DOI: 10.29050/harranziraat.1635868
Harran Tarım ve Gıda Bilimleri Derg. 2025, 29(2): 228-236

Research Article/Araştırma Makalesi

Development of Meloidogyne chitwoodi resistant potato lines

Meloidogyne chitwoodi'ye dayanıklı patates hatlarının geliştirilmesi

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To cite this article:
Özkaynak, T. (2025). Development of Meloidogyne chitwoodi resistant potato lines. Harran Tarım ve Gıda Bilimleri Dergisi, 29(2): 228-236

DOI: 10.29050/harranziraat.1638568

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Received Date:
12.02.2025
Accepted Date:
16.04.2025

ABSTRACT

Meloidogyne chitwoodi is an important root-knot nematode in some potato production regions of Türkiye. Crop rotation and some chemical and cultural control methods remain ineffective in controlling this nematode. Developing resistant varieties is an environmentally safe and most effective solution for *M. chitwoodi*. The current study was aimed at the development of *M. chitwoodi*-resistant potato lines and varieties adapted to different regions. Two breeding populations (‘22-99: PP-89 X Maradonna’, ‘22-108: PP-85 X Banba’) and two offspring populations (‘32-12: 22-99-33 X Provento’; ‘32-10: 22-108-24 X Saturna’) were used as genetic material. *Meloidogyne chitwoodi* molecular marker and field pathogenicity tests were used for investigation of *M. chitwoodi*-resistant lines and varieties. The results of the molecular marker and field tests were compatible. A total of 112 potato breeding lines were evaluated In two populations and 81 were resistant to *M. chitwoodi*. Consequently, potato line of number 33 from the 22-99 population and potato line of number 24 from the 108-24 population, along with their superior agronomic and yield characteristics, were chosen as *M. chitwoodi*-resistant lines according to the marker and field test studies. Overall the results suggest that resistant varieties can be improved by large-scale molecular markers and field tests.

Key Words: Potato lines, Molecular marker, Root-knot nematode, *Solanum tuberosum* L., Nematode resistance

ÖZ

Meloidogyne chitwoodi Türkiye'nin bazı patates üretim bölgelerinde önemli bir kök ur nematodudur. Ekim nöbeti, bazı kimyasal ve kültürel uygulamalar bu nematodla mücadelede etkili olmamıştır. *Meloidogyne chitwoodi*'ye karşı çevre açısından güvenli ve etkili en iyi yöntem dayanıklı çeşitlerin geliştirilmesidir. Bu araştırmanın amacı, *M. chitwoodi*'ye karşı farklı bölgelere adapte olmuş dayanıklı patates hatları ve çeşitleri geliştirmek olmuştur. Denemede genetik materyal olarak iki ıslah popülasyonu (22-99: PP-89 X Maradonna, 22-108: PP-85 X Banba) ve ikinci döl popülasyonu (‘32-12: 22-99-33 X Provento’; ‘32-10: 22-108-24 X Saturna’) kullanılmıştır. Nematod dayanıklı hat ve çeşitlerin geliştirilmesinde *M. chitwoodi* moleküler markeri ve tarla patolojik testleri kullanılmıştır. Moleküler marker testi ve tarla testlerinin sonuçları uyumlu gözükmüştür. İki popülasyonda 112 patates ıslah hattı değerlendirilmiş olup bunların 81'i *M. chitwoodi* nematoduna dayanıklıdır. Araştırma sonucunda 22-99 popülasyonuna ait 33 numaralı patates hattı ve 108-24 popülasyonuna ait 24 numaralı hatlar, üstün agronomik ve verim özelliklerine sahip olmaları yanında, marker ve tarla deneme çalışmalarına göre *M. chitwoodi*'ye dayanıklı hat olarak seçilmiştir. Bu çalışmanın devamında, geniş çaplı moleküler marker test ve tarla denemeleri sonrasında dayanıklı çeşitler geliştirilebilecektir.

Anahtar Kelimeler: Patates hattı, Moleküler Marker, Kök-ur Nematod, *Solanum tuberosum* L., Nematod dayanıklılığı

Introduction

Meloidogyne chitwoodi is an important nematode in potatoes and some vegetables and field crops in western Europe, the Netherlands, the United States, Belgium and Germany (Nyczepir et al., 1982; Molendijk & Mulder, 1996; Karssen & Moens, 2006; Graebner et al., 2018; Bali et al., 2022). *Meloidogyne chitwoodi* was first reported from Türkiye during 2007 in Niğde and Nevsehir (Ozarslandan et al., 2007). *Meloidogyne* root-knot nematode species, including *M. chitwoodi*, were identified and determined using morphological and molecular analyses (Devran et al., 2009; Ozarslandan et al., 2009). *Meloidogyne chitwoodi* race 1 was detected only in Türkiye (Evlice & Bayram, 2019).

Meloidogyne chitwoodi is a major pest of potato crop in the countries having intensive potato production. It has been recorded from Türkiye recently and readily spread to 11 provinces in 4 regions of the country. These provinces constitute 48.6% of Türkiye's potato production areas and 56.8% of the total production indicating severe consequences of infestation (Evlice & Bayram, 2016). Infestation is rising in Türkiye since its first appearance, and producers continue to cultivate potato with intensive nematicide applications. Despite being one of the world's major potato-producing countries, Türkiye has no native commercial pest-resistant variety that can be used for commercial production. Considering the climate, soil and potato production regions in Türkiye, it is quite difficult to develop varieties resistant to race-specific pests in these regions (Avci et al., 2018).

Meloidogyne chitwoodi causes necrotic spotting under the tuber skin and vigorous galling formation on the tuber surface, which collectively reduce the quality of fresh and/or processed potato tubers (Castagnone-Sereno et al., 1999; Mitkowski & Abawi 2003; Devran et al., 2009; Graebner et al., 2018). Currently, growers/farmers have a limited number of management options against *M. chitwoodi*. There are many limitations of crop rotation to control *M. chitwoodi* nematode. First, *M. chitwoodi* has a wide host range and low damage threshold. Even in the most effective rotation, unacceptable losses can occur in the long warm season in low populations at the residue level (Graebner et al., 2018; Bali et al., 2019; Bali et al., 2022; Hu et al., 2023).

Tolerance to *M. chitwoodi* may vary among potato varieties (Van Riel, 1993). Several studies have determined the resistance of *M. chitwoodi* in *Solanum* species, including *S. hougasii, S. bulbocastanum* and *S. fendleri* (Brown et al., 1989; 1991; Janssen et al., 1998; Brown et al., 2004). In addition, two resistance genes, i.e., RMc1 (blb) and RMctuber (blb) preventing root development and tuber infections, respectively have been identified (Brown et al., 1996; 2009). Candidate varieties resistant to *M. chitwoodi* race 1 have been improved in recent years (Brown et al., 1991; 2004; 2006; 2009; Norshie et al., 2011; Dinh et al., 2014). However, commercial production and distribution of candidate resistant potato varieties remain limited (Bali et al., 2019; Bali et al., 2022). The resistant gene RMc1 (blb) from *S. bulbocastanum* was transferred to the potato gene pool. This gene provides resistance against *M. chitwoodi* race 1, and enables the growth and development of potato roots. Thus, the tubers get rid of nematode damage. The RMc1 (blb) gene obtained from PA99N82–4 material has been genetically characterized and it provides resistance against *M. chitwoodi*. It was a single dominant gene and mapped on the 11th chromosome. The resistance mechanism is understood to a limited extent (Bali et al., 2019; Bali et al., 2022, Hu et al., 2023).

This study used *M. chitwoodi*-resistant and susceptible potato materials and populations. The study aimed to determine the resistance of the tested potato material to *M. chitwoodi* nematode using molecular marker under natural field infestation.

Material and Methods

Plant material

Two tetraploid M. chitwoodi resistant materials (‘PP-85’, ‘PP-89’), two susceptible commercial varieties (‘Granola’ and ‘Marabel’), other commercial varieties (Table 1 and 2), four population (‘22-99: PP-89 X Maradonna’, ‘22-108: PP-85 X Banba’, ‘32-12: 22-99-33 X Provento’; ‘32-10: 22-108-24 X Saturna’) were used as plant materials in the experiment.

Biological tests, field experiments and marker tests

22-99 (PP-89 X Maradonna) and 22-108 (PP-85 X Banba) Populations

Granola, Marabel, Maradonna and Banba commercial potatoes varieties were grown in the experiment field in 2015 in Niğde / Konaklı (38 10’ 33”N,34° 50’39”E, 1347 m above sea level). The field was determined as infected with M. chitwoodi and the varieties grown were susceptible (data not presented). This field was used for resistance testing experiment in 2017 only Konaklı location.

Resistant materials (‘PP-89’ and ‘PP-85’) were crossed with ‘Maradonna’ and ‘Banba’ varieties during 2016 spring season. A total of 100 seeds from each population were sown and grown under greenhouse conditions during 2016 autumn season. A total of 60 genotypes in ‘22-99’ population and 52 genotypes in ‘22-108’ population were selected and used in the field experiment. The ‘22-99’ and ‘22-108’ populations were used in the M. chitwoodi breeding program. Tubers were planted on May 27, 2017, and harvested on October 25, 2017. Two resistant materials, two populations, and ten commercial varieties were used in the field experiment (Table 1). A total of 112 genotypes from two populations, commercial varieties, and resistant materials were planted in the M. chitwoodi-infected field. Breeding genotypes belonging to ‘22-99’ and ‘22-108’ populations were planted using one tuber for each genotype. Resistant materials and commercial varieties were planted in the field using 50 tubers. The tubers were planted without replication as 70 cm distance between rows and 30 cm among rows. Necessary irrigation, fertilization, and plant protection practices were carried out during the season.

Figure 1. *Meloidogyne chitwoodi* resistant potato lines (22-99 population lines: 2, 23, 48, 51 and 57; 22 108 population lines: 31, 36 and 49)

In genetic materials (‘PP89’, ‘PP85’, commercial varieties, ‘22-99’ and ‘22-108’ populations) samples were taken from the true leaves of plants in the field and were screened with *M. chitwoodi* molecular marker test. The molecular marker developed by Zhang et al.

(2007) was used in the research. Potato genotypes, populations, commercial varieties were determined as resistant or susceptible according to the marker. Observations were determined phenotypically for nematodes in the field at harvest time and compared with marker test results (Figure 1 and 2).

Figure 2. *Meloidogyne chitwoodi* susceptible potato lines (line 51 is belonging to 22-108 population)

32-10 (22-108-24 X Saturna) and 32-12 (22-99-33 X Provento) Populations

Commercial varieties used in potato breeding in the second phase of the research. Genetic materials, and *M. chitwoodi*-resistant materials determined by marker test in two offspring populations developed from 64 lines (‘32-10’, ‘32-12’) were included in the second phase. The ‘32-10’ and ‘32-12’ breeding populations were planted in the non-nematode infected field as 400 single tubers in the spring season of 2018. A total of 64 lines selected according to their tuber characteristics, were grown in pots in the autumn season of 2018. One tuber in 64 lines, and two tubers of commercial varieties and other genetic materials were used for planting. Leaf samples were taken from plants and screened for molecular marker testing to determine whether they were resistant to *M. chitwoodi*.

Results and Discussion

The results obtained by planting tubers in the infected field are included in Table 1. Marker test results and the phenological observations were in harmony with each other. Resistance was indicated as (+) and susceptible as (-) in the current study. Resistant potato genotypes provided resistance in field conditions and *M. chitwoodi* damage was not observed in tubers (Figure 1). All commercial varieties were found to be susceptible in both marker testing and field phenological observations. Out of 60 breeding lines, 43 were resistant (+, presence) and 17 were susceptible (-, absence) in ‘22-99’ population. In ‘22-108’ population, 38 lines were resistant and 14 were susceptible.

In the second phase of the study, commercial varieties were used in potato breeding studies, and advanced genotypes, ‘32-10’, and ‘32-12’ populations formed from resistant genotypes were screened with marker test. All commercial varieties were found susceptible. The first tuber generation plants (64 lines) of the 2 populations were screened by marker test. As a result of the screening, 40 lines in the ‘32-12’ population and 37 lines in the ‘32-10’ population were found resistant (+) (Table 2). The resistant/susceptible ratio was found to be approximately 1: 2 in both populations.

Table 1. *Meloidogyne chitwoodi* resistance of potato lines

	22-99					22-108					
	$R_{Mc1(blb)}$	Field		$R_{Mc1(blb)}$	Field		$R_{Mc1(blb)}$	Field		$R_{Mc1(blb)}$	Field
1	+	+	31	+	+	1	+	+	33	+	+
2	-	-	32	+	+	2	+	+	34	+	+
3	+	+	33	+	+	3	+	+	35	-	-
4	+	+	34	+	+	4	+	+	36	+	+
5	+	+	35	+	+	5	+	+	37	+	+
6	+	+	36	+	+	6	+	+	38	+	+
7	+	+	37	+	+	7	+	+	39	-	-
8	+	+	38	+	+	8	+	+	40	+	+
9	+	+	39	-	-	9	+	+	41	-	-
10	+	+	40	+	+	10	+	+	42	-	-
11	-	-	41	+	+	11	+	+	43	+	+
12	-	-	42	-	-	12	-	-	44	+	+
13	-	-	43	-	-	13	+	+	45	+	+
14	-	-	44	+	+	14	+	+	46	+	+
15	+	+	45	-	-	15	+	+	47	+	+
16	+	+	46	-	-	16	+	+	48	+	+
17	+	+	47	+	+	17	+	+	49	+	+
18	+	+	48	+	+	18	+	+	50	-	-
19	+	+	49	+	+	19	+	+	51	-	-
20	-	-	50	-	-	20	-	-	52	-	-
21	-	-	51	+	+	21	+	+	Agata	-	-
22	+	+	52	-	-	22	-	-	Agria	-	-
23	+	+	53	-	-	23	-	-	Blondine	-	-
24	+	+	54	+	+	24	+	+	Granola	-	-
25	+	+	55	+	+	25	-	-	Madeline	-	-
26	+	+	56	+	+	26	-	-	Marabel	-	-
27	+	+	57	+	+	27	+	+	Melody	-	-
28	+	+	58	-	-	28	-	-	Natasha	-	-
29	+	+	59	-	-	29	+	+	Maradonna	-	-
30	+	+	60	+	+	30	+	+	Banba	-	-
						31	+	+	PP-89	+	+
						32	+	+	PP-85	+	+
	Marker presence (+) : 43						Marker presence (+): 38				
	Marker absence (-) : 17						Marker absence (-): 14				

$R_{Mc1(blb)}$: *Meloidogyne chitwoodi molecular marker*, PP-89 and PP-85: Resistant genotypes; 22-99: PP-89 X Maradonna, 22-108: PP-85 X Banba

Table 2. *Meloidogyne chitwoodi* resistance of commercial varieties and potato breeding lines

	$R_{Mc1(blb)}$	32-12	$R_{Mc1(blb)}$	32-12	$R_{Mc1(blb)}$	32-10	$R_{Mc1(blb)}$	32-10	$R_{Mc1(blb)}$
-Cara	-	1	+	33	+	1	-	33	+
Hermes	-	2	+	34	-	2	+	34	+
Saturna	-	3	-	35	+	3	+	35	-
Safrane	-	4	+	36	+	4	-	36	+
Innovator	-	5	-	37	-	5	+	37	-
Markies	-	6	+	38	+	6	+	38	+
Alexia	-	7	-	39	-	7	+	39	+
Solist	-	8	+	40	+	8	-	40	+
Fontane	-	9	-	41	+	9	+	41	-
Actress	-	10	+	42	-	10	+	42	-
Musica	-	11	-	43	+	11	+	43	-
Doremi	-	12	+	44	-	12	-	44	+
Sante	-	13	+	45	+	13	-	45	-
Saline	-	14	+	46	+	14	+	46	+
Colomba	-	15	-	47	+	15	-	47	-
Van Gogh	-	16	-	48	+	16	-	48	-
Ivory Rus.	-	17	+	49	-	17	+	49	+
Orcestra	-	18	+	50	+	18	+	50	-
Nazca	-	19	+	51	-	19	-	51	+
Concordia	-	20	+	52	+	20	+	52	-
Victory	-	21	-	53	-	21	-	53	+
Triomphe	-	22	+	54	+	22	+	54	+
Touareg	-	23	+	55	-	23	-	55	+
Provento	-	24	-	56-	-	24	+	56	+
		25	+	57	+	25	-	57	+
		26	+	58	+	26	+	58	-
		27	-	59	+	27	-	59	+
		28	+	60	-	28	+	60	-
		29	-	61	+	29	-	61	+
		30	+	62	-	30	+	62	+
		31	-	63	+	31	-	63	-
		32	+	64	+	32	+	64	+
		Marker presence (+): 40					Marker presence (+): 37		
		Marker absence (-): 24					Marker absence (-): 27		

$R_{Mc1(blb)}$: *Meloidogyne chitwoodi molecular marker*, 32-12: 22-99-33 X Provento; 32-10: 22-108-24 X Saturna

Root-knot nematode infestation is increasingly being reported from potato produced in Türkiye. Two strategies seem useful against *M. chitwoodi* nematode. The first strategy is using nematicides and the second is the use of resistant varieties. Today, chemical control is carried out against the nematode in the nematode-infected fields by spraying during the flowering period. This method is both costly and harms the environment and human health. Therefore, it is increasingly necessary to develop and utilize resistant varieties. For this purpose, breeding studies were initiated. Results of the preliminary studies indicated a large amount of nematode infection almost every part of the experiment field, due to the use of nematode-infected susceptible 'Granola' variety (unpublished data). Advanced lines evaluated as nematode resistant were planted in infected field, and their resistance reactions were determined. In the study, the results of field resistance and marker test (presence/absence) were found to be compatible.

Norshie et al. (2011) concluded that the single resistance gene provides 97% partial resistance against *M. chitwoodi*. It has been demonstrated that at this tolerance level, yield losses decrease and tuber quality increases at high population density. *Meloidogyne chitwoodi* is distributed in Türkiye is almost all agriculture regions. Potato and tomato were the main host of *M. chitwoodi*, which is the most important agricultural product, to make damage in crops such as wheat and barley increased the importance of Türkiye even more (Evlice &

Bayram, 2016). In the potato, many studies have been conducted on obtaining varieties and variety candidates resistant to *M. chitwoodi* (Brown et al., 1989; Brown et al., 1991; Janssen et al., 1998, Brown et al., 1999; Brown et al., 2004). The results of these studies, resistance genes and markers were identified, hopeful field trial results were obtained (Brown et al., 2006, Zhang et al., 2007; Brown et al., 2009; Norshie et al., 2011; Dinh et al., 2014). However, there is no commercially produced resistant variety yet (Bali et al., 2022; Hu et al., 2023). Studies have shown that the injury caused by *M. chitwoodi* on potato tubers varies depending on the varieties (Evlice & Bayram, 2016). Van Riel (1993) reported that the symptoms on tubers varied between 3-21% at the end of the study comparing 20 different potato varieties. It has been stated by Suffert & Giltrap (2012) that tubers of some potato varieties have much less damage from *M. chitwoodi* than other varieties. For example, 'Agria' variety has been reported to be more tolerant than variety 'Hansa'. In the field trial conducted by Van Riel (1994) using 20 different varieties, symptoms on the tuber varied between 3.4-34.1% depending on the varieties, and the highest population density in the soil was 2778 and 4167 J2 / 100 cm^3, respectively, in early and late varieties. The fact that 'Marabel' variety is early and susceptible to *M. chitwoodi* in the field experiment and marker test, refutes the concept that early varieties are resistant. The damage caused by *M. chitwoodi* on potato tuber skin varies depending on the varieties used (Evlice & Bayram, 2016; Van Riel 1993, 1994). While resistant lines were not shown any nematode symptoms, susceptible lines obtained lots of *M. chitwoodi* symptoms on the skin of tubers.

Crop rotation with the use of non-host plants is one of the most important methods used in controlling root-knot nematodes globally (Sikora et al., 2005; Bali et al., 2022). However, due to the wide host distribution, correct crop rotation is extremely difficult to implement. Commonly cultivated crops in Türkiye like tomato, potato, wheat, oats, barley, corn, sugar beets, sunflowers, some of the ornamental plants and fruit trees are host for *M. chitwoodi* and there are no available resistant varieties of these plants. The crop rotation, which will be made using tolerant/resistant varieties, will be very important in the future, but current information on the subject is extremely limited. There are records of less damage and even unharmed products from early potato varieties (Van Riel, 1993). Bali et al. (2022) stated that the STS marker used by Zhang et al. (2007) for *M. chitwoodi* resistance gave false fingerprints across populations or in initial populations. They suggested that this situation may be due to these markers not being tightly linked with the R*MC1(blb)* locus and may lead to losses in recombination in advanced selections. In this study, the marker results were found to be compatible with the results in the infected field in initial and advanced selections.

Consequently, developing resistant varieties, crop rotation is necessary to avoid the spread of *M. chitwoodi*.

Conclusion

The root-knot nematode damage and infection of potato planted in Türkiye is increasing every year. Studies on the root-knot nematode continue in the form of chemically control and bean-wheat crop rotation. Potato planting will be eliminated in some regions due to nematodes in the coming years. In the current study, resistance was determined in some potato genotypes by using the marker developed by Zhang et al. (2007). Single resistance gene to *M. chitwoodi* resulted in high resistance, improved tuber quality and tolerance for yield reduction.

It has emerged that the resistant materials obtained, and the population created from them must be tested in the infected field and the resistance must be confirmed. The resistance determined by the marker test has been carried out in line with this purpose and has provided resistance in the field. *M. chitwoodi*-resistant commercial variety or candidates will be developed by choosing advanced lines among durable materials. At the end of the research, the potato line of number 33 from the 22-99 population, along with its good agronomic, plant, and tuber characteristics, was determined as *M. chitwoodi*-resistant line according to molecular marker and field phenotypic test results. The '22-99-33' potato line was found successful in registration trials. However, it could not be registered because it was very susceptible to PVY (Potato Virus Y). It was used as a parent in the breeding of PVY and *M. chitwoodi* resistant varieties. The variety candidates developed from this line was applied for registration in 2025.

Acknowledgements

We thank the financial support of the TUBITAK (Project number: 1140133)

Compliance with ethical standards and conflict of interest

The authors state in the article that no unethical studies were conducted on humans or animal.

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author's Contributions

The author declares that he has contributed to the article.

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