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Orijinal araştırma (Original article)

Screening resistance level of Brassicaceae plants to cabbage cyst nematode, *Heterodera cruciferae* Franklin, 1945 (Tylenchida: Heteroderidae)[1]

Lahana kist nematodu *Heterodera cruciferae* Franklin, 1945 (Tylenchida: Heteroderidae)'ye Brassicaceae bitkilerinin dayanıklılık seviyelerinin araştırılması

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Summary

Although cabbage cyst nematode is one of the most common pests in the cabbage growing areas of Samsun, there were no records related to resistance of cabbages. The aim of this research was to screen cabbage cultivars resistance level for their potential to be included in breeding programs for resistance against *Heterodera cruciferae*. Franklin, 1945 (Tylenchida: Heteroderidae) Thirty cabbage cultivars were tested in controlled greenhouse trials and 3 cultivars were determined susceptible, 11 cultivars were partially susceptible, 9 cultivars were resistant and 7 cultivars were highly resistant. White head cabbage cultivars were the most resistant; 7 of them were highly resistant and 5 of them were resistant. Forty % of kale were resistant and 60% were partially susceptible. Chinese cabbage and Brussels sprouts were all classified as partially susceptible.

Key words: *Heterodera cruciferae,* cabbage, resistance

Özet

Lahana kist nematodu, Samsun'un lahana yetiştirilen alanlarındaki en yaygın zararlılardan biri olmasına rağmen lahana çeşitlerinin dayanıklılığı ile ilgili bir bilgi mevcut değildir. Bu nedenle, lahana çeşitlerinin *Heterodera cruciferae* Franklin, 1945 (Tylenchida: Heteroderidae)'ye dayanıklılık seviyelerinin değerlendirilmesi ile ıslah programlarında dayanıklılık kaynağı olarak kullanılabilme potansiyelinin belirlenmesi amaçlanmıştır. Otuz lahana genotipi kontrollü seralarda testlenmiştir. Değerlendirilen genotipler arasından 3'ü hassas, 11'i kısmen hassas, 9'u dayanıklı ve 7'si yüksek oranda dayanıklı olarak tespit edilmiştir. Dayanıklılığın en yüksek olduğu beyaz baş lahana çeşitlerinin 7 tanesi yüksek dayanıklı, 5 tanesi ise dayanıklı olarak bulunmustur. Yaprak lahanaların % 40'ı dayanıklı, % 60'ı ise kısmen hassas iken, Çin lahanası ve Brüksel lahanası kısmen hassas olarak belirlenmiştir.

Anahtar sözcükler: *Heterodera cruciferae,* lahana, dayanıklılık

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Introduction

Heterodera cruciferae Franklin, 1945 (Tylenchida: Heteroderidae), the cabbage cyst nematode, has been reported from Europe, the United States of America (California), South Australia, Eastern Azarbaijan and Iran, especially in cabbage growing areas (Stone & Rowe, 1976; Whitehead, 1998; Sturhan & Liskova, 2004; Jabbari & Niknam 2008). The nematode infects only crucifers and persists in the soil even in the absence of a host crop; and two to three generations may be completed in one year on a long-growing host. In severe infestations, long crop rotations of more than 6 years are required. This nematode causes stunting, chlorosis and reddish colors on leaves (Thorne, 1961). Infected plants can retard growth by placing plants under stress, although crop yields are rarely affected. Initial symptoms of *H. cruciferae* infection include small and undernourished appearance in the host plant. As the infection progresses, leaves may wilt or curl, especially during hot weather. In the soil the invaded roots branch effusively, while the taproots remain small. Some plants may die prematurely; the survivors produce loose small heads and discolored root systems. Invasion of infected roots by fungi is a common secondary problem. Circular patches of affected plants may appear in the field. The best diagnostic characteristic, however, is the appearance of the pearly white, tan or reddish bodies of females dotting the root surface. More than three quarters of the cabbage growing areas (78%) in Samsun province, a middle Black Sea Region famous for cabbage production in Turkey, are infested with *H. cruciferae* (Mennan & Handoo, 2006; Mennan et al., 2009).

As a member of the Heteroderidae family, this species has a protective cyst that makes this pest more difficult to control with nematicides and cultural practices. Developing new chemical nematicides is difficult because of ecological and economical restrictions on nematicide use for control of plant parasitic nematodes (Chitwood, 2002). Soil fumigation, suitable planting time, selection of varieties and destruction of weed hosts assists in controlling this nematode (Stone & Rowe, 1976). Using resistant cultivars is considered one of the most effective and environmentally safe alternative control method (Roberts, 1992). Resistance to nematodes in plants is generally characterized by both the failure of the nematodes to produce functional feeding sites in the host after invasion and to develop subsequently as reproducing females (Williamson & Kumar, 2006). In contrast, susceptible plants let the nematode reproduce, making them good hosts (Trudgill, 1991).

The aims of this research were to screen the resistance of 30 *Brassica* cultivars to *H. cruciferae*, so as to be able to provide advice to farmers about resistant varieties and to select suitable lines for breeding programs.

Materials and Methods

Plant material

Resistance / susceptibility of 30 Brassica cultivars including 16 white head cabbages, 10 kale, 2 red head cabbages, a Chinese cabbage and a Brussels sprout to *H. cruciferae* were evaluated. Individual cabbage seedlings were transplanted at the 3-4 leaf stage to 300 cm^3 plastic pots filled with steam sterilized sandy loam soils in a controlled greenhouse at 25±1°C temperature and 60±10% relative humidity.

Nematode culture

Heterodera cruciferae cysts were obtained from infested fields. For mass culture, cabbage seedlings were inoculated with those cysts. *Heterodera cruciferae* numbers were multiplied on white head cabbage cv. Yalova-1 grown in steam-sterilized sandy loam soil: sand (1:1) in a controlled greenhouse at 25±1°C temperature and 60±10% relative humidity.

Resistance testing

All plants were inoculated with 2500 second stage juveniles 3 days after transplanting. Pots were arranged in a completely randomized block design with four replicates and watered daily and fertilized weekly with A-Crop®, a water-soluble fertilizer (18% N, 18% P and 18% K). This experiment was repeated twice under the same controlled conditions. All plants were harvested 8 weeks after the inoculations. Fresh root and shoot weights were measured. Dry shoot weights, were measured after drying tissue at 80 °C for 48 hours. The leaf area of each cabbage plant was also measured with a planimeter (LI-3000C Portable Area Meter, LI-COR Biosciences). Cysts were extracted from both the soil of each pot and roots of treated plants; roots were stained with acid fuchsine for determination of nematode population densities (Hussey, 1990). Females were counted under a stereo binocular microscope (Leica, S6D).

Data analysis

Plant growth data were analyzed with the t test by using the SAS statistical program to determine the relationship between cultivar and nematode (SAS Institute, 1985). To compare the number of females per root between cultivars were used by Tukey test using SAS statistical program. Resistance level of the cabbage cultivars was determined by using a scale for evaluation of potato cultivars for potato cyst nematode depending on numbers of females per root and a female index calculation formula explained below (Table 1) and reported by Noel et al. (1990). A multivariate cluster analysis of the nematode resistance measurement for number of *H. cruciferae* females per plant was used to create dendograms of similarity matrix data by applying the unweighted pair-group method with arithmetic average (UPGMA) cluster analysis using the SAS-JMP package.

Table 1. Scale of resistance / susceptibility level of cabbage cultivars to *Heterodera cruciferae*

Scale	Female/plant	*Female index	Level of resistance
0	0	0	Highly resistant
1	1-15	0,10-0,60	Resistant
2	16-50	0,61-2,00	Partially susceptible
3	>50	> 2,00	Susceptible

*Female index= (number of females/ number of initial juveniles) x100

Results and Discussion

There were significant differences ($P \leq 0.05$) between *Brassica* cultivars for all evaluated parameters, including female *Heterodera cruciferae* per g root and reproduction factor. It significantly affected shoot fresh and dry weight and also root weight.

On the basis of the number of females present, seven cabbage cultivars, 524 Ç, 166 T, 519 Ç, 542 Ç, 508 Ç, 115 T and 530 T, showed high resistance. No females were found in the roots and soils of these cultivars which were all white head cabbages (Tables 2 & 3). Nine cabbage cultivars, 5 of which were white head cabbage cultivars, were resistant to cyst nematode, and only 3 cabbage cultivars, 195 T, 526 Ç and *B. oleracea* var. c*apitata* subvar. *rubra* cvs. Uludağ, were susceptible. The highest numbers of females were found on these white head cabbages. Sixty percent of *B. oleracea* var. *acephala* (Kale) cultivars were partially susceptible and forty percent of *B. oleracea* var. *acephala* (Kale) cultivars were resistant. Two *B. oleracea* var. c*apitata* subvar. *rubra* (red head cabbage) cultivars were screened; while one of them was only partially susceptible, the other cultivar was susceptible. Both *B. campestris* subsp. *pekinensis* (Chinese cabbage) and *B. oleracea* convar. *oleraceae* var. *gemmifera* (Brussels sprout) were classified as partially susceptible, but Brussels sprout had more females than other cultivars.

Table 2. The effects of *Heterodera cruciferae* on shoot height (cm), fresh and dried shoot weight (g), leaf number, leaf area and fresh root weight (g) of 30 Brassica cultivars

Species	Cultivar	Nematode applied (+) or not (-)	Shoot height (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Leaf number	Leaf area	Fresh root weight (g)
B. oleracea var. *capitata* subvar. *alba*	Yalova-1	+	17,25 b	11,93 a	1,31 a	7,75 a	300,45 a	1,20 a
		-	19,75 a	14,06 a	1,68 a	7,50 a	321,50 a	0,16 b
	524 Ç	+	16,87 a	8,18 a	1,54 a	5,00 a	132,33 a	1,76 a
		-	16,75 a	8,49 a	1,72 a	7,50 a	147,65 a	1,54 a
	241 Ç	+	16,75 b	8,89 a	1,49 b	6,25 a	153,00 a	1,36 a
		-	19,25 a	11,19 a	2,34 a	6,50 a	184,50 a	1,93 a
	145 Ç	+	13,00 a	5,66 a	1,19 a	6,00 a	100,02 a	1,63 a
		-	11,50 a	3,63 b	0,83 a	5,50 a	62,70 b	2,04 a
	166 T	+	16,75 a	7,98 a	1,26 a	6,00 a	120,53 a	1,73 a
		-	16,50 a	5,92 a	1,01 a	5,50 a	100,70 a	1,19 a
	519 Ç	+	15,12 a	9,40 a	1,66 a	6,75 a	125,30 a	2,62 a
		-	14,00 a	6,00 a	1,46 a	5,00 b	115,95 a	1,11 b
	542 Ç	+	16,50 a	10,55 a	2,09 a	6,75 a	179,15 a	1,94 a
		-	16,25 a	11,37 a	2,31 a	7,50 a	196,15 a	2,23 a
	144 Ç	+	18,37 a	15,66 a	2,58 a	6,50 a	251,63 a	3,91 a
		-	20,00 a	10,13 b	1,39 b	9,00 a	158,00 a	2,74 a
	165 Ç	+	22,87 a	18,96 a	2,77 a	7,50 a	307,20 a	4,16 a
		-	19,25 a	8,42 b	1,69 b	6,00 b	164,30 b	0,93 b
	508 Ç	+	21,62 a	13,05 a	2,22 a	6,00 a	173,75 a	2,15 a
		-	18,25 b	10,81 a	2,19 a	6,50 a	43,60 b	1,80 a
	148 Ç	+	24,50 a	10,61 a	1,84 a	4,75 a	139,98 a	1,69 a
		-	21,50 a	7,48 a	1,67 a	4,50 a	121,60 a	1,75 a
	115 T	+	24,75 a	17,91 a	2,49 a	5,75 a	239,33 a	2,21 a
		-	19,25 b	8,74 b	1,23 b	4,50 a	98,00 b	0,63 b
	530 T	+	21,12 a	14,17 a	2,21 a	6,75 a	175,58 a	2,22 a
		-	18,00 a	8,08 b	1,78 a	5,50 a	128,75 a	0,95 b
	195 T	+	21,12 b	26,15 a	2,04 a	9,75 a	368,20 a	2,00 a
		-	28,75 a	24,67 a	1,61 a	10,00 a	459,00 a	2,11 a
	526 Ç	+	23,17 a	17,67 a	1,18 a	7,25 a	284,48 a	1,64 a
		-	26,00 a	15,37 a	0,77 a	7,50 a	234,85 a	2,32 a
	140 T	+	18,50 a	21,01 a	1,76 a	9,75 a	342,43 a	2,34 a
		-	16,75 a	12,52 b	1,11 b	9,00 a	270,65 a	1,88 a
B. oleracea var. *acephala*	OR-49	+	20,62 a	8,60 b	0,57 b	8,75 a	262,75 b	0,30 a
		-	23,50 a	12,45 a	1,13 a	9,00 a	340,95 a	0,01 b
	OR-37	+	18,50 a	10,99 a	0,67 a	11,50 a	309,90 a	0,28 a
		-	23,00 a	13,64 a	0,99 a	10,50 a	420,00 a	0,01 b
	OR-51	+	21,00 a	9,90 b	1,08 a	7,50 b	256,25 b	0,40 a
		-	22,75 a	18,40 a	1,71 a	12,00 a	438,65 a	0,15 b
	OR-46	+	22,12 a	12,39 a	0,98 a	11,50 a	334,75 a	0,23 a
		-	24,50 a	13,10 a	1,00 a	8,00 b	299,05 a	0,18 a
	S-6	+	31,25 a	17,17 a	1,65 a	6,50 a	379,33 a	0,86 a
		-	27,25 a	20,87 a	1,83 a	8,00 a	470,95 a	0,09 b
	T-20	+	23,37 a	13,38 a	1,09 a	6,25 a	358,53 a	0,46 a
		-	19,75 a	7,71 a	0,52 a	5,00 a	224,45 a	0,01 a

Table 2 (continued)

Species	Cultivar	Nematode applied (+) or not (-)	Shoot height (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Leaf number	Leaf area	Fresh root weight (g)
	T-4	+	20,12 a	14,90 a	0,96 a	8,50 a	373,50 a	0,37 a
		-	21,00 a	14,89 a	0,35 b	8,50 a	393,30 a	0,11 a
	Karadeniz	+	20,62 a	12,30 a	1,45 a	7,50 a	295,48 a	1,84 a
		-	20,75 a	13,86 a	1,52 a	7,50 a	333,00 a	0,49 a
	OR-38	+	16,75 a	8,00 a	0,88 a	6,50 a	229,00 a	1,51 a
		-	16,25 a	7,52 a	0,99 a	6,50 a	203,45 a	0,30 b
	OR-39	+	14,50 b	7,80 a	0,97 a	6,25 a	202,43 a	1,14 a
		-	17,00 a	9,69 a	1,34 a	7,00 a	258,15 a	0,74 a
B. oleracea var. *capitata* sbvar. *rubra*	Mahrenkopf	+	18,50 a	12,49 a	1,14 a	8,00 a	271,28 a	1,04 a
		-	20,00 a	13,14 a	1,44 a	8,00 a	305,70 a	0,24 b
	Uludağ	+	21,17 a	14,65 a	1,55 a	7,25 a	266,95 a	1,78 a
		-	24,70 a	12,53 a	1,11 a	7,00 a	255,40 a	1,63 a
B. campestris subsp. *pekinensis*	Tokat-2	+	20,87 a	11,33 b	0,57 a	11,0 a	217,23 a	0,12 a
		-	24,75 a	13,48 a	0,62 a	15,0 a	268,95 a	0,01 a
B. o. convar. *oleraceae* var.	Star F_1	+	12,10 a	16,12 a	1,39 a	12,75 a	230,05 a	1,69 a
		-	13,50 a	9,07 a	0,70 b	9,50 a	133,10 a	1,90 a

*Means within a column followed by the same letter were not different according to t test (P≤0.05).

Studies related to *H. cruciferae* have mostly been about its morphology, biology, distribution and population density (Sykes & Winfield, 1966; Winfield et al., 1970; Lewis, 1971; Ecevit, 1975; Mennan & Handoo, 2006; Jabbari & Niknam, 2008; Mennan et al., 2009). The present study is the first to report on the resistance levels of commonly grown cabbage cultivars to *H. cruciferae*. The resistance of Brassicaceae plants to root-knot nematodes has been reported (Khan & Khan, 1991; McSorley & Frederick, 1995; Pattison et al., 2006), but there were no reports on *H. cruciferae*. Mennan & Handoo (2006) reported differences in infection rates among cabbage cultivars from a survey in Samsun province. They found the highest numbers of cysts on red head and white head cabbages. This is consistent with our findings that the highest numbers of females were on the white head cabbage (195 T) and red head (Uludağ) cultivars that are preferred by the growers in the Samsun region and the rest of Turkey (Table 4).

Table 3. Number of females, female index and resistance level of 30 Brassica cultivars after inoculation with 2500 second stage juveniles of *Heterodera cruciferae*

Species	Cultivar	Female	Female index	Level of resistance
B. oleracea var. *capitata* subvar. *alba*	Yalova-1	21,00 c-g*	0,84	Prt.** susceptible
	524 Ç	0,00 g	0	Highly resistant
	241 Ç	5,00 fg	0,20	Resistant
	145 Ç	15,00 e-g	0,60	Resistant
	166 T	0,00 g	0	Highly resistant
	519 Ç	0,00 g	0	Highly resistant
	542 Ç	0,00 g	0	Highly resistant
	144 Ç	4,00 fg	0,16	Resistant
	165 Ç	4,00 fg	0,16	Resistant
	508 Ç	0,00 g	0	Highly resistant
	148 Ç	2,00 g	0,08	Resistant
	115 T	0,00 g	0	Highly resistant
	530 T	0,00 g	0	Highly resistant
	195 T	74,50 a	2,98	Susceptible
	526 Ç	55,25 a-c	2,21	Susceptible
	140 T	49,50 a-d	1,98	Prt. susceptible
B. oleracea var. *acephala*	OR-49	17,50 d-g	0,70	Prt. susceptible
	OR-37	5,50 fg	0,22	Resistant
	OR-51	32,50 b-g	1,30	Prt. susceptible
	OR-46	14,00 e-g	0,56	Resistant
	S-6	17,50 d-g	0,70	Part. susceptible
	T-20	4,50 fg	0,18	Resistant
	T-4	10,50 e-g	0,42	Resistant
	Karadeniz	19,50 d-g	0,78	Prt. susceptible
	OR-38	19,00 d-g	0,76	Prt. susceptible
	OR-39	31,00 b-g	1,24	Prt. susceptible
B. oleracea var.	Mohrenkopf	42,50 a-e	1,70	Prt. susceptible
c*apitata* subvar. *rubra*	Uludağ	60,75 ab	2,43	Susceptible
B. campestris subsp. *pekinensis*	Tokat-2	18,00 d-g	0,72	Prt. susceptible
B. oleracea convar. *oleracea* var. *gemmifera*	Star F_1	37,50 b-f	1,50	Prt. susceptible

*Means within a column followed by a common letter are not different according to the Tukey test (P≤0.05). ** Prt.: Partially

Table 4. Resistance / susceptibility levels (%) of *Brassica* cultivars to *Heterodera cruciferae*

Cabbage group	Cultivars	Highly resistant (%)	Resistant (%)	Partially susceptible (%)	Susceptible (%)
White head	16	7 (43,75)	5 (31,25)	2 (12,50)	2 (12,50)
Red head	2	0	0	1 (50,00)	1 (50,00)
Kale	10	0	4 (40,00)	6 (60,00)	0
Chinese cabbage	1	0	0	1 (100)	0
Brussels sprout	1	0	0	1 (100)	0
Total	30	7 (23,34)	9 (30,00)	11 (36,66)	3 (10,00)

Among 30 cultivars, 3 cultivars (10%) were determined susceptible, 11 cultivars (36.66%) were partly susceptible, 9 cultivars (30%) were resistant and 7 cultivars (23.34%) were highly resistant (Tables 3 & 4). Using the number of females per plant for comparison, the dendogram in Figure 1 was developed from the relative resistance of *Brassica* cultivars to *H. cruciferae*. The investigated *Brassica* cultivars were differentiated into three large groups regarding their resistance / susceptibility. These groups had 0-5.50, 10.50-42.50 or 49.50-74.50 females per plant. According to this dendogram, cultivars could be divided into three different resistance / susceptibility categories - resistant, moderately susceptible/resistant or resistant. All partially susceptible cultivars, except *B. oleracea* var. c*apitata* subvar. *alba* 140 T, could be grouped in the first group from Yalova 1 to Star F1, which also included the three resistant cultivars 145 Ç, OR-46 and T-4, which showed resistance levels near to the partially susceptible cultivars. Two small groups can be seen within the second big group. These two small groups are the cultivars with high

resistance and resistance. Four cultivars (195 T, 526 Ç, Uludağ and 140 T) are included in the third group. Three of them are susceptible and one genotype displayed partially susceptibility.

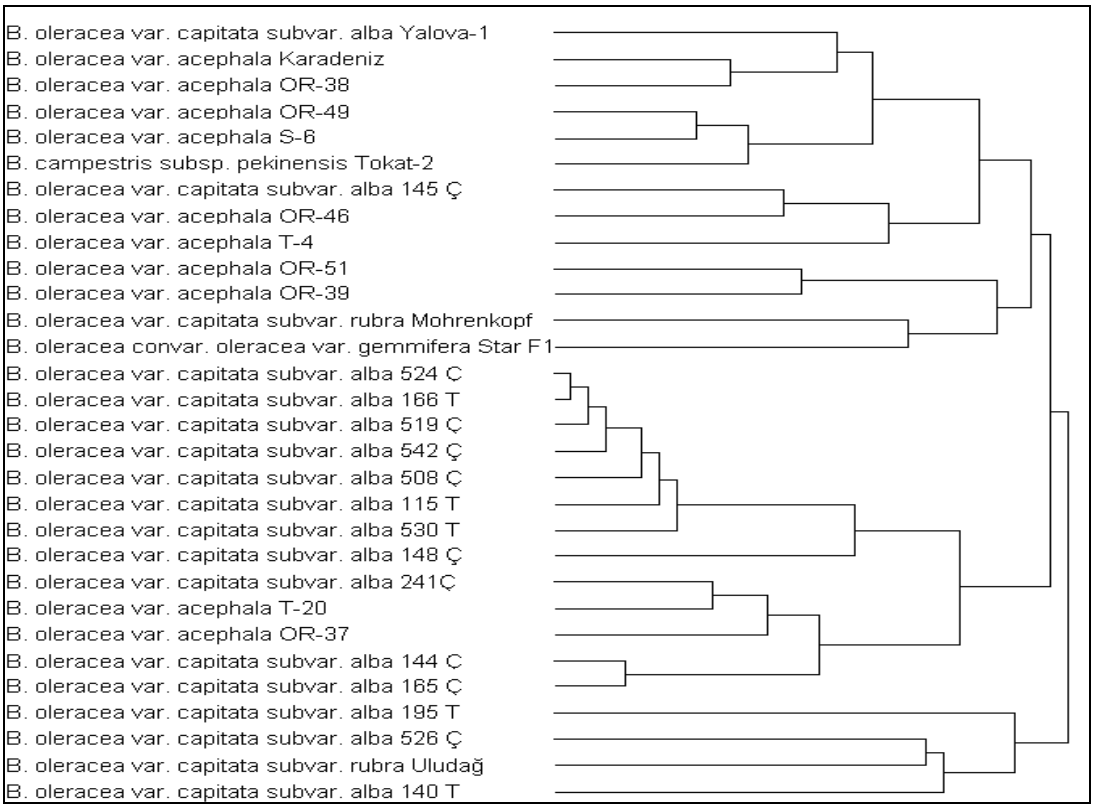

Figure 1. Dendrogram displaying groupings in a similarity matrix from UPGMA cluster analysis of resistance measurements determined from numbers of female *Heterodera cruciferae* per plant for 30 Brassicaceae cultivars.

Chemicals are not effective against cyst nematodes because of their protective cysts. Therefore, resistant cultivars may be the solution for cyst nematode problems when *Brassica* are grown in infested soil. Thus, by describing the resistance of 30 *Brassica* cultivars to *H. cruciferae*, this study makes a contribution to the selection of lines for resistance breeding programs.

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