RESEARCH NOTE/NOTA DE INVESTIGACIÓN

MELOIDOGYNE CHITWOODI RACES IN FIELDS OF CENTRAL ANATOLIA, TURKEY

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ABSTRACT

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The Columbia root-knot nematode, *Meloidogyne chitwoodi,* is of economic importance to potato because it reduces tuber quality and quantity. *Meloidogyne chitwoodi* was first identified in Turkey from potato tubers collected in Nigde. *Meloidogyne chitwoodi* is currently reported to consist of two host races and two pathotypes, which cannot be distinguished morphologically or by utilizing molecular methods. Fifty-eight geographic isolates of *M. chitwoodi* were collected from Niğde (45), Nevşehir (12), and Aksaray (1) and used in this study. All isolates were evaluated for their ability to reproduce on carrot, alfalfa, and *Solanum bulbocastanum* SB22 for race and pathotype determination. Carrot was a good host for all populations (Reproductive factor (Rf): 2.01- 4.66), while alfalfa was classified as a poor host for 29 populations (Rf: 0.10-0.75) and as non-host for 29 populations (Rf: 0-0.09). None of the populations reproduced on *S. bulbocastanum* SB22. Based upon these results, only *M. chitwoodi* race 1 was found in Turkey with no evidence for the existence of host race 2 and its associated pathotypes.

Key words: Potato, root-knot nematode, *Meloidogyne chitwoodi*, *Solanum bulbocastanum* SB22

RESUMEN

Evlice, E., y Ş. Bayram. 2019. *Meloidogyne chitwoodi* carreras en campos del centro de Anatolia, Turquía. Nematropica 49:157-165.

El nematodo del nudo de la raíz de Columbia, *Meloidogyne chitwoodi*, es de importancia económica para varios cultivos, especialmente la papa, que reduce seriamente la calidad y cantidad del tubérculo. *Meloidogyne chitwoodi* se describió por primera vez a partir de tubérculos de papa infectados en Estados Unidos de América se identificó por primera vez en Turquía a partir de tubérculos de papa recolectados en Nigde. Actualmente se informa que *M. chitwoodi* consta de dos razas huésped y dos patotipos que no se pueden distinguir morfológicamente o utilizando métodos moleculares. En este estudio se utilizaron 58 aislamientos geográficos de *M. chitwoodi* que fueron recolectados de Niğde (45), Nevşehir (12) y Aksaray (1). Todos los aislamientos fueron evaluados por su capacidad de reproducirse en zanahoria, alfalfa y *Solanum bulbocastanum* SB22 para la determinación de la raza y el patotipo. Los resultados documentaron que la zanahoria es un buen huésped para todas las poblaciones (Factor reproductivo (Fr): 2.01-4.66), mientras que la alfalfa se clasificó como un huésped pobre para 29 poblaciones (Fr: 0.10-0.75) y como no

huésped para 29 poblaciones (Fr: 0-0.09). Ninguna de las poblaciones se reprodujo en *S. bulbocastanum* SB22, por lo tanto, esta planta se clasificó como no huésped para todas las poblaciones. De acuerdo con estos resultados, solo se encontró la raza 1 de *M. chitwoodi* en Turquía sin evidencia de la existencia de la raza huésped 2 y los patotipos en Turquía en este momento.

Palabras clave: Patata, *Meloidogyne chitwoodi*, nematodo de nudo de raíz, *Solanum bulbocastanum* SB22

The Columbia root-knot nematode, *Meloidogyne chitwoodi* Golden *et al.*, 1980, is of economic importance to several crops, especially potato because it reduces tuber quality. The nematode causes small pimple-like galling on the tuber surface, which renders tubers unacceptable for processing or fresh market even when these spots are as few as 10% (Al-Rehiayani and Hafez, 1998). *Meloidogyne chitwoodi* was first described from potatoes (*Solanum tuberosum*) in the Pacific Northwest (USA) (Golden *et al*., 1980). *Meloidogyne chitwoodi* was first reported in Turkey in 2009 from potato tubers (Özarslandan *et al.,* 2009). Additionally, *M. chitwoodi* has been reported on four continents: Africa (South Africa, Mozambique), Asia (Turkey), Europe (Belgium, France, Germany, the Netherlands, Portugal, Sweden), and North and South America (USA, Mexico, and Argentina) (EPPO, 2019; Özarslandan *et al*., 2009). *Meloidogyne chitwoodi* was in Europe and America much earlier than first reported (Golden *et al*., 1980; Brinkman *et al.*, 1994), thus the nematode's place of origin is unclear (Waeyenberge and Moens, 2001; Humphreys-Pereira and Elling, 2013). The nematode has a wide host range across several families, including both crop plants, weeds, and possibly some un-documented host plants (Santo *et al*., 1980; O'Bannon *et al*., 1982; den Nijs *et al*., 2004; Rich *et al.*, 2009; van der Gaag *et al.*, 2011). Although *M. chitwoodi* is a quarantine pest in many regions of the world (e.g., EPPO, EU, NAPPO), it continues to spread within and outside Europe (EPPO, 2019).

Host races of *Meloidogyne* species have been defined according to the nematode's ability to reproduce on a set of host plants from different genera (Hartman and Sasser, 1985; Rammah and Hirschmann, 1990; Carneiro *et al*., 2003; Mojtahedi *et al*., 2007; Robertson, 2009). To date, two races and two pathotypes of *M. chitwoodi* are recognized; however, these races and pathotypes cannot be identified using molecular methods or morphological characteristics. Identification of pathotype depends on reproduction on a set of differential host plants.

Differences in virulence and reproduction of *M. chitwoodi* populations as well as resistance against *M. chitwoodi* races and pathotypes remain important factors in the development of broad and durable resistance (provide citation). Breeding for resistance in potato against *M. chitwoodi* with geographic populations is appropriate because of the high molecular and morphological variation found in the nematode (Humphreys-Pereira and Elling, 2013). Use of resistant varieties against *Meloidogyne* spp. is one of the most effective and environmentally safe control measures. Sources of resistance from wild *Solanum* species to *M. chitwoodi* have been identified, and it has been determined that resistance levels differ among races of *M. chitwoodi* (Brown *et al*., 1989; 1991). Although commercial potato varieties do not have significant resistance against *M. chitwoodi*, several breeders are currently working toward developing new potato cultivars with resistance to *M. chitwoodi* (Brown *et al*., 2006; 2009; Boydston *et al*., 2007; Norshie *et al*., 2011; Dinh *et al*., 2014). As a result of the studies carried out in recent years, genetic resistance against races of *M. chitwoodi* has been established to be independent from each other and also some breeding lines with resistance to *M. chitwoodi* produced commercially acceptable tubers (Brown *et al*., 1999; 2006; 2009; Norshie *et al*., 2011).

The aim of this study was to define the race and pathotype of *M. chitwoodi* found in Turkey. This knowledge is important to determine which resistance source can be included in commercial potato varieties and deployed against *M. chitwoodi*, which has recently spread in potato-producing areas in Turkey.

For this research, 58 geographic isolates of *M. chitwoodi* were obtained from the Niğde (45), Nevşehir (12), and Aksaray (1) districts in Turkey (Table 1). All populations were identified as *M. chitwoodi* using morphological, morphometrical,

and molecular methods as previously described (Evlice and Bayram, 2016).

The rearing of *M. chitwoodi* populations, and pot experiment were conducted in a climate chamber at 23°C (±2) with a 14-hr photoperiod. The plants were established in pots (760 ml, 10x10x11 cm), filled with an autoclaved (Smith and Onions, 1994) soil mixture (85% silver sand, 15% soil). Osmocote® (Scotts, Marysville, OH, USA) (18-6-12) was applied at 1 g/kg soil mixture. Plants were watered every 2-3 days.

The nematode inoculum was collected from tomato roots. To extract eggs, galled roots were rinsed with tap water to remove soil. Washed roots were chopped into 1- to 2-cm pieces and placed in conical flasks. Roots were placed in 200 ml of 0.52% sodium hypochlorite (NaOCl) solution and eggs were obtained by vigorously shaking for 3.5 min. Extracted eggs were gently rinsed with tap water to remove NaOCl and passed through 75- and 20-µm aperture sieves. Eggs collected on the 20-µm aperture sieve were washed into a beaker (Hartman and Sasser, 1985). Nematode concentrations were adjusted to 1,000 eggs/1 ml and stored at 4°C until used. Inoculum was used within 24 hr.

Carrot cv. Red Core Chantenay and alfalfa cv. Prosementi seeds were sown in pots filled with the soil mixture. After germination, five seedlings for carrot and ten seedlings for alfalfa were selected. To obtain *in vitro* plants of *S. bulbocastanum*, shoots were cut every 2-3 wk and transferred into tissue culture vessels (8 cm high) filled with MS medium containing 30 g/L sucrose (Murashige and Skoog, 1962). One potato seedling, 8 to 10 cm, was transplanted per pot. During the first 3 days after transplanting, pots were covered with transparent plastic to maintain 100% humidity. Four weeks after sowing, pots were inoculated with 5,000 eggs of *M. chitwoodi* per population. The egg inoculum

Table 1. Isolates and sources of *Meloidogyne chitwoodi* populations from Turkey.

Population code	GN[z]	Location	Population Code	GN	Location
NIG-1	1	Niğde/Tırhan	NIG-33	30	Niğde/Bağlama
NIG-2	2	Niğde/Tırhan	NIG-34	31	Niğde/Alay
NIG-3	3	Niğde/Tırhan	NIG-35	32	Niğde/Konaklı
NIG-5	4	Niğde/Hasaköy	NIG-37	33	Niğde/Konaklı
NIG-6	5	Niğde/Ağcaşar	NIG-38	34	Niğde/Konaklı
NIG-7	6	Niğde/Yeşilgölcük	NIG-39	35	Niğde/Edikli
NIG-8	7	Niğde/Alay	NIG-40	36	Niğde/Edikli
NIG-9	8	Niğde/Tırhan	NIG-41	37	Niğde/Edikli
NIG-10	9	Niğde/Tırhan	NIG-43	38	Niğde/Edikli
NIG-11	10	Niğde/Hasaköy	NIG-45	39	Niğde/Konaklı
NIG-12	11	Niğde/Hasaköy	NIG-46	40	Niğde/Konaklı
NIG-13	12	Niğde/Hasaköy	NIG-47	41	Niğde/Konaklı
NIG-14	13	Niğde/Bağlama	NIG-48	42	Niğde/Hasaköy
NIG-15	14	Niğde/Bağlama	NIG-49	43	Niğde/Orhanlı
NIG-16	15	Niğde/Bağlama	NIG-50	44	Niğde/Orhanlı
NIG-17	16	Niğde/Alay	NIG-52	45	Niğde/Orhanlı
NIG-18	17	Niğde/Bağlama	NEV-1	46	Nevşehir /Acı Göl
NIG-19	18	Niğde/Kiledere	NEV-2	47	Nevşehir /Acı Göl
NIG-20	19	Niğde/Kiledere	NEV-3	48	Nevşehir/ Derinkuyu
NIG-21	20	Niğde/Kiledere	NEV-4	49	Nevşehir/ Derinkuyu
NIG-22	21	Niğde/Kiledere	NEV-5	50	Nevşehir/ Derinkuyu
NIG-23	22	Niğde/Kiledere	NEV-8	51	Nevşehir /Derinkuyu
NIG-24	23	Niğde/Kiledere	NEV-9	52	Nevşehir /Derinkuyu
NIG-25	24	Niğde/Kiledere	NEV-10	53	Nevşehir /Derinkuyu
NIG-26	25	Niğde/Kiledere	NEV-11	54	Nevşehir /Derinkuyu
NIG-27	26	Niğde/Ağcaşar	NEV-15	55	Nevşehir/Ürgüp
NIG-29	27	Niğde/Kiledere	NEV-17	56	Nevşehir/Acı Göl
NIG-30	28	Niğde/Kiledere	NEV-18	57	Nevşehir/Acı Göl
NIG-31	29	Niğde/Kiledere	AKS-1	58	Aksaray/Gülağaç

[z]Codes used in biplot graph.

was delivered into 3-4 cm deep holes made around root system and covered lightly with fresh soil mixture. Host suitability was assessed 55 days after inoculation (Santo *et al*., 1988; Brown *et al*., 1989; Mojtahedi *et al*., 1989). At this time, plants were gently lifted from the soil and rinsed with tap water to remove soil from the roots. Afterwards the eggs were extracted from roots (Hartman and Sasser 1985), and the final population (Pf) and reproductive factors (RF=Pf/Pi) were determined. Based on RF values, the cultivars were grouped into four host suitability categories: RF=0-0.09, nonhost; RF=0.1- 0.9, poor host; RF=1-2, moderate host; RF>2, good host (Santo *et al*., 1988). Tomato (Tueza F1) served as a susceptible control. The experiments were carried out in a completely randomized design with five replications The pot position was changed each week so that all pots spent equal time in the front, at the back, and on the sides during the experiment to avoid unintentional side effects. Comparison biplot was performed to determine the relationship between nematode populations and plant hosts by using GenStat (14 ed.) software.

Average RF of *M. chitwoodi* on tomato was 59.8 and ranged from 37.8 to 83.4. This result indicated that the collected *M. chitwoodi* populations were viable and infective.

All 58 populations of *M. chitwoodi* reproduced well on carrot (RF=2.01-4.66) (Table 2). Reproduction factor values ranged from 2.01 to 2.99 for 37 populations, from 3.00 to 3.99 for 15 populations, and from 4.00 to 4.66 for 6 of the nematode populations.

Reproduction factor values on alfalfa ranged between 0.004 to 0.09 for 29 populations and 0.10 to 0.75 for 29 populations (Table 2). No reproduction was observed on alfalfa in 2 populations. On the basis of the RF values, alfalfa was a nonhost for 27 *M. chitwoodi* populations and a poor host for 29 populations of *M. chitwoodi*. No reproduction of *M. chitwoodi* was found in at least 2 replicates per population and no reproduction was observed in a total of 167 replicates across populations.

The roots of *S. bulbocastanum* SB22 were stained with Phloxine B and evaluated for nematode egg masses and galling with the use of a microscope. None of the populations of *M. chitwoodi* from Turkey were able to reproduce on *S. bulbocastanum* SB22.

The comparison biplot graphs showed good discrimination for some populations (Fig. 1). These populations, specifically NIG-50 (#44) and NIG-34 (#31) were clearly distinguished from the other *M. chitwoodi* populations. These populations also had high reproduction on both carrot and alfalfa in contrast to the other populations. Also, the biplot clearly separated *M. chitwoodi* populations that had high reproduction on both carrot (NIG-49 #43) and alfalfa (NIG-48 #42).

Only *M. chitwoodi* race 1 was found in Turkey. There was no evidence for the existence of host race 2 or pathotype 1 of race 1. These results are similar to studies performed in Europe. Carrot was a good host for all populations. Alfalfa was a poor host for 29 populations and a non-host for 29 populations. *Solanum bulbocastanum* SB22 was a non-host for all populations. All populations reproduced moderately well on carrot, but some populations failed to reproduce in some replicates. Similar results were obtained in previous studies (van Der Beek *et al*., 1999; Wesemael and Moens, 2008). In alfalfa, there was no reproduction of *M. chitwoodi* in several of the replications. This is an expected result for *M. chitwoodi* race 1, but some *M. chitwoodi* race 1 populations may potentially reproduce on alfalfa (Pinkerton *et al*., 1986). Thus, attention should be paid to where infestations of *M. chitwoodi* race 1 occur, and when an alfalfa rotation is used.

Meloidogyne chitwoodi has two races and two pathotypes identified using the host differential test. The races and pathotypes are morphologically and molecularly indistinguishable (Mojtahedi *et al*., 2007). The discrimination of races is made according to the reproduction status of *M. chitwoodi* populations on carrot and alfalfa. It has been reported that *M. chitwoodi* race 1 reproduces well on the Red Cored Chantenay carrot cultivar whereas race 1 reproduces weakly on the Thor alfalfa cultivar. *Meloidogyne chitwoodi* race 2 does not reproduce on carrot but reproduces well on alfalfa, which is the basic distinction between the two races (Mojtahedi *et al*., 1988). Pathotype 1 of race 1 and pathotype 1 of race 2 are able to reproduce on *S. bulbocastanum,* which is resistant to both races of *M. chitwoodi,* and these pathotypes are determined according to the reproductive status on *S. bulbocastanum* (Mojtahedi *et al*., 1998; 2007). The second race of *M. chitwoodi*, had the ability to reproduce on alfalfa cv. Thor, which is a

Table 2. The reaction of carrot (Red Core Chantenay), alfalfa (Prosementi) and *Solanum bulbocastanum* SB22 to *Meloidogyne chitwoodi* isolates from Turkey.

Population code	Carrot[y]	Alfalfa	*S. bulbocastanum* SB22	Population ccode	Carrot	Alfalfa	*S. Bulbocastanum* SB22
NIG-1	2.45±1.14[z]	0.23±0.32	0.00	NIG-33	3.59±0.82	0.22±0.25	0.00
NIG-2	2.21±1.47	0.08±0.09	0.00	NIG-34	4.41±0.82	0.43±0.43	0.00
NIG-3	2.06±1.45	0.13±0.14	0.00	NIG-35	2.61±2.61	0.20±0.33	0.00
NIG-5	2.38±2.33	0.12±0.15	0.00	NIG-37	2.32±1.43	0.02±0.03	0.00
NIG-6	3.28±0.97	0.42±0.44	0.00	NIG-38	2.12±2.01	0.04±0.06	0.00
NIG-7	2.70±0.60	0.18±0.29	0.00	NIG-39	3.84±2.50	0.01±0.01	0.00
NIG-8	2.59±1.75	0.07±0.13	0.00	NIG-40	4.31±1.24	0.11±0.14	0.00
NIG-9	2.46±1.58	0.13±0.26	0.00	NIG-41	3.27±2.06	0.03±0.04	0.00
NIG-10	2.73±0.58	0.20±0.38	0.00	NIG-43	2.01±1.88	0.08±0.08	0.00
NIG-11	2.90±0.83	0.09±0.13	0.00	NIG-45	2.02±1.50	0.04±0.03	0.00
NIG-12	2.22±1.47	0.17±0.29	0.00	NIG-46	3.64±0.88	0.01±0.01	0.00
NIG-13	2.79±1.61	0.04±0.06	0.00	NIG-47	3.49±1.20	0.03±0.05	0.00
NIG-14	2.50±1.65	0.03±0.06	0.00	NIG-48	2.75±2.03	0.75±1.54	0.00
NIG-15	2.66±0.52	0.10±0.19	0.00	NIG-49	4.53±1.63	0.01±0.02	0.00
NIG-16	2.78±1.74	0.05±0.06	0.00	NIG-50	4.66±1.42	0.51±1.06	0.00
NIG-17	2.58±1.99	0.04±0.06	0.00	NIG-52	4.02±1.03	0.08±0.18	0.00
NIG-18	2.57±1.71	0.02±0.04	0.00	NEV-1	3.51±1.36	0.40±0.46	0.00
NIG-19	2.67±1.83	0.04±0.05	0.00	NEV-2	2.25±1.54	0.00±0.01	0.00
NIG-20	3.15±1.94	0.00±0.00	0.00	NEV-3	3.08±1.49	0.08±0.18	0.00
NIG-21	2.41±1.72	0.21±0.36	0.00	NEV-4	2.21±2.66	0.02±0.02	0.00
NIG-22	3.21±0.99	0.23±0.30	0.00	NEV-5	2.51±0.96	0.25±0.36	0.00
NIG-23	2.91±1.84	0.12±0.21	0.00	NEV-8	4.05±1.69	0.02±0.05	0.00
NIG-24	2.72±1.93	0.40±0.58	0.00	NEV-9	3.01±1.63	0.22±0.34	0.00
NIG-25	2.91±1.17	0.00±0.00	0.00	NEV-10	3.02±1.15	0.03±0.05	0.00
NIG-26	2.71±2.17	0.04±0.09	0.00	NEV-11	2.10±2.33	0.20±0.31	0.00
NIG-27	2.14±1.55	0.22±0.39	0.00	NEV-15	2.31±0.77	0.27±0.27	0.00
NIG-29	2.20±1.59	0.06±0.11	0.00	NEV-17	3.17±1.20	0.10±0.11	0.00
NIG-30	3.28±1.06	0.21±0.23	0.00	NEV-18	2.38±1.71	0.01±0.02	0.00
NIG-31	3.16±2.15	0.27±0.43	0.00	AKS-1	2.82±1.89	0.29±0.65	0.00

[y]Data are Mean ± Standard error.

[z]Reproductive factors (Rf=Pf/Pi).

non-host for race 1, and was identified in the USA (Santo and Pinkerton, 1985). Pathotype 1 of *M. chitwoodi* race 2 was found by Mojtahedi *et al.* (1994) as a new race, *M. chitwoodi* race 3, afterward it was recognized as a new pathotype (Mojtahedi *et al*., 1998). Pathotype 1 of *M. chitwoodi* race 1, which has overcome the *M. chitwoodi* resistance gene Rmc1(blb), was found in the Pacific Northwest of the United States (Mojtahedi *et al*., 2007).

The first determinations of different *M. chitwoodi* races and pathotypes were done in the USA (Golden *et al*., 1980; Santo and Pinkerton 1985; Mojtahedi *et al*., 1998, 2007). Studies have shown that *M. chitwoodi* has very broad genetic and morphological variations in isolates from the USA (van der Beek and Poleij, 2008; Humphreys-Pereira and Elling, 2013; 2014). In Mexico, race 2 was found to be the predominant isolate in Tlaxcala State (Cuevas, 1990). The hypothesis that *M. chitwoodi* is of American origins and that it has come to Europe from the Americas has been accepted (Schmitz *et al*., 1998; Waeyenberge and Moens, 2001; Humphreys-Pereira and Elling 2013). Additionally, it is known that *M. chitwoodi* was already in Europe much earlier than when it was first identified in the USA. The nematode also has broad genetic diversity in Europe and Turkey (Brinkman *et al*., 1994; Waeyenberge and Moens, 2001; Devran *et al.*, 2009). Up to now, only *M. chitwoodi* race 1 has been detected in Europe, and no results have been obtained about the existence of different races and pathotypes (van Der Beek *et al*., 1999; Waeyenberge and Moens, 2001; van der

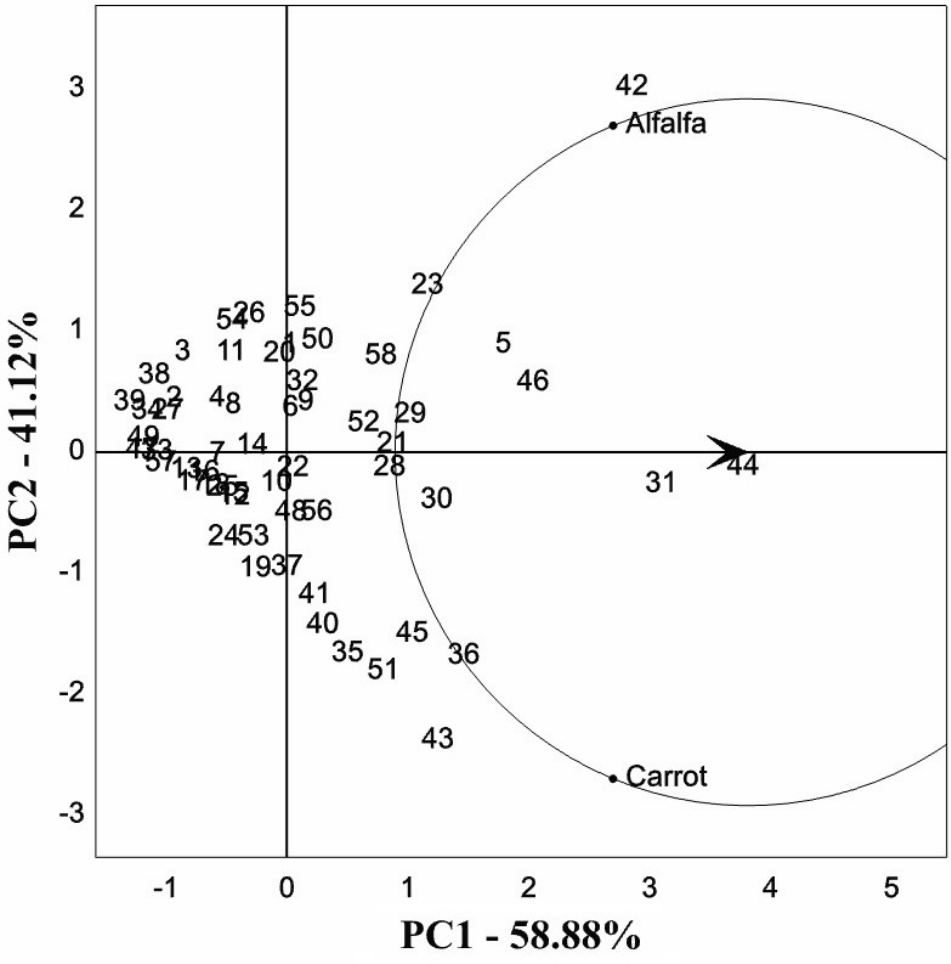

Figure 1. Biplot graph of the reproduction factors of *Meloidogyne chitwoodi* populations on carrot (Red Core Chantenay) and alfalfa (Prosementi). Numbers represent different populations of *M. chitwoodi* collected across Turkey.

Beek and Poleij, 2008; den Nijs *et al.*, 2016). The Dutch population was reported as a new race of *M. chitwoodi* due to the differences in morphology and isozyme patterns (van Meggelen *et al*., 1994), but later this population was defined as a new species, *M. fallax* (Karssen, 1996).

Tolerances of potato varieties to *M. chitwoodi* differ (van Riel, 1993). Resistance to *M. chitwoodi* has been identified in some wild *Solanum* species, such as *S. bulbocastanum*, *S. hougasii*, and *S. fendleri* (Brown *et al*., 1989; 1991; 2004; Janssen *et al*., 1998). Also, it has been determined that resistance genes for root reproduction and tuber infection exist in $R_{Mc1(blb)}$ and $R_{Mctuber(blb),}$ respectively (Brown *et al*., 1996; 2009). Recently, resistant potato varieties against *M. chitwoodi* race 1 have been developed (Brown *et al*., 1991; 2004; 2006; 2009; Norshie *et al*., 2011; Dinh *et al*., 2014). These varieties may prove to be useful for controlling *M. chitwoodi* in Turkey, where we detected the presence of only race 1.

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