strategy, some parts had intensive necrosis and discoloration in epidermis and exodermis (Figure 4c, d) and it covered major regions. Since Adana-99 has *Cre1* and *Cre3* genes (Table 1), syncytia occurred in limited numbers and consisted of only few cells (Figures 1a and 4b, d) on this cultivar. Figure 4d shows juveniles migrated in root cell of the resistant cv. Silverstar. They had large vacuoles and hyperthyroid cell around syncytia. Syncytia joined more cells as wall disintegrated but xylem and phloem were unaffected. Compared to wheat with *Cre1* and *Cre3* genes, Silverstar had smaller syncytia than Adana-99.

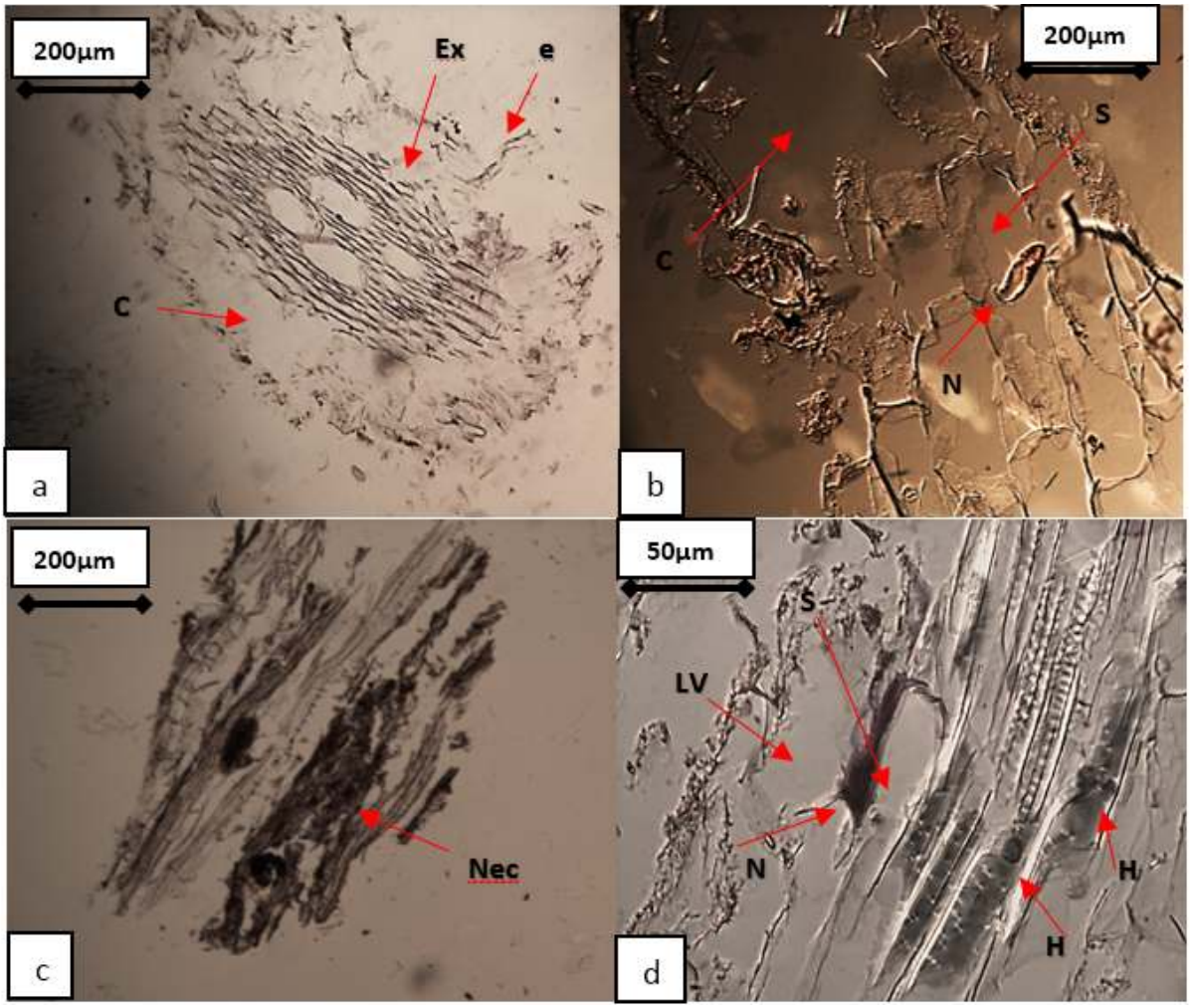


Figure 4. a &b) Cellular reaction to *Heterodera avenae* on Adana-99; c & d) Silverstar.
LV: Large vacuole, E: epidermis, Ex: exodermis, N: Nematode, Nec: Necrosis, C: Cavity, H: Hypertrophied nuclei, S: Syncytium.

Discussion

Juveniles penetrated roots cells of resistant and susceptible wheat cultivars in this study. *H. avenae and P. thornei* avoided the endodermis after infection. However, *P. neglectus* juveniles fed on the endodermal layer. It is known that *P. thornei* always migrates through the epidermal and cortical layers in chickpea (Castillo et al., 1998). No nematodes were found in the stele. Based on these observations, these nematodes infected Adana-99, Ceyhan-99, Porsuk-2800, Atlı-2002 and Seri-82. However, Adana-99, Porsuk-2800, Atlı-2002 were resistant to cyst and root lesion nematodes (Table 1).

Pratylenchus thornei and *P. neglectus* are included into the group of migratory endoparasitic nematodes. Motions of *P. thornei* and number of deposited eggs were limited in resistant wheat cultivars. It is known that nematode multiplication was greatly inhibited by resistance because only 10% juveniles developed in roots of resistant compared to the susceptible plants. Egg deposition was up to 30%, which was lower in resistant cultivars than susceptible cultivars. In addition, the effects of root exudates inhibited migration, hatching and reproduction (Linsell et al., 2014). In our study, it was observed that *P. thornei* and *P. neglectus* damaged the cells in tunnel shapes. The resistant genotype Porsuk-2800 sustained different cavity sizes. However, there were no coiled or straight individuals of *P. thornei*. Nevertheless, *P. thornei* and *P. neglectus* developed colonies in Atlı-2002, Ceyhan-99 and Seri-82. Moreover, Porsuk-2800 retained hyperthyroid nuclei and eggs. It was reported that *P. penetrans* lay eggs in cortical tissues as colonies form (Vovlas & Troccoli, 1990).

Histological studies indicated that *H. avenae, P. thornei* and *P. neglectus* readily penetrated these wheat cultivars. Nematodes were never observed to move in the stele. *Pratylenchus neglectus* penetrated roots in groups and clustered in the cortex. *Pratylenchus neglectus* penetrated equally in resistant and susceptible cultivars. However, the number of nematodes in resistant wheat was lower than in susceptible wheat cultivars due to initial population in soil (Farsi et al., 1996). Although wheat cultivars were resistant to nematodes, it was observed that *P. thornei* and *H. avenae* fed in the cells. Most probably, some of juveniles were not able to develop and were unsuccessful in syncytium zone. However, other juvenile development appeared in syncytial root cells in susceptible and resistant wheat cultivars (Williams & Fisher, 1993). It is known that degeneration of syncytia started 13 d after inoculation in host tissue. Also, the syncytia in wheat with *Cre1* or *Cre3* genes were extensively vacuolated and less metabolically active; syncytia developed later than in susceptible wheat cultivars (Grymaszewska & Golinowski, 1991; Seah et al., 2000). Silverstar has *Cre1* and *Cre3* genes, as a result small syncytium and less activity was observed. Cell wall thickenings and breakage were found in syncytia close to nematode heads.

Plant parasitic nematode damage to cells also allows pathogens such as fungi and bacteria to infect the same sites. This can result in large necrotic lesions in the cortex. The damage of cells which the nematode fed and migrate can result from both biochemical and physical factors (Farsi, 1996; Gheysen & Mitchum, 2011; Linsell et al., 2014; Göze Özdemir et al., 2018). Plants can release secretions as reaction or defense mechanism to kill plant parasitic nematodes (Kepenekçi et al., 2016; Aydınlı et al., 2019). Therefore, the different strategies may have developed in the resistance response in wheat or other crops. This study provides information on the histopathological of the interaction of nematodes with the host plants. It will be helpful in understanding the mechanisms of nematode damage.

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