Screening for susceptibility and tolerance to *Meloidogyne incognita* and *M. javanica* in okra cultivars in Iraq

Basil H. Kandouh[1], Alaa E. Hasan[1], Asmaa M. Abd-Al-Rasoul[2] and Brent S. Sipes[3]

(1) Department of Plant Protection, Faculty of Agriculture, University of Kufa, Iraq, Email: basilh.noamani@uokufa.edu.iq;
(2) Department of Plant Protection, College of Agriculture and Forestry, University of Mosul, Iraq;
(3) Department of Plant Entomology and Plant Pathology,
College of Tropical Agriculture and Human Resources, University of Hawaii, Manoa, USA

Abstract

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Ten okra cultivars were screened for their susceptibility and resistance/tolerance to the root-knot nematodes *Meloidogyne incognita* and *M. javanica*in in a two-trial experiment under ambient conditions. 14 days old okra seedlings were inoculated with 3000 egg/J2 of *M. incognita* or *M. javanica*. The nematode reproduction factor (Rf) was used to determine susceptibility or resistance among tested cultivars, and cultivars were considered highly susceptibleif Rf ≥ 2, susceptible if 2 >Rf ≥ 1 and resistant if Rf< 1. Whereas, magnitude of reduction in plant shoot weight (ShW) over the control indicated tolerance and the categories tolerant, moderately tolerant and intolerant were established, when ShW reduction was <10%, 10-20% and >20%, respectively. Most of the cultivars tested were susceptible at various levels. Although Rf of *M. javanica* was always higher than that of *M. incognita*, but the plant growth (Shoot weight) was rather more highly affected by *M. incognita* than by *M. javanica*. Lahluba was the only resistant cv. to both nematode species, tolerant to *M. javanica* but intolerant *to M. incognita* (>30% ShW reduction). Taiwan Hybrid F1 was resistant/intolerant (shoot and root weight were decreased) to *M. javanica* and susceptible/moderately tolerant to *M. incognita*. Although *M. incognita* infected Musoliya, it showed high rates of egg masses (EMR) and galling (GR), thus it was moderately resistant and highly tolerant. Betraa was the most susceptible to both nematode species with moderate tolerance to *M. javanica* and intolerant to *M. incognita*. Clemson Spineless was susceptible/tolerant to *M. incognita* despite of EMR, GR and Rf high values. All the other cultivars failed in the category of susceptible/intolerant. The galling index did not always reflect the reproduction index or the rate of shoot weight reduction. Nematode reproduction Rf was reliable as susceptibility-resistance indicator as well as the extent of ShW reduction for tolerance assessment. The severe root weight reduction due to nematode infection may also indicate plant intolerance. Different levels of resistance and tolerance to nematode infection can be found among okra varieties. Selecting okra cultivars resistant and tolerant to *Meloidogyne* spp. is an effective approach to maintain high yield, mitigate yield costs and manage the nematode population.

Keywords: Okra, RKN, resistance, *M. javanica*, *M. incognita*, tolerance.

Introduction

Okra (*Abelmoschus esculentus* L. Moench) is an important summer vegetable crop grown almost in all parts of Iraq. The acreage cultivated with okra is usually comparable to that of watermelon and cucumber, and comprises 14% of total area cultivated with major vegetable crops (CSOI, 2016). Although Iraq is among the first ten biggest okra producing countries in terms of total production, its productivity (ton/hectare) does not exceed 50% of that produced in India and Egypt or 75% of Jordan and Pakistan (FAO, 2016). Root-knot nematode (RKN) in Iraq, as in most world countries, is the most prevalent nematode associated with vegetable crops causing serious yield loss (Stephan, 1988; Stephan *et al.*, 1998; Taylor and Sasser, 1982,). *Meloidogyne*-infected okra plants show poor growth, stunting, premature wilting and leaf chlorosis and the root system exhibits round to elongated galls of different sizes mostly coalesced and appear on both main and secondary roots, leading to plant death or yield reduction (Sikora and Fernandez, 2005).

Although the importance of okra in Iraq and middle-east, studies on nematode problems associated with its cultivation are few, even in the case of the most problematic RKN. *Meloidogyne* spp. were found to be very damaging to okra in Egypt (Ibrahim *et al.*, 1982) and north of Iraq (Al-Sabie and Ami, 1990). Most of okra varietal screening for resistance to RKN studies were done in India, Nigeria and Pakistan, where okra is widely cultivated (Darekar and Ranade, 1990; Hussain *et al.*, 2014; Jain and Gupta, 1996; Kedarnath *et al.*, 2017; Mahajan and Sharma, 1979; Martinello *et al.*, 2001; Mohanta and Mohanty, 2012; Mukhtar *et al.*, 2014; Ramakrishnan *et al.*, 1993; Rekha and Gowda, 2000; Sharma and Trivedi, 1990; Sheela *et al.*, 2006; Singh *et al.*, 1993; Thies and Fery 1994). Most of these studies evaluated susceptibility of okra to the common *M. incognita* which is the most dominant in tropical and subtropical humid climate. *M. javanica* was tested on some Indian okra varieties by Jain and Gupta (1996). Thirty six okra accessions and landraces were screened against *M. javanica* in Jordan, some of them showed moderate resistance, whereas the rest were susceptible at different levels (Karajeh and Salameh, 2015). Information on okra varietal screening to both RKN species is limited and scarce (Martinello *et al.*, 2001). Both *M. incognita* and *M. javanica* were found in mid and south of Iraq on vegetable

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crops with predominance of *M. javanica* (60-70%) over *M. incognita* populations (30-40%) (Alwaily *et al.*, 2011; Kandouh, 2018; Taylor and Sasser, 1982).

Recently, several imported and local okra cultivars are available to farmers in the Iraqi markets. Their responses in terms of susceptibility and tolerance to *M. incognita* or *M. javanica* are not determined in Iraq. Absence of fumigant nematicides makes the control of nematodes much more difficult and challenging (Haydock *et al.*, 2006). Appropriate post planting non-fumigants, if available, are not only costly and toxic with long waiting period but also requiring well trained farmers for their application. Practically, resistant cultivars provide the best economical and environment friendly nematode control approach. Screening for resistance and tolerance may be slow, but finding a stable resistant plant cultivar is worth the time spent and effort (Brown *et al.*, 1996; McDonald and Linde, 2002). The objective of this study, therefore, was to investigate susceptibility and tolerance of okra cultivars in Iraq to the root-knot nematodes *M. incognita* and *M. javanica*.

Materials and Methods

Inoculum

Root-knot nematodes were collected from infested okra fields. Species identity was confirmed according to Eisenback (1985) using the perennial pattern technique (Taylor and Netscher, 1974) and pure cultures were established from single egg masses. Egg masses of *M. incognita* or *M. javanica* were inoculated to tomato plants *Solanum lycopersicum* cultivar Mermande or okra *A. esculentus* cultivar Hussainawiya (a local cultivar) and plants were maintained in the green house. 90 days later, plants were uprooted, washed and *Meloidogyne* eggs and second stage juvenile J2 were extracted and collected using the NaOCl method (Hussey and Barker, 1973).

Seed sources of okra cultivars

Okra seeds of 10 cultivars were collected from different market places of Najaf and some other parts of Iraq. Seeds of cultivars 'Clemson spineless 1' (USA), 'Clemson spineless 2 (Turkey)' and 'Taiwan Hybrid F1' (Taiwan) are sold in the Iraqi market in sealed packs treated with pesticide. Cultivar 'Musoliya' seeds were obtained from Musol province through the Faculty of Agriculture, University of Musol. 'Hilawiya' and 'Btera' seeds were from Al-Hilla local market. The other four cultivars 'Husainawiya', 'Lahluba', 'Betraa' and 'Egypt Red' were locally purchased from an agriculture dealer in Najaf.

Experimental design

Two liter pots were filled with steam sterilized soil mix [regular field soil, river sand and coarse sand at 2:1:1 ratio (v/v)]. Pots were planted with 3 to 5 okra seeds each. Two weeks after planting, the emerged plants were thinned to one seedling per pot and inoculated with initial population (Pi) of *M. incognita* or *M. javanica* at population density of 3000 eggs/pot diluted in 10 ml aliquots. Pots were placed on benches and maintained in a cage under ambient temperature of 30-42 °C. Pre-plant pots were incorporated with fertilizer (DAP) at the rate of 5 g per pot, followed later by two additional applications during the growing period. Irrigation was applied as needed. The ten chosen okra cultivars were screened for their resistance and tolerance to the RKN *M. incognita* and *M. javanica*. This experiment was repeated in two trials, April 1-June 15, 2017 for the 1st trial and May 1 - July 15, 2017 for the 2nd trial. Experimental units were arranged in a complete randomized design (CRD) with eight replicates.

Data collection

Sixty days after inoculation, all plants of the ten okra cultivars were uprooted and each root system was carefully washed to remove soil. To determine number of J2 from plant soil, a 100 ml from each plant pot was taken and subjected to floatation and centrifugation extraction (Barker, 1985). Plant's vegetative growth parameters (weight and length of shoot and root) were recorded for tolerance comparison among cultivars across nematode species. Roots were stained (Thies *et al.*, 2002) and numbers of galls and egg masses on the entire root system were counted. The roots were then subjected to NaOCl egg extraction (Hussey and Barker, 1973). Resistance was assessed based on Galling index (GI), egg mass index (EMI) and reproduction factor (Rf), where Rf = Pf (final population)/Pi (initial population).The galling and egg masses indices were rated using a scale of 0 to 5, where: 0= no galls/egg masses; 1=1-2 galls/egg masses; 2= 3-10 galls/egg masses; 3= 11-30 galls/egg masses; 4= 31-100 galls/egg masses; 5= more than 100 galls/egg masses (3). Rf values were used to determine cultivars susceptibility or resistance. Cultivars with $Rf \geq 2$ were considered highly susceptible, those with $2 > Rf \geq 1$ were considered susceptible, whereas cultivars with $Rf < 1$ were considered resistant.

Statistical Analysis

Data obtained from the two trials were subjected to analysis of variance (ANOVA) and means were compared (Noe, 1985). The Waller-Duncan's multiple range test was used for means separation, wherever appropriate ($P \leq 0.05$). Data analysis was carried out using SAS (SAS, Inc., Cary, NC).

Results

Screening for resistance and tolerance

Data from trial 1 were consistent with those collected from trial 2, and they were combined for analysis. The okra cultivars showed various reactions to different *Meloidogyne* species. Shoot weight (ShW) and shoot length (ShL) was reduced in a similar manner due to nematode infection (Figure 1). In case of *M. incognita* infection, all the cultivars, except Clemson Spineless and Musoliya, were intolerant and showed ShW reduction range from 11% to 34%, while ShL of all the cultivars showed higher reduction ranged from 13 to 41% over the control plants. ShW and ShL were generally less affected by *M. javanica* infection. Btera and Lahluba were the only tolerant cultivars with ShW and ShL reduction ≤ 10%. Clemson Spineless and

Clemson Turkey had the highest ShW reduction (24-34%), whereas most cultivars showed moderate ShW reduction that ranged from 10 to 17%. Similar responses were observed in case of ShL. ShL of Clemson Spineless was the most reduced (31%), whereas ShL of Lahluba was the least affected (2%). Regarding root weight (RW), RKN infection almost always resulted in a RW higher than that of non-infected plants. Generally, RW increased as the gall rating index (GRI) increased (Table1). Plants infected with *M. incognita* had much higher RW compared to those infected with *M. javanica* of the same cultivar. However, RW was reduced over the control only in Lahluba and Tiawan Hybrid cvs. due to *M. javanica* infection (Figure 1). *M. javanica* reduced RL by 42% in cv. Clemson Spineless and 31% in Clemson Turkey, whereas Lahluba was less affected with 5 % RL reduction, as compared to the controls. *M. incognita* infected plants of all cultivars showed RL reduction that ranged from 2 to 27% over their control plants. Mosuliya, Clemson Spineless and Hillawiya showed low RL reduction (2-7%) due to *M. incognita* infection, whereas all other cultivars showed higher RL reduction (≥10%).

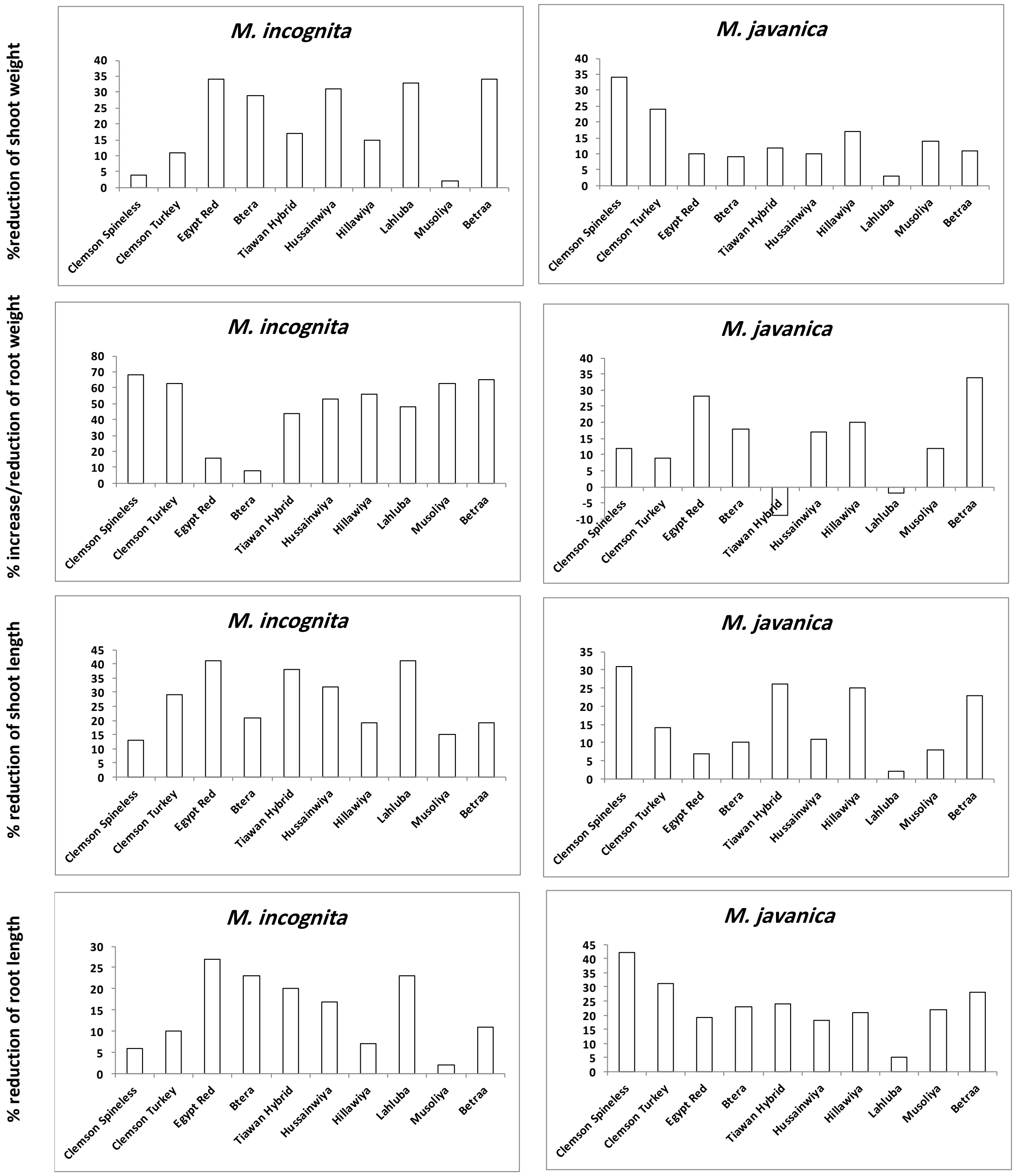

Figure 1. Effect of *Meloidogyne incognita* and *M. javanica* infection on shoot and root weight and length of different okra cultivars. Values are means of eight replicates.

The two RKN species behaved differently on different okra cultivars. Egg mass rating (EMR) values were generally higher in the *M. javanica* with a range from 2.62 to 3.5, whereas *M. incognita* infected plants had EMR that ranged from 2.25 to 3. However, within cultivar, *Meoidogyne* species did not produce high differences in EMR. The highest EMR was recorded on Clemson Spineless infected with *M. javanica* (3.5), whereas the lowest EMR was on Lahluba (2.25-2.62), regardless of the RKN species. Taiwan Hybrid showed moderate EMR for both nematode species compared to all other cultivars. Root galls rating index (GR) on the other hand was generally higher in all *M. incognita* infected cultivars ranging from 3.75 to 4.25, but GI in case of *M. javanica* infected plants ranged from 2.12 in Taiwan Hybrid to 3.87 in Clemson Turkey. GI values did not perfectly reflect the reproduction factor (Rf) values in some of the tested cultivars. In this study, Rf was used to determine susceptibility or resistance among cultivars.

Regardless of the RKN species, most of the cultivars were found to be susceptible or highly susceptible with Rf higher than one (Rf≥1). Unlike GI which was higher in all the cultivars infected with *M. incognita*, Rf of all cultivars, except for Taiwan Hybrid, was higher in case of infection with *M. javanica*. Cultivar Betraa was the most susceptible to both nematode species, while Lahluba was resistant to both species.

Taiwan Hybrid was resistant to *M. javanica* (Rf<1), but at the same time was among the most susceptible ones to *M. incognita*. Cultivar Mosuliya was resistant (Rf<1) to *M. incognita*, but it was susceptible to *M. javanica* infection. All the other cultivars, regardless of the nematode species, were either susceptible or highly susceptible to infection.

Discussion

Gall rating index (GR) can be a reliable measure in varietal screening to evaluate plant resistance or susceptibility (Mukhtar *et al.*, 2014), especially if it perfectly reflects the nematodes reproduction rate (Cook and Evans, 1987; Roberts, 2002). The GR index in our study reflected plant response to infection, but it was not a perfect indicator for plant resistance or susceptibility. The cultivar Lahluba, based on the GR only, could be identified as moderately resistant to *M. javanica* and susceptible to *M. incognita*. However, this does not support our findings as Lahluba (based on Rf index) was the only resistant cultivar to both nematode species with relatively same Rf values (Rf<1). Another approach was used previously (Sasser *et al.*, 1984; Canto-Saenz, 1985) based on GR and Rf to identify resistant, tolerant, hyper susceptible and susceptible genotypes. Based on this approach, plant genotypes with Rf ≥1 and GR ≤ 2 are considered tolerant. However, some plant species or cultivars within a species may support high nematode reproduction Rf>1 with few or no galls. Taiwan Hybrid in this study was among most susceptible/intolerant cultivars to *M. incognita* with Rf ≥1 and ShW reduction > 10%, but it was resistant (Rf<1) to *M. javanica* infection with reduction in ShW and RW. Mosuliya also was not a good fit to this approach, as it was resistant/tolerant to *M. incognita* with Rf<1 and ShW that did not differ from the control. The GR approach could not determine susceptibility or resistance in some evaluated cultivars and could not reflect tolerance level. It is already known that nematode reproduction and root galling are two different independent phenomena in the plant-nematode interaction (Williamson and Roberts, 2009). Generally, resistant tomato plants that possess *Mi-1* gene or cowpea plants with *Rk* gene suppress nematode reproduction and reduce root galling (Das *et al.*, 2008). However, other types of plant resistance were also observed where root galling was greatly suppressed but the nematode reproduction was not affected and vice versa. Genes that are involved in suppression of root galling but do not suppress nematode reproduction were identified earlier (Garcia *et al.*, 1996; Roberts *et al.*, 2008). This study confirmed that the reproduction factor is the most reliable criteria to determine plant susceptibility or resistance, whereas plant growth and yield parameters are good indicators for plant tolerance.

Table 1. Egg mass rating (EMR), gall rating (GR) and reproduction factor (Rf) of *Meloidogyne incognita* and *M. javanica* on ten different okra cultivars

Okra cultivars	Egg mass rating (EMR)		Gall rating (GR)		Reproduction factor (Rf)	
	M. incognita	***M. javanica***	***M. incognita***	***M. javanica***	***M. incognita***	***M. javanica***
Clemson spineless	3.0 a	3.3 a	3.7 b	3.8 a	1.1 ab	3.0 ab
Clemson Turkey	2.8 a	2.8 abc	4.2 a	4.0 a	1.3 ab	2.3 bcd
Egyptian Red	2.8 a	2.8 abc	4.0 ab	4.0 a	1.5 a	2.5 abc
Btera	3.0 a	2.7 bc	4.0 ab	3.8 a	1.1 ab	2.1 cd
Taiwan Hybrid	2.6 ab	2.6 cd	4.0 ab	2.1 d	1.3 ab	0.8 e
Husainawiya	2.7 ab	2.7 bc	4.1 a	3.4 b	1.2 ab	2.2 cd
Hillawiya	2.7 ab	2.8 abc	4.1 a	3.3 b	1.3 ab	2.4 abcd
Lahluba	2.2 b	2.2 d	4.2 a	2.8 cd	0.3 c	0.8 e
Musoliya	3.0 a	3.0 ab	4.2 a	3.1 bc	0.8 b	1.8 d
Betraa	2.8 a	3.0 ab	4.3 a	3.3 b	1.4 ab	3.1 a

M. incognita completes a full life cycle in approximately 20 days at 30°C and higher temperatures of 30-35°C may result in delayed adult phase and thus egg laying (Ploeg and Maris, 1999; Ploeg and Stapleton, 2001). The overall summer temperature in Iraq often exceeds 30°C and for more than five months from mid-April to late August. This may explain the overall low final nematode population and thus the reproduction factor of this nematode in this study. Both RKN species used in this study were affected by the high temperature, as plants were grown in plastic pots where soil had higher temperature than under field conditions (Thomason and Lear, 1961). Time may also be an important factor affecting nematode population density. In a comparative study, it was found that the number of *M. incognita* was lower than that of *M. javanica* or *M. arenaria* during the first 30 to 60 days of the study, but there were no differences in the final population after 90 days (Loubser and Meyer, 1987). *M. javanica* was more tolerant to higher temperature (up to 36.5°C) even under soil conditions with rapid succession of humidity and drought periods (Trudgill *et al.*, 2005). Thus, it is not surprising that *M. javanica* reproduced at much higher rate under optimum condition of 25±3°C resulting in Rf that ranged from 1.9 to 13 on okra grown under controlled growth room (Karajeh and Salameh, 2015). Similarly, *M. incognita* resulted in higher Rf that ranged from 1.09 to 8.2 under optimum temperature (25±2°C) on okra in a pot experiment under field conditions (Mukhtar *et al.*, 2014). Similar rates of reproduction were found in our study although the low reproduction factor of both nematode species due to high temperature conditions for the same period (60 days). Although some okra cultivars evaluated in this study were found to be resistant to one or both RKN species, they showed various degrees and types of resistance and tolerance as RKN species differed (Jacquet *et al.*, 2005). Cultivars are susceptible to nematodes when the plant supports high levels of nematode reproduction (Canto-Saenz, 1985; Williamson, 1998). Thus, susceptibility can be measured by the pathogen reproductive factor or the final population in an infected host plant. Unlike susceptible cultivars, tolerant cultivars have less or no differences from un-inoculated plants in terms of growth or yield in the presence of nematodes (Cook and Starr, 2006). A tolerant cultivar can be either resistant or susceptible and vice versa. A combination of both resistance and tolerance can be measured and found in some economic and cash crops (Cook and Evans, 1987). Selecting okra cultivars resistant and tolerant to *Meloidogyne* spp. is an effective approach to maintain high yield, mitigate yield costs and manage the nematode population. Such cultivars may also be introduced to breeding programs to produce new cultivars resistant/tolerant to nematodes infection.

الملخص

كندوح، باسل، علاء حسن، أسماء عبد الرسول وبرنت سايبس. 2019. الكشف عن الحساسية والتحمل للإصابة بديدان تعقد الجذور *Meloidogyne incognita* و *M. javanica* في أصناف البامية المزروعة في العراق. مجلة وقاية النبات العربية، 37(3): 279−285.

تمت غربلة عشرة أصناف من البامية لمقاومتها/قابليتها وتحملها للاصابة بنيماتودا تعقد الجذور وذلك بإلقاح شتلات بعمر 14 يوماً بـ 3000 بيضة/يافعة من *M. incognita* أو *M. javanica*. استخدم معامل تكاثر النيماتودا (Rf) لتحديد القابلية للإصابة بين الأصناف ونسبة الخفض في وزن المجموع الخضري (ShW) مقارنة بمعاملة الشاهد كمقياس للتحمل. كانت معظم الأصناف التي تم اختبارها قابلة للإصابة عند مستويات مختلفة. تأثر نمو النبات بدرجة أكبر بـ *M. incognita* عن *M. javanica* على الرغم من أن دليل التكاثر كان الأعلى للثانية عن الأولى. كان الصنف لهلوبة هو الوحيد المقاوم لنوعي النيماتودا ، متحملاً لـ *M. javanica* ولكنه غير متحمل لـ *M. incognita*. كان صنف هجين تايوان F1 مقاوماً/غير متحمل لـ *M. javanica* بينما كان الصنف موصلية مقاوماً/متحملاً لـ *M. incognita*. كان صنف بتراء أعلى الاصناف قابلية للإصابة بكلا النوعين، حيث كان متوسط التحمل لـ *M. javanica* وغير متحمل لـ *M. incognita*. كان صنف كليمسون سباينلس قابل للإصابة/متحمل لـ *M. incognita*، اما الأصناف الأخرى فكانت قابلة للإصابة غير متحملة. يمكن الاعتماد على نسبة الخفض بمؤشرات النمو (الوزن الخضري) بسبب الإصابة كمؤشر لقياس التحمل. اثبت البحث امكانية العثور على مستويات مختلفة من القابلية والتحمل للإصابة بنيماتودا تعقد الجذور بين أصناف الباميا المختلفة. يعتبر اختيار أصناف مقاومة ومتحملة من الباميا للإصابة بـ *Meloidogyne* spp. طريقة فعالة لزيادة الإنتاج وخفض التكاليف ومكافحة النيماتودا.

كلمات مفتاحية: باميا، ديدان تعقد الجذور، المقاومة، *M. incognita*، *M. javanica*، التحمل.

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