Research Article/Araştırma Makalesi

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MEDITERRANEAN AGRICULTURAL SCIENCES (2020) 33(2): 195-199
DOI: 10.29136/mediterranean.691432

Resistance reactions of onion landraces collected from Turkey to the stem and bulb nematode *Ditylenchus dipsaci*

Türkiye'den toplanan yerel soğan çeşitlerinin soğan sak nematodu *Ditylenchus dipsaci*'ye dayanıklılık reaksiyonları

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ARTICLE INFO

Received 19 February 2020
Received in revised form 28 March 2020
Accepted 30 March 2020

***Keywords*:**

Allium cepa
Growth chamber testing
Nematode reproduction
Plant parasitic nematode
Resistance

ABSTRACT

A total of 27 accessions of different onion landraces from Turkey were screened for their resistance to stem and bulb nematode. The study was carried out in a growth chamber at 20°C, with a 16/8 h (light/dark) photoperiod and at 70% relative humidity. The plants were grown in 7 X 8 cm diameter plastic pots filled with a mixture of 45% sand, 45% clay loam soil, 10% organic matter. Two hundred nematodes in 10 µl nematode suspension were inoculated to each plant at the 3-4 leaves stage. Plants were harvested six weeks after inoculation and number of nematodes was counted. Onion landraces that had low nematode reproduction were subjected to a second screening test. The landraces were classified from moderately resistant to highly susceptible according to their nematode reproduction in comparison to susceptible standard cultivar Betapanko. Accession 30 had the lowest number of nematodes in the both experiments and was classified moderately resistant and moderately susceptible in the first and second experiments, respectively. The accessions 23 and 25 had lower number of nematodes relative to standard cultivar and were classified as moderately susceptible in conclusion.

MAKALE BİLGİSİ

Alınış tarihi 19 Şubat 2018
Düzeltilme tarihi 28 Mart 2020
Kabul tarihi 30 Mart 2020

***Anahtar Kelimeler*:**

Allium cepa
Bitki paraziti nematod
Büyütme dolabı testlemesi
Dayanıklılık
Nematod üremesi

ÖZ

Türkiye' den toplanmış olan toplam olarak 27 farklı yerel soğan çeşidinin soğan sak nematoduna dayanıklılıkları taranmıştır. Çalışma 20°C' de %70 nem koşullarında 16/8 saat (aydınlık/ karanlık) periyodunda büyütme dolabında gerçekleştirilmiştir. Bitkiler 7 X 8 cm boyutlarında plastik saksılarda %45 kum, %45 killi milli toprak, %10 oranında organik madde karışımında yetiştirilmiştir. İki yüz nematod içeren 10µl nematod süspansiyonu 3-4 yaprak aşamasında bitkilere bulaştırılmıştır. Bitkiler nematod inokulasyonundan 6 hafta sonra hasat edilmiş ve bitkilerdeki nematodlar sayılmıştır. Düşük nematod üremesi görülen bitki çeşitleri ikinci kez *Ditylenchus dipsaci* ile inokule edilmiştir. Bitki çeşitlerinde nematod üremesine göre hassas standart çeşit Betopanko ile karşılaştırmalı olarak orta dayanıklıdan yüksek oranda hassas reaksiyona kadar gruplandırılmıştır. Otuz numaralı çeşit iki denemede de en düşük nematod üremesi göstermiştir ve birinci denemede orta dayanıklı, ikinci denemede ise orta hassas olarak gruplandırılmıştır. Yirmi üç ve 25 numaralı çeşitler hassas kontrole göre düşük sayıda nematod üremesine sahip olmuş ve nihai olarak orta hassas olarak gruplandırılmıştır.

1. Introduction

Ditylenchus dipsaci is one of the most important plant-parasitic nematodes affecting plant production in temperate climate regions (Sikora and Fernandez 2005). The nematode is a species complex which multiplies on more than 500 plant species and contains high intra specific variation. Thirty biological races within the species were identified according to their host preferences. One of the most devastating races of *D. dipsaci* is onion race. The onion race has a wide host spectrum and the main hosts are onion, garlic and bulbous plants (Sturhan and Brzeski 1991). The economic damage threshold for *D. dipsaci* on onion is very low; 1 nematode in 100 g of soil (Seinhorst 1956). Onion yield losses caused by the *D. dipsaci* were reported up to 80% in Turkey and in the world (Sturhan and Brzeski 1991; Mennan and Ecevit 2002; Yavuzaslanoglu et al. 2015).

In earlier studies, synthetic nematicides were used to perform the effective control of *D. dipsaci* on host plants (Gray and Soh 1989; Andres and Lopez-Fando 1996). However, use of chemical nematicides is mostly restricted due to their adverse effects on the environment and human health (Devran et al. 2008; 2013; Moens et al. 2009).

Instead, application of more eco-friendly control mechanisms is needed for the control of nematodes. Therefore, cultural practices such as deep ploughing, rotation, use of the fallow system, and use of resistant and tolerant plant cultivars with biological control agents need to be used widely. There are no registered nematicides to *D. dipsaci* for onions in Turkey. Mennan (2005) studied the effect of alteration of the sowing time of the onion on *D. dipsaci* damage and showed that early sowing of onions decreased the nematode population level. Plant damage was decreased by dipping onion bulbs in hot water at 44-45°C for 3 h before sowing (Bridge and Hunt 1986). Control of *D. dipsaci* by rotation applications is limited due to the wide host range of the nematode species complex. There have been reports on resistant and tolerant oat, rye, beans, potato and clover cultivars which are host of *D. dipsaci* (Plowright et al. 2002; Mwaura et al. 2015). Previous studies reported commercial onion cultivars that provided low nematode reproduction and tolerance to *D. dipsaci*, under controlled and field conditions (Yavuzaslanoglu et al. 2015; Yavuzaslanoglu 2019).

Resistance and tolerance are useful control mechanisms for nematode damage. Resistance reduces the nematode population and prevents nematode penetration and/or multiplication on the plant while tolerance preserves plant yield in the presence of nematodes (Cook and Evans 1987).

Local onion varieties are advantageous for their adaptations to prevalent climatic conditions and provide higher yield and quality. Additionally, landraces possess high genetic variation. Therefore, potential of local varieties for resistance and/ or tolerance to biotic stress agents is higher.

In the present study, Turkish onion landrace cultivars were tested for their resistance to stem and bulb nematode *D. dipsaci* under the growth chamber conditions.

2. Material and Methods

2.1. Plant material

A total of 27accessions of onion landraces were tested for their resistance reactions to *Ditylenchus dipsaci* under the growth chamber conditions (Table 1). Twenty six accessions and one accession of onion landraces were provided by Pamukkale University Plant Genetics and Agricultural Biotechnology Application and Research Center (PAUBIYOM) and the Turkish Ministry of Agriculture and Forestry, Aegean Agricultural Research Institute, National Gene Bank (AARINGB), İzmir, Turkey, respectively. The standard cultivar Betapanko was provided by Atatürk Horticultural Central Research Institute (AHCRI), Yalova, Turkey and included as susceptible control for reference.

2.2. Nematode source

The stem and bulb nematode *D. dipsaci* was originally isolated from garlic grown in Karaman Province (N: 37.111592, E: 33.112628), Turkey. Pure culture from one nematode was reared on sterile carrot discs. Nematodes extracted from carrot cultures were used for plant inoculations (Kühnhold et al. 2006).

2.3. Experimental design

The landrace onion were grown in 7 x 8 cm diameter plastic pots filled with a mixture of sterilized sand, clay loam field soil and organic matter (w: w: w; 45: 45: 10). The pots were arranged in a completely randomized design in a growth chamber, at 20°C, with a 16:8 h (light:dark) photoperiod and 70% relative humidity.

Nematode inoculum was applied between two onion leaves of seedlings at the 3-4 leaves stage. Ten seedlings (one per pot) of each accession were inoculated with an average of 200 nematodes (Pi) in 10 µl carboxymethylcellulose solution (1%) (Kühnhold et al. 2006). Six weeks after inoculation, nematodes were extracted from the plants overnight (Hallmann and Subbotin 2005) and then counted to determine the final nematode population density (Pf). The nematode reproduction factor (RF) was calculated as nematodes per plant (Pf) divided by initial inoculum density (Pi= 200). The cultivars had the low number of nematodes in the first experiment were tested in the second experiment. Experimental conditions were similar to those described for the first experiment.

2.4. Statistical analysis

Kolmogorov-Smirnov (KS) goodness-of-fit test was performed to test whether the data comes from the normal distribution. According to KS test, the data transformed to ln(x+1) values to provide normality. The homogeneity of the variables was tested using Levene's test. Analysis of variance (ANOVA) and Tukey HSD test were conducted to detect statistically significant differences among the number of nematodes of the cultivars at the end of experiments. Statistical analyses were performed using JMV package based on R Packages (The R Project for Statistical Computing 2019).

Reproduction factor (RF) and relative susceptibility (RS) was used for evaluation of the resistance of onion accessions. Relative susceptibility was calculated as the number of nematodes on the accession plant divided by the number of nematodes on a susceptible standard cultivar Betapanko times by 100. Accessions were designated into resistance groups according to their RF and RS values. The RF values lower than one was accepted as resistant, while higher than one was accepted as susceptible. Level of resistance and susceptibility was determined according to RS values of the accessions (Mwaura et al. 2015).

3. Results

3.1. Experiment 1

Transformed number of nematodes was significantly different among the landrace cultivars tested ($P<0.001$). Reproduction factor of the two accessions (Accessions 25 and 30) were lower than one (0.80 and 0.50, respectively) and accepted resistant to *D. dipsaci*. Level of resistance of these accessions was designated as moderately resistant (MR) according to their RS values (35.46 and 22.24, respectively) (Table 1).

Table 1. Accession name, origin of onion landrace cultivars, ln(x+1) transformations ± standard error of mean and statistical difference according to Tukey HSD test and non transformed means (NTM) of *Ditylenchus dipsaci* per plant, reproduction factor (RF), relative susceptibility (RS) values and resistance designation of the accessions in the first experiment.

Accession name	Source[1]	*D. dipsaci* plant^{-1}		RF	RS	Resistance Designation
		ln(x+1) ± Std. Error Statistical difference	NTM			
1	PAUBIYOM	6.04± 0.38 abc	506	2.53	111.45	S
2	PAUBIYOM	7.19± 0.29 a	1.506	7.53	331.71	HS
3	PAUBIYOM	6.25± 0.38 abc	856	4.28	188.54	HS
5	PAUBIYOM	6.17± 0.38 abc	521	2.60	114.75	S
6	PAUBIYOM	6.32± 0.31 abc	661	3.30	145.59	S
7	PAUBIYOM	6.11± 0.38 abc	536	2.68	118.06	S
8	PAUBIYOM	6.10± 0.27 abc	531	2.65	116.96	S
9	PAUBIYOM	6.10± 0.44 abc	494	2.47	108.81	S
10	PAUBIYOM	6.61± 0.31 ab	851	4.25	187.44	HS
11	PAUBIYOM	5.38± 0.44 abc	321	1.60	70.70	MS
13	PAUBIYOM	6.53± 0.31 ab	881	4.40	194.05	HS
14	PAUBIYOM	5.90± 0.29 abc	438	2.19	96.47	S
15	PAUBIYOM	6.04± 0.34 abc	473	2.36	104.18	S
16	PAUBIYOM	6.42± 0.38 abc	766	3.83	168.72	HS
17	PAUBIYOM	5.68± 0.38 abc	466	2.33	102.64	S
18	PAUBIYOM	6.67± 0.34 ab	917	4.58	201.98	HS
19	PAUBIYOM	6.10 ± 0.44 abc	561	2.80	123.56	S
20	PAUBIYOM	6.85± 0.44 ab	961	4.80	211.67	HS
21	PAUBIYOM	6.30± 0.44 abc	834	4.17	183.70	HS
22	PAUBIYOM	6.48± 0.38 abc	866	4.33	190.74	HS
23	PAUBIYOM	5.67± 0.44 abc	341	1.70	75.11	MS
25	PAUBIYOM	4.83± 0.44 bc	161	0.80	35.46	MR
27	PAUBIYOM	7.09± 0.34 a	1.389	6.94	305.94	HS
28	PAUBIYOM	6.96± 0.34 ab	1.637	8.18	360.57	HS
29	PAUBIYOM	6.37± 0.44 abc	654	3.27	144.05	S
30	PAUBIYOM	4.38± 0.44 c	101	0.50	22.24	MR
Balıkesir Landrace (TR79943)	AARINGB	7.18± 0.38 a	1.336	6.68	294.27	HS
Betapanko	AHCRI	5.95± 0.44 abc	454	2.27	100	S
F Probablity		<0.001				

[1]: PAUBIYOM: Pamukkale University Plant Genetics and Agricultural Biotechnology Application and Research Center, AHCRI: Atatürk Horticultural Central Research Institute, AARINGB: Aegean Agricultural Research Institute National Gene Bank.

Reproduction factor of other tested landraces was higher than one and accepted as susceptible to *D. dipsaci*. Susceptible cultivars were classified moderately susceptible (MS) to highly susceptible (HS) according to their RS to standard cultivar Betapanko.

Reproduction factor of accessions 11 and 23 were 1.60 and 1.70, respectively. They had RS values of 70.70 and 75.11, respectively and designated into MS group.

Eleven accessions (1, 5, 6, 7, 8, 9, 14, 15, 17, 19 and 29) was designated as susceptible (S) as with RS values from 96 to 145.59.

The 12 cultivars with RS values higher than 168.72 (2, 3, 10, 13, 16, 18, 20, 21, 22, 27, 28 and Balıkesir Landrace TR79943) were designated into highly susceptible (HS) group (Table 1).

3.2. Experiment 2

Four accessions which had lower number of nematodes in the first experiment were tested secondly. Transformed number of nematodes of four accessions was not statistically different in the second experiment. All of the tested accessions had reproduction factor of higher than one, it ranged from 9.62 to 49.83. Number of nematodes was greater in the second experiment (mean of 3.896 nematodes plant^{-1}) than that in the first experiment (mean of 757 nematodes plant^{-1}). However, accession 30 had 67% lower number of nematodes than standard cultivar Betapanko. Relative susceptibility rate of accession 30 was 32.77 and designated as MS in the second test. Similarly, accessions 23 and 25 were MS with about 50% lower number of nematodes than standard cv. Betapanko. The RS values were 50.24 and 50.36, respectively. Accession 11 had higher number of nematodes than standard cv. Betapanko and was HS with a 169.70 RS value in the second experiment (Table 2).

Table 2. Accession name, origin of onion landrace cultivars, ln(x+1) transformations ± standard error of mean and statistical difference according to Tukey HSD test and non transformed means (NTM) of *Ditylenchus dipsaci* per plants, reproduction factor (RF), relative susceptibility (RS) values and resistance designation of the accessions in the second experiment.

Accession name	Source[1]	*D. dipsaci* plant^{-1}		RF	RS	Resistance Designation
		ln(x+1) ± Std. Error Statistical difference	NTM			
11	PAUBIYOM	9.17± 0.64 a	9.967	49.83	169.70	HS
23	PAUBIYOM	7.59± 0.55 a	2.951	14.75	50.24	MS
25	PAUBIYOM	7.62 ± 0.42 a	2.958	14.79	50.36	MS
30	PAUBIYOM	7.37± 0.37 a	1.925	9.62	32.77	MS
Betapanko	AHCRI	7.99± 0.49 a	5.873	29.36	100	S
F Probablity		1.578				

[1]: PAUBIYOM: Pamukkale University Plant Genetics and Agricultural Biotechnology Application and Research Center, AHCRI: Atatürk Horticultural Central Research Institute.

4. Discussion and Conclusion

Resistance of Turkish onion landrace cultivars to *D. dipsaci* was investigated in the current study. Resistance in cross pollinated plants is defined as proportion of the resistant background in the heterogeneous plant populations since they contain both susceptible and resistant genetic background (Roberts 2002). Cross-pollinated onion plants require a strategy for nematode resistance breeding in which plants are selected that have less nematode damage and lower nematode multiplication. In the current study, number of nematodes in accession 30 was lower as a proportion of the standard cultivar in both experiments. Even the nematode numbers were much greater in the second experiment; accession 30 had 67% less nematodes than standard cv. Betapanko in the second experiment. Therefore, accession 30 is suggested as a good onion source for resistance to *D. dipsaci*.

The stem and bulb nematode causes stunning, swelling and deterioration of plant tissues (Sikora and Fernandez 2005). Hosting ability of different plant species to the stem and bulb nematode was determined using plant symptoms in many studies reviewed by Janssen (1994). Beside, nematode resistance was described as a plant genetic mechanism which does not allow nematode penetration or multiplicationby Cook and Evans (1987). The plant symptoms arising from nematode multiplication need to be supported by nematode data for resistance evaluation.

Seedling screening of plants allow evaluation of large numbers of plant materials in a short time period for nematode resistance. Moreover, the seedling resistance reactions of red clover and lucerne to *D. dipsaci* were in agreement with mature hosting suitability data (Bingefors 1970; Lundin and Jonsson 1975).

Pang et al. (2009) identified resistant onion cultivars to *Pratylenchus penetrans* and *Meloidogyne hapla* among onions grown in Idaho. However, there are few studies focused on resistance of the onion to *D. dipsaci*. Yavuzaslanoglu et al. (2015, 2019) reported some commercial cultivars providing lower nematode multiplication and tolerance to *D. dipsaci*.

The current study is a preliminary survey of Turkish onion landraces for their resistance reactions to *D. dipsaci*. Future studies need to be focused on the field performance of the cultivars as well as with collaboration on onion breeding to improve market yield and quality.

Acknowledgements

This study was supported by Turkish Scientific and Technical Research Council (Project number: 111O222). Onion materials for the study provided by Pamukkale University Plant Genetics and Agricultural Biotechnology Application and Research Center (Denizli, Turkey), the Turkish Ministry of Agriculture and Forestry, Aegean Agricultural Research Institute, National Gene Bank (İzmir, Turkey) and Atatürk Horticultural Central Research Institute (Yalova, Turkey).

References

Andres MF, Lopez–Fando S (1996) Effect of granular nemticide applications on the population density of *Ditylenchus dipsaci* in garlic. Nematropica 2: 167-170.

Bingefors S (1970) Resistance against stem nematodes, *Ditylenchus dipsaci* (Kuhn) Filipjev. European and Mediterranean Plant Protection Organization Publication Series A No 54: 63-75.

Bridge J, Hunt D (1986) Nematodes. Tropical Development and Research Institute and Office of Overseas Development Administration Pest Control in Tropical Onion. Tropical Development and Research Institute, London.

Cook R, Evans K (1987) Resistance and Tolerance. In: Brown RH, Kerry BR (Eds.), Principles and Practice of Nematode Control in Crops. Academic Press, Sydney, pp. 179-231.

Devran Z, Söğüt MA, Gözel U, Tör M, Elekcioglu IH (2008) Analysis of genetic variation between populations of *Meloidogyne* spp. from Turkey. Russian Journal of Nematology 16: 143-149.

Devran Z, Başköylü B, Taner A, Doğan F (2013) Comparison of PCR-based molecular markers for identification of *Mi*gene. Acta Agriculturae Scandinavica, Section B – Soil and Plant Science 45: 395-402.

Gray FA, Soh DH (1989) A Nematicide seed treatment to control *Ditylenchus dipsaci* on seedling Alfalfa. Journal of Nematology 21: 184-188.

Hallmann J, Subbotin SA (2005) Methods for Extraction, Processing and Detection of Plant and Soil Nematodes. In: Luc M, Sikora RA, Bridge J (Eds.), Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CABI Publishing, London, pp. 87-119.

Janssen GJW (1994) The relevance of races in *Ditylenchus dipsaci* (Kühn) Filipjev, the stem nematode. Fundamentals of Applied Nematology 17: 469-473.

Kühnhold V, Kiewnick S, Sikora R (2006) Development of an in vitro bioassay to identify sugar beet resistance to the stem nematode *Ditylenchus dipsaci*. Nematology 8: 641-645.

Lundin P, Jonsson HA (1975) Weibull'sVertus, a Lucerne variety with high resistance to stem nematodes and *Verticillium* wilt. Agri Hortique Genetica 33: 17-32.

Mennan S, Ecevit O (2002) Investigations on effectiveness of different preparations on onion race of *Ditylenchus dipsaci*. Journal of Ondokuz Mayıs University Faculty of Agricultre 17: 20-24.

Mennan S (2005) Influence of planting date and initial population density on damage to onion (*Allium cepa* L.) caused by stem and bulb nematode (*Ditylenchus dipsaci*) (Kühn, 1857) (Tylenchida: Anguinidae). Turkish Journal of Entomology 29: 215-224.

Moens M, Perry RN, Starr JL (2009) *Meloidogyne* Species a Diverse Group of Novel and Important Plant Parasites, 1-17". In: Root-Knot Nematodes (Eds. Perry RN, Moens M, Starr JL). CABI, London, pp. 488.

Mwaura P, Niere B, Vidal S (2015) Resistance and tolerance of potato varieties to potato rot nematode (*Ditylenchus destructor*) and stem nematode (*Ditylenchus dipsaci*). Annals of Applied Biology 166: 257-270.

Pang W, Hafez SL, Sundararaj P (2009) Screening of onion cultivars for resistance and tolerance to *Prtylenchus penetrans* and *Meloidogyne hapla*. Nematropica 39: 47-55.

Plowright RA, Caubel G, Mizen KA (2002) *Ditylenchus* species. In: Starr JL, Cook R, Bridge J (Eds.), Plant resistance to parasitic nematodes. CABI Publishing, Wallingford, pp. 107-139.

Roberts PA (2002) Concepts and consequences of resistance, In: Starr JL, CookR, Bridge J (Eds.), Plant Resistance to Parasitic Nematodes. CABI Publishing, Wallingford, pp. 23-41.

Seinhorst JW (1956) Population studies on stem eelworms (*Ditylenchus dipsaci*). Nematologica 1: 159-164.

Sikora AR, Fernandez E (2005) Nematode parasites of vegetables, In: Luc M, Sikora R, Bridge J (Eds.), Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CABI Publishing, UK, pp. 319-393.

Sturhan D, Brzeski MW (1991) Stem and Bulb Nematodes, *Ditylenchus* spp., In: Nickle WR (ed.) Manual of Agricultural Nematology, Marcel Dekker Publications, New York, pp. 423-465.

The R Project for Statistical Computing (2019) statistical analysis packages. https://cran.r-project.org/web/packages. Accessed 15 November 2019.

Yavuzaslanoglu E, Dikici A, Elekcioglu IH (2015) Effect of *Ditylenchus dipsaci* Kühn, 1857 (Tylenchida: Anguinidae) on onion yield in Karaman Province, Turkey. Turkish Journal of Agriculture and Forestry 39: 227-233.

Yavuzaslanoglu E (2019) Resistance and Tolerance of Commercial Onion Cultivars to Stem and Bulb Nematode, *Ditylenchus dipsaci*. Journal of Agricultural Sciences 25: 409-416.