Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi
Journal of Agricultural Faculty of Gaziosmanpasa University
http://ziraatdergi.gop.edu.tr/

Araştırma Makalesi/*Research Article*

JAFAG
ISSN: 1300-2910
E-ISSN: 2147-8848
(2019) 36 (2), 117-123
doi:10.13002/jafag4518

Reactions of Some New Cotton Genotypes (F_7) to *Meloidogyne incognita* Chitwood and *Meloidogyne javanica* Chitwood

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Alındığı tarih (Received): 25.07.2018 Kabul tarihi (Accepted): 05.04.2019
Online Baskı tarihi (Printed Online): 27.08.2019 Yazılı baskı tarihi (Printed): 31.08.2019

Abstract: Cotton is an important industrial plant because of its fiber and seed. Root-knot nematodes (*Meloidogyne* spp.) are among the harmful of cotton due to its parasitic effects, as in other plants. On account of the adverse effects of nematicides used in this struggle against to humans and environmental health, it is necessary to develop durable cotton genotypes as an alternative to chemical struggle. This study was conducted in Kahramanmaraş province in 2016 for determine the reactions of five new cotton genotypes (F_7) which obtained from Nazilli 84 S $_{(♀)}$ x Ashgabat 100 $^{(♂)}$ cross combination against to race 1 and race 2 of *M incognita* and race 1 of *M javanica*. *M. incognita* and *M. javanica* are root-knot nematodes. For this purpose, an experiment with 5 replications was carried out in the climate chamber, with a randomized plot design.
As a result of study, G_1, G_4 and G_5 cotton genotypes were found resistant to both race 1 and race 2 of *M. incognita*. Whereas G_2 and G_3 genotypes were found susceptible to race 1 of *M. incognita*, but resistant to race 2. On the other hand, all cotton genotypes found to be resistant to race 1 of *M. javanica*.

Keywords: Cotton, *Meloiodogyne incognita*, *Meloiodogyne javanica*

Bazı Yeni Pamuk Genotiplerinin (F_7) *Meloidogyne incognita* Chitwood ve *Meloidogyne javanica* Chitwood Kök-Ur Nematodlarına Reaksiyonları

Öz: Pamuk, lifi ve tohumu ile önemli bir endüstri bitkisidir. Birçok kültür bitkisinde sorun olan bitki paraziti kök-ur nematodları (*Meloidogyne* spp.), pamuğun da zararlıları arasındadır. Bu zararlıyla mücadelede kullanılan nematisitlerin insan ve çevre üzerindeki olumsuz etkilerden dolayı, kimyasal mücadeleye alternatif, dayanıklı pamuk genotiplerinin geliştirilmesi önem taşımaktadır. Bu çalışma, Nazilli 84 S $_{(♀)}$ x Aşkabat 100 $^{(♂)}$ melez kombinasyonundan geliştirilen beş adet yeni pamuk genotipinin (F_7), kök-ur nematodlarından *M. incognita'nın* ırk 1 ve ırk 2'si ile *M. javanica'nın* ırk 1'ine karşı reaksiyonlarını belirlemek amacıyla 2016 yılında, Kahramanmaraş ilinde, tesadüf parselleri deneme desenine göre 5 tekerrürlü olarak iklim odasında yürütülmüştür.
Çalışma sonucunda, pamuk genotiplerinin *M. Javanica* nematodunun ırk 1'ine karşı dayanıklı olduğu saptanırken, G_1, G_4 ve G_5 pamuk genotiplerinin *M. incognita* nematodunun ırk 1 ve ırk 2'sine karşı dayanıklı, G_2 ve G_3 pamuk genotiplerinin ise *M. incognita* nematodunun ırk 1'ine duyarlı, ırk 2'sine ise dayanıklı olduğu belirlenmiştir.

Anahtar kelimeler: Pamuk, *Meloiodogyne incognita*, *Meloiodogyne javanica*

1. Introduction

Due to the benefits to human life of cotton, it is grown about eighty countries with Turkey in the world. Turkey has got three important areas for cotton such as Aegean, Mediterranean and Southeastern Anatolia Region (Anonymous 2018). The cotton which is an industrial plant, and supporting to both textile and oil industries, it is exposed to a large number of biotic and abiotic stress factors during the growing season. For this reason, the loss of yield is 5-15 % due to diseases and harms in cotton and can reach 30-50 % if not enough fighting is done (Erdoğan 2011). The basis of the successful productions management is cultivar practices such as crop rotation, good tillage, certified seed use, fertilization program based on soil analysis, into ridge sowing, proper sowing time and sowing frequency as well as selection of durable/tolerant variety for decrease of diseases and harmful effect and density (Mart 2005). Plant parasitic nematodes have recently been accepted as one of the causes of economic damage on cotton. It was reported that *M. incognita, Rotylenchulus reniformis, Hoplolaimus columbus* and *Belonolaimus longicaudatus* were the most harmful species in the United States (Blasingame 1993a; Koenning et al. 1999; Starr et al. 2005; Blasingame 2006), and both public and private sector have been conducting a number of studies from 1990 on the development of resistant varieties for controlling against these harms (Starr et al. 2007). *M. incognita* is regarded as the common and main pest of cotton in the world (Anwar and Khan 1973, Kinlock and Sprenkel 1994, Martin et al. 1994, Baird et al. 1996, Bateman et al. 2000). In addition, the interaction of root-knot nematode with fungal pathogens can be causes the cotton to be affected by various diseases (Blasingame 1993b). While root-knot nematodes facilitate the entry of fungi through by wounds they open in cotton roots, it also has increased the violence of the fusarium in cotton (Atkinson, 1892 and 1899). Today, new and alternative methods are needed in order to prevent the adverse effects of nematicides which used in the struggle of root-knot nematodes on human health and environment, and use of resistant cotton varieties to against root-nematode (*M. incognita*) is a widespread and economical method (Colyer et al. 2000). While the productivity is increase significantly with the cultivations of the resistant varieties in the cotton areas where infected with root-knot nematode, the yield reduces about 30 % in the first three years if used non-resistant varieties (Ogallo et al. 1999). On the other hand, if used resistant cotton varieties to against races of *M. incognita*, the females of *M. incognita* can't be produce much eggs on the roots of cotton (McClure et al. 1974). Other some studies clearly shown that, Bikaneri Narma, Sel 11-111 (Pankaj et al. 1996); Stoneville LA887, Paymaster 1560, CPCSD Acala Nem X (Robinson et al. 1998, Koenning et al. 2001, Star et al. 2007); TX-1828, TX-25 and TX-1860 (Robinson et al. 2004), Auburn 623, Auburn 623 RNR, N6072 and Cleve (Veech 1978, Viglierchio 1978) cotton varieties found resistant to *M. incognita* populations, but Deltapine 16 susceptible.

In addition, while the Auburn 634 RNR (Kırkpatrick and Sasser 1983), TE-94-4, FQ 92-19, CY889, AG4869 with DF885 (Kutywayo et al. 2006) cultivars is reporting as resistant to race 1 of *M. incognita*, the Auburn 634 RNR genotype is determined as resistant to race 2 of *M. incognita* (Kırkpatrick and Sasser 1983). Moreover, Auburn 623, Auburn 634 (Kirkpatrick and Sasser 1983), M315 (Creech et al. 1995), Acala NemX and Stoneville LA887 cotton varieties is determined to be resistant against to race 3 of *M. incognita* (Zhou and Starr 2003).

This study was conducted in climate chamber conditions at Kahramanmaraş in 2016 for determine reaction of five new cotton genotypes (F_7) to both races 1 and 2 of *M. incognita* and race 1 of *M. javanica* which are root-knot nematodes.

2. Material and Method

In the study, G_1, G_2, G_3, G_4 and G_5 cotton genotypes (F_7) and their parents [Nazilli 84 S ♀ (*Gossypium hirsutum* L.) and Ashgabat 100 ♂ (*Gossypium barbadense* L.)] used as plant materials having good properties such as higher yield, lint percentage, fiber length and fiber strength (Table 6). Besides that, Rutgers and Falcon (tomatoes), Deltapine 16 (cotton), California wonder (Pepper), Florunner (Groundnut) and NC 95 (tobacco) cultivars used as others plant materials for replicate and identify species and races of *M. incognita* and

M. javanica. Species and races of nematodes were determined from larvae and mature female populations on the root of this plants. Furthermore, While Rutgers tomatoes used for the identify species and races of nematodes, but Falcon tomatoes was used for determine of pure culture and duplication of this races.

Pure culture formation of root-knot nematodes

For this, firstly, the egg mass of nematodes which regularly was replicated and left to the root region of the Falcon tomato, after purified in 65 days were used in cotton plant.

Identification of species of root-knot nematodes

Species identification of root-knot nematodes was performed by polyacrylamide gel electrophoresis (PAGE), and perineal section method where was a morphological character. Mature female individuals were used in both methods. Firstly, after the female subjects which obtained from the roots of susceptible Falcon tomato plant were kept in 45 % lactic acid, then the body fluids were drained and then, female body was cut from posterior straightly to leave 1/3 of the cuticle.

The perineal forms were compared with the type of diagnostic table prepared by Eisenback et al. (1981).

In the PAGE method, the species was identified by comparing the esterase band of the females running on polyacrylamide gels with the protein band ruler of Esbenshade and Triantaphyllou (1989) (Table 1).

Table 1. Egg mass-reaction scale (Triantaphyllou 1981; Sasser and et al. 1984)

Numbers of eggs mass on root	Scala values	Results
No egg mass	0	Resistance
1-2	1	Resistance
3-10	2	Resistance
11-30	3	Susceptible
31-100	4	Susceptible
101->upper	5	Susceptible

Identification of races of root-knot nematodes

Northern Carolina test was used for identity root-knot nematodes in climate chamber (16 hours light 8 hours dark and 25 + 1 ^{o}C) (Table 2). The pot experiment was carried out in randomized plot design with 5 replications and cotton (Deltapine 16), tomato (Rutgers), pepper (California wonder), peanut (Florunner) and tobacco (NC95) plants. When the test plants reached 15 cm, 3000 second-larvae were inoculated per plant and after 65 days from inoculation the test plants were removed from the pots. Then, the roots were kept in the red food stain so that the egg clusters found at the roots of susceptible test plants could be seen more clearly. After this process, the egg clusters were counted and egg cluster was adjusted to the reaction scale of 0-5 (Triantaphyllou 1981, Sasser et al. 1984). While the test plants with 0-2 scale values at the roots were identified as (-) the plants with 3-5 scale value were identified as (+), and racial diagnoses were made (Hartman and Sasser 1985, Rammah and Hirschmann 1990, Carneiro et al. 2003, Robertson et al. 2009).

Replications of Root-knot nematodes

When obtaining the infective juvenile larvae of root-knot nematodes, the roots of susceptible Falcon tomato plants were washed with water for not be damaged of egg clumps of root-knot nematodes. The egg clumps on the roots had been taken into the water one by one and the eggs were expected to open in the incubator at 28 ºC. The second stage larvae obtained, counted under light microscope and stored in a refrigerator at + 4 ºC for use in experiments.

Pot trial of cotton new cotton (F_7) genotypes

The study was conducted in climate room conditions (16 hours light, 8 hours dark, 25 ± 1 ° C temperature) in 2016 and repeated twice.

First, the seeds of the cotton genotypes (G_1, G_2, G_3, G_4 and G5) were planted on the viols containing the turf and perlite mixture. Afterwards, when the plants reached 2-4 leaf conditions, were planting 5 replicates, according to randomized plot design in pots (0.7 liter) which containing with autoclaved the sand (80%) + turf (20%) mixture. Susceptible Falcon tomato variety was used in determining the inoculum viability. Inoculation of root-knot nematodes to roots of seedling cotton in pots was done one week later. During inoculation, 1000 second-larval larvae were placed in four hole that were opened at a distance of 3-4 cm and 2 cm deeply from root-strait of the plant, and cotton plants were removed 65 days after inoculation. The root of each plant has been dismantled and washed with tap water for to away of soils on them, and were kept in the water with red food stain. Then, the egg masses were counted and evaluated according to the 0-5 egg mass-reaction scale (Triantaphyllou 1981, Sasser et al. 1984).

Table 2. North Carolina differential host test (Hartman and Sasser 1985, Rammah and Hirschmann 1990, Robertson 2009)

Nematodes and their races	Test Plants				
	Tabaco (NC95)	Cotton (Delta Pine 16)	Pepper (California Wonder)	Tomato (Rutgers)	Groundnut (Florunner)
Meloidogyne incognita					
Race 1	-	-	+	+	-
Race 2	+	-	+	+	-
Race 3	-	+	+	+	-
Race 4	+	+	+	+	-
Race 5	-	-	-	+	-
Race 6	+	-	-	+	-
Meloidogyne javanica					
Race 1	+	-	-	+	-
Race 2	-	-	+	+	-
Race 3	+	-	-	+	+
Race 4	+	-	+	+	+
Race 5	-	-	-	+	-

+: Positive -: Negative

3. Findings and Discussion

As a result of the study, while the G_1, G_4 and G_5 cotton genotypes were found resistant to race 1 of *M. incognita*, the G_2 and G_3 genotypes were determined susceptible. Furthermore, the lowest egg scale values at mass-reaction (1.50 ± 0.22-1.50 ± 0.15) obtained from G_1 and G_5 genotypes. Moreover, while the G_2 genotype determined as having most susceptible with the highest score (3.30 ± 0.20), susceptible value of the G_2 and G_3 genotypes were determined 3 and above (Table 3).

Table 3. The results of *M. incognita* race 1 according to 0-5 egg mass reaction scale

Cotton genotypes	*Meloidogyne incognita* race 1		
G_1	1.50 ± 0.22	c	R
G_2	3.30 ± 0.20	a	S
G_3	3.00 ± 0.00	a	S
G_4	2.20 ± 0.12	b	R
G_5	1.50 ± 0.15	c	R

R: resistant S: Susceptible

Although investigators reported that *M. incognita* race 3 and race 4 can only grow in few cotton genotypes (Sasser 1979, Veech and Starr 1986), whereas our findings indicated that the race 1 of *M. incognita* can replicated in cotton. While this situation indicates that different races belonging to *M. incognita* can't replicate same rate in each cotton genotype, the finding of some research (Kutywayo et al. 2006, Kırkpatrick and Sasser 1983) had been supported to our results. Moreover, these researchers reported that TE-94-4, FQ 92-19, CY889, AG4869, DF885 (Kutywayo et al. 2006) and Auburn 634 RNR cotton cultivars were found resistant to race 1 of *M. incognita*.

As seen as in Table 4, it was determined that all cotton genotypes which in study were found resistant to race 2 of *M. incognita*, and have an egg mass reaction scale below 3, and ranked as G_1, G_4, G_5, G_3 and G_2, respectively. Kırkpatrick and Sasser (1983) reported that race 2 of *M. incognita* did not proliferate in susceptible Deltapine 16 and durable Auburn 634 RNR cotton varieties.

It is understanding from Table 5, because of no egg mass and root-knot were found in their roots, all cotton genotypes which used in this study had been found resistant to race 1 of *M. javanica* (0.00 ± 0.00), and attend in same statistical group.

Table 4. The results of *M. incognita* race 2 according to 0-5 egg mass reaction scale

Cotton genotypes	*Meloidogyne incognita* race 2		
G_1	0.20 ± 0.12	b	R
G_2	2.60 ± 0.18	a	R
G_3	2.50 ± 0.15	a	R
G_4	0.70 ± 0.20	b	R
G_5	0.70 ± 0.20	b	R

R: resistance; S: Susceptible

Table 5. The results of *M. javanica* race 1 according to 0-5 egg mass reaction scale

Cotton genotypes	*Meloidogyne javanica* race 1	
G_1	0.00 ± 0.00	R
G_2	0.00 ± 0.00	R
G_3	0.00 ± 0.00	R
G_4	0.00 ± 0.00	R
G_5	0.00 ± 0.00	R

R: Resistance, S: Susceptible

Table 6. Some properties of cotton genotypes which used as plant materials

Genotypes	Seed cotton yield (kg da^{-1})	Gin out turn (%)	Fiber		
			Fineness (micronaire)	Length (mm)	Strength (g tex^{-1})
Nazilli 84 S	425.51	43.52	4.68	28.89	27.61
Ashgabat 100	218.20	36.48	3.95	36.41	36.65
G_1	398.65	40.32	4.08	35.21	36.19
G_2	453.55	42.39	4.54	32.16	31.87
G_3	432.67	44.65	4.20	34.56	32.78
G_4	265.74	42.23	3.64	33.15	35.34
G_5	326.32	40.21	4.44	35.09	34.46

4. Results

At the present time, there are few commercial cotton varieties resistant to root-knot nematode which is *M. incognita*. İt is understand that in the result of this study, cotton genotypes in the experiment found to react differently to root-knot nematodes, despite being from the same parents. These cotton genotypes showed resistance to a single nematode race, as well as resistance to more than one nematode race. For example, while the G_1, G_4 and G_5 genotypes show resistance to race 1 of *M. incognita*, the G_1, G_2, G_3, G_4 and G_5 genotypes shown resistance to race 2 of *M. incognita*. Moreover, G_1, G_4 and G_5 genotypes have been shown resistance to both race 1 and race 2 of *M. incognita*, respectively. However, it was found that the race 1 of *M. javanica* couldn't form any egg masses on the cotton genotypes while all of the cotton genotypes were resistant to race 1of *M. javanica*.

As a result of this study, it is can be said that new cotton genotypes which having resistance with both singular and plural durability can be developed by breeding methods against to root-knot nematodes. In these terms, it can be said that this cotton genotypes are the potential candidates for the prevention of economic losses due to root-knot nematodes.

In particular, the finding about defiance of the G_1, G_4 and G_5 cotton genotypes to both race 1 and race 2 of *M. incognita* and race 1 of *M. javanica* shown importance of breeding studies to against root-knot nematodes. As a result, it can be said that G_2, G_4 and G_5 cotton genotypes can be very important for planting areas where infected with both race 1 and race 2 of *M. incognita* as well as race 1 of *M. javanica*.

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