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Evaluation of Okra Landraces and Accessions Response to the Root-Knot Nematode, *Meloidogyne javanica*

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ABSTRACT

Thirty-seven landraces and accessions of okra were evaluated under controlled growth conditions; seven of them were further evaluated under open field conditions for their response to the root-knot nematode, *Meloidogyne javanica* in Jordan. Their degree of susceptibility or resistance ranged from highly susceptible to moderately resistant. International landraces; TOT 581 (from Bangladesh), Egypt Green (from Egypt), TOT 7963 (from Guatemala), TOT 2739 (from Malaysia), Palestine landrace, TOT 7957 (from USA), TOT 7346 (from Vietnam) and USA red and some local Jordanian landraces (Jordan 12, Jordan 34, and Jordan 169) were found moderately resistant (root galling index (RGI) is 2 and reproductive factor (RF) ranged from 1-5). Egypt Red, Jordan 3, Jordan 8, Jordan 48, Jordan 52, Jordan 84, TOT 1767 (from India), India Prabhani, India Sade, TOT 7159 (from Malaysia), TOT 7164 (from Myanmar), TOT 7101 and 7102 (from Philippines), TOT 7343 and 7345 (from Vietnam), and TOT 7966 (from Yugoslavia) were moderately susceptible (RGI=3 and RF ranged from 3 to more than 5). Egypt Green, Egypt Red, Jordan 8 and USA lee gave moderately resistant reactions under open field conditions. Therefore, more efforts should be directed toward intensive breeding of okra for high resistance to the root-knot nematode.

Keywords: *Abelmoschus esculentus*, host-nematode interactions, plant resistance.

INTRODUCTION

Okra (*Abelmoschus esculentus* L. Moench) formerly *Hibiscus esculentus* L. is an annual self-pollinated plant with hermaphrodite flowers, belonging to malvaceae family (Düzyaman, 1997). Its origin is from African (Sigmonds, 1976). Okra is one of the warm season crops and cultivated in the tropical and subtropical regions (Rahsid *et al.,* 2002). It is an important vegetable crop commercially grown in most of tropics and subtropics with an extension to the Mediterranean climate (Düzyaman and Vural, 2003). It provides an important input of vitamins and mineral salts including calcium, which are often lacking in the diet of developing countries (Hamon *et al*., 1990). Despite being a minor crop, okra has gained considerable interest as an alternative to more traditional vegetables in many countries throughout the world (Düzyaman, 2006). Recently, the genome size of okra has been measured using Flow Cytometry to be between 3897 and 17321 Mbp (Salameh, 2014a). Small-holder local farmers usually grow okra for local consumption in Jordan and it occupies an area of 974 hectare, which comprise 2.7% of the total area planted with vegetables in 2012 (Department of General Statistics, 2012).

Root-knot nematodes (*Meloidogyne* spp., RKNs) are

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one of the three most economically damaging genera of plant parasitic nematodes and distributed globally. They are obligate parasites of the roots of a wide range of plant host species. Annually, about 5% of the total world crop production is destroyed by *Meloidogyne* species (Sasser *et al*., 1983; Barker *et al*., 1985; Sasser, 1987). Abu-Gharbieh (1994) estimated the average annual losses of irrigated vegetable crops cultivated in the Jordan Valley due to RKNs infection by 15%.

Abu-Gharbieh *et al.* (2005) conducted a survey to cover most of the irrigated agricultural areas of Jordan; their results indicated that the three *Meloidogyne* species (*M. javanica, M. incognita,* race 1 and 2, and *M. arenaria,* race 2) with predominance of *M. javanica* were existed. Among the collected samples, three populations of *M. javanica* were found virulent to the *Mi* gene of tomato resistant cultivar Better Boy as indicated by their ability to multiply significantly on its root (Karajeh *et al*., 2005).

There is an escalating reliance on environmentally friendly means for the management of plant diseases, thus non-chemical strategies are likely to involve genotypic interactions between the nematode and its host (Hayman and Wipple, 1996). Plant resistance is currently the most effective and environmentally safe method to control root-knot nematodes (Castagnone-Sereno, 2002).

Comprehensive information on responses of various okra landraces and accessions is imperative for effective management of RKNs in Jordan. Therefore, the objective of this study was to evaluate response (degree of resistance or susceptibility) of the different collected accessions and landraces against the predominant RKN (*M. javanica*) in Jordan.

MATERIALS AND METHODS

Thirty-seven landraces and accessions of okra were evaluated under controlled conditions for their resistant or susceptibility reactions to the Jordanian population of *M. javanica*. Seven of them were further evaluated under open field conditions. Of which, twenty okra accessions were brought from World Vegetable Center (WVC), Shanhua, Tainan, Taiwan and seventeen were obtained from the National Center for Agricultural Research and Extension (NCARE), Amman, Jordan (Table 1). The nematode population was collected from an eggplant field in Karak Valley of Jordan (31°11′0″N 35°42′0″E/31.18333°N). It had previously been identified as *M. javanica* through morphological and host preference characteristics and confirmed by species-specific SCAR-PCR (Karajeh, 2004).

Table 1. Okra landraces and accessions that were used for screening for resistance to the root-knot nematode, *Meloidogyne javanica*:

Number	Landrace and Accession	Country of Origin	Collection Center
1	Egypt AliG	Egypt	WVC*
2	Egypt Green **	Egypt	WVC
3	Egypt Red **	Egypt	WVC
4	Fairooz	Jordan	NCARE***
5	India Prabhani	India	WVC
6	India Sade	India	WVC
7	Jordan 1	Jordan	NCARE
8	Jordan 2	Jordan	NCARE

Number	Landrace and Accession	Country of Origin	Collection Center
9	Jordan 3	Jordan	NCARE
10	Jordan 8**	Jordan	NCARE
11	Jordan 12	Jordan	NCARE
12	Jordan 34	Jordan	NCARE
13	Jordan 42	Jordan	NCARE
14	Jordan 48	Jordan	NCARE
15	Jordan 52**	Jordan	NCARE
16	Jordan 84	Jordan	NCARE
17	Jordan 169	Jordan	NCARE
18	Palestine**	Palestine	WVC
19	USA lee**	USA	WVC
20	USA red	USA	WVC
21	TOT 581	Bangladesh	WVC
22	TOT 1767	India	WVC
23	TOT 2739	Malaysia	WVC
24	TOT 5616	Thailand	WVC
25	TOT 6214	Thailand	WVC
26	TOT 7101	Philippines	WVC
27	TOT 7102	Philippines	WVC
28	TOT 7159	Malaysia	WVC
29	TOT 7164	Myanmar	WVC
30	TOT 7343	Vietnam	WVC
31	TOT 7345	Vietnam	WVC
32	TOT 7346**	Vietnam	WVC
33	TOT 7442	LAO	WVC
34	TOT 7697	LAO	WVC
35	TOT 7957	USA	WVC
36	TOT7963	Guatemala	WVC
37	TOT 7966	Yugoslavia	WVC

* World Vegetable Center, Shanhua,Tainan, Taiwan.

** Okra landraces and accessions were also tested under open field conditions.

***The National Center for Agricultural Research and Extension, Amman, Jordan.

Okra seeds were sown in nursery polystyrene trays filled with a pasteurized mixture of peat-moss, perlite, and clay soil (1:2:1, v/v). Each replication (1 three-week old plant /200-ml pot) was inoculated with 3000 eggs of *M.*

javanica. The pots were transferred into a controlled growth room at a temperature of 25 ± 3 ^{o}C and 16 h of artificial daylight. Each treatment was replicated 5 times and arranged in a randomized complete block design. Sixty days after inoculation, plants were removed from pots and roots were gently washed to remove adhesive soil particles. Shoot and root fresh and dry weights were recorded. Each root system was indexed for root galling (Abu-Gharbieh *et al*., 1978; Barker *et al*., 1985) according to the number of visible galls on the entire root system: 0 (immune) = no galling; 1 (resistant) = 1-9 galls/ plant; 2 (slightly susceptible) = 10-19 galls/ plant; 3 (moderately susceptible) = 20-29 galls/ plant; 4 (susceptible) = 30-39 galls/ plant; and 5 (highly susceptible) = more than 40 galls/ plant. Then, each root system was cut in small species of 5 cm and blended in a 0.5% NaOCl solution to extract eggs and 2nd stage juveniles, J2s (Hussey and Barker, 1973). The solution was poured directly through a 200-mesh (75 µm) sieve nested on a 1l beaker, then into a 500-mesh (26 µm) sieve. The number eggs+J2 per plant were counted and the (RF) was then calculated according to the formula RF = Pf /Pi, where Pi represents the nematode initial population (3000 eggs), and Pf represents the final population of eggs+J2 recovered at the end of the experiment. Value of RF was considered low, medium, high, and very high when the RF ≤1, 1-2.9, 3-5, ≥ 5, respectively. . The experiment was repeated once using similar conditions except that 1000 freshly emerged second-stage juveniles from surface-sterilized egg-masses were used as an inoculum instead of 3000 eggs of *M. javanica.*

A field trail was conducted to evaluate some of the tested landraces and accessions of okra in Karak Valley of Jordan previously planted with susceptible cucumber under drip irrigation and highly infested with the root-knot nematode, *M. javanica* with an average initial population number of 1200 second stage juveniles/ 200 cm^3 soil (Table 1). Okra seeds were sown directly into previously prepared rows with 30 cm planting space, 20 cm row width and 50 cm spacing between the rows arranged into a randomized complete block design with three replicates (5 plants/ replicate) during okra summer growing season on the beginning of May, 2013. Plants were drip irrigated and fertilized once with a N-P-K fertilizer after three weeks from sowing. Four weeks after sowing, plants were uprooted and washed gently with tap water. Root galling index was assessed as previously described.

Data were analyzed using the GLM procedure of SPSS for Windows System Version 11.5 (SPSS Inc., Chicago, USA). The least significant difference (LSD) test was used to separate the means at P≤0.05.

RESULTS

None of the tested landraces and accessions was recorded to be highly resistant *M. javanica*. Egypt Green, Jordan 12, Jordan 34, Jordan 169, Palestine, TOT 581 (Bangladesh), TOT 2739, TOT 7346, TOT 7957, TOT 7963, and USA red were found to be moderately resistant (RGI = 2) and the RF of *M. javanica* ranged from moderate to high 1-5 (Table 2).

Egypt Red, Jordan 3, Jordan 8, Jordan 48, Jordan 52, Jordan 84, India Prabhani, India Sade, TOT 1767, TOT 7101, TOT 7102, TOT 7159, TOT 7164, TOT 7343, TOT 7345 and TOT 7966 were found to be moderately susceptible (RGI=3) and RF ranged from high to very high (RF = 3 to more than 5).

The Okra commercial cultivar Fairooz besides as well as the following landraces and accessions; Jordan 1, Jordan 2, Jordan 42, TOT 6214, TOT 7442, and TOT 7697 were found susceptible (RGI = 4) and the nematode RF ranged from high to very high (RF= 3 to more than 5). Egypt AliG, TOT 5616, and USA lee gave high susceptible reaction, RGI = 5 and RF of above 5 (Table 2).

Table 2. Response of some okra landraces and accessions of different country origins to the root-knot nematode, *Meloidogyne javanica* under controlled growth room:

Landrace and accession	Plant Growth				Plant-Nematode interaction			
	SFW [2] (g/plant)	RFW (g/plant)	SDW (g/plant)	RDW (g/plant)	GI [2] (0-5)	RG [3]	RF [4]	RF level [5]
Egypt AliG	2.95 [6] bc [7]	0.31 d	0.29 bc	0.14 bc	5	HS	4.0	VH
Egypt Green	3.29 bc	1.12 cd	0.29 bc	0.23 bc	2	MR	2.1	M
Egypt Red	2.47 bc	1.02 cd	0.31 bc	0.09 c	3	MS	7.8	VH
Jordan 1	2.49 bc	2.28 cd	0.30 bc	0.21 bc	4	S	7.6	VH
Jordan 2	0.57 bc	6.78 a	0.23 bc	0.46 a	4	S	12.3	VH
Jordan 3	3.59 ab	0.51 d	0.13 bc	0.43 ab	3	MS	5.1	VH
Jordan 8	1.88 bc	0.87 d	0.12 c	0.03 c	3	MS	5.2	VH
Jordan 12	1.43 bc	1.04 cd	0.17 bc	0.21 bc	2	MR	2.7	M
Jordan 34	1.20 bc	1.29 cd	0.22 bc	0.06 c	2	MR	4.3	H
Jordan 42	0.28 c	2.40 cd	0.11 c	0.18 bc	4	S	6.0	VH
Jordan 48	1.14 bc	0.78 cd	0.12 c	0.15 bc	3	MS	7.0	VH
Jordan 52	2.05 bc	1.14 cd	0.33 a	0.05 c	3	MS	6.3	VH
Jordan 84	2.07 bc	1.17 cd	0.22 bc	0.14 bc	3	MS	4.6	H
Jordan 169	0.82 bc	1.96 cd	0.21 bc	0.10 bc	2	MR	2.4	M
Fairooz	1.26 bc	0.26 d	0.03 c	0.17 b	4	S	4.9	H
India Prabhani	2.13 bc	2.01 cd	0.32 b	0.23 bc	3	MS	10.1	VH
India Sade	2.37 bc	3.23 b	0.24 bc	0.54 a	3	MS	9.6	VH
Palestine	1.61 bc	3.01 bc	0.31 bc	0.23 bc	2	MR	2.6	M
TOT 581	3.90 a	0.34 d	0.52 a	0.19 bc	2	MR	2.0	M
TOT 1767	1.72 bc	2.41 cd	0.34 bc	0.17 bc	3	MS	6.0	VH
TOT 2739	1.77 bc	1.34 cd	0.33 bc	0.08 c	2	MR	4.3	H
TOT 5616	4.16 a	1.25 cd	0.41 a	0.08 c	5	HS	4.0	VH
TOT 6214	0.50 bc	4.81 a	0.40 a	0.10 bc	4	S	4.6	H
TOT 7101	0.57 c	3.71 b	0.25 bc	0.23 bc	3	MS	4.9	H
TOT 7102	4.48 a	1.73 cd	0.55 a	0.17 bc	3	MS	8.5	VH
TOT 7159	1.03 bc	2.03 cd	0.21 bc	0.14 bc	3	MS	11.2	VH
TOT 7164	1.55 bc	0.13 d	0.03 c	0.05 c	3	MS	4.3	H
TOT 7343	4.67 a	0.46 d	0.31 b	0.05 c	3	MS	3.3	H
TOT 7345	1.12 bc	1.70 cd	0.21 bc	0.03 c	3	MS	5.4	VH
TOT 7346	0.42 c	3.25 b	0.27 bc	0.48 a	2	MR	2.1	M

Landrace and accession	Plant Growth				Plant-Nematode interaction			
	SFW [2] (g/plant)	RFW (g/plant)	SDW (g/plant)	RDW (g/plant)	GI [2] (0-5)	RG [3]	RF [4]	RF level [5]
TOT 7442	0.36 c	2.39 cd	0.35 ab	0.15 bc	4	S	7.7	VH
TOT 7697	1.53 bc	1.92 cd	0.28 bc	0.10 bc	4	S	6.0	VH
TOT 7957	2.05 c	3.84 b	0.50 a	0.09 c	2	MR	1.9	M
TOT 7963	2.28 bc	1.74 cd	0.13 c	0.20 bc	2	MR	3.9	H
TOT 7966	0.92 bc	4.03 b	0.17 bc	0.25 b	3	MS	13.0	VH
USA lee	0.80 bc	4.72 ab	0.19 bc	0.53 a	5	HS	10.4	VH
USA red	4.63 a	1.67 cd	0.37 b	0.25 b	2	MR	2.6	M

(1) SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight, RDW: root dry weight, GI: root galling index, RG: plant resistance grading, RF: nematode reproductive factor.

(2) Root galling index: 0 (immune) = no galling; 1 (resistant) = 1-9 galls/ plant; 2 (moderately resistant) = 10-19 galls/ plant; 3 (moderately susceptible) = 20-29 galls/ plant; 4 (susceptible) = 30-39 galls/ plant; and 5 (highly susceptible) = more than 40 galls/ plant.

(3) Resistance grading: I: immune, R: resistant, MR: moderately resistant, MS: moderately susceptible, S: susceptible, HS: highly susceptible.

(4) RF: Reproductive factor = final nematode population number divided on the initial nematode population number.

(5) Reproductive factor level grading: below 1 = low (L); 1-2.9 = medium (M); 3-5 = high (H), and above 5 = very high (VH).

(6) Averages of two growth room trials (each of five replicates).

(7) Means followed by the same letters are not significantly different using least significant difference (LSD) test.

Shoot fresh weight was significantly different in Jordan 3, TOT 581, TOT 5616, TOT 7102, TOT 7343, and USA red when compared to other landraces. Root fresh weight was significantly different in Jordan 2, TOT 6214, and USA lee. Jordan 52, TOT 581, TOT 5616, TOT 6214, TOT 7102, and TOT 7957 gave the highest shoot dry weight and Jordan 2, Jordan 3, India Sade, TOT 7346, and USA lee gave the highest root dry weight as shown in (Table 2). There was a significant negative correlation between plant root dry weight and root galling index caused by the nematode and positive correlation between root galling index and nematode reproduction factor (data are not shown).

Under open field conditions of Jordan, Egypt green, Egypt Red, Jordan 8 and USA lee gave a moderate resistant reaction to *M. javanica* (RGI = 2). The other three landraces Palestine, Jordan 52, TOT 7346 showed moderately susceptible (RGI = 3), susceptible (RGI = 4), and highly susceptible (RGI = 5), respectively (Table 3).

Table 3.Response of some okra landraces and accessions of different country origins to the root-knot nematode, *Meloidogyne javanica* under open field conditions:

Landrace and accession	Galling Index [1] (0-5)	Plant resistance grading [2]
Egypt green	2	MR
Egypt Red	2	MR
Jordan 8	2	MR
Jordan 52	4	S
Palestine	3	MS
TOT 7346	5	HS
USA lee	2	MR

(1) Root galling index: 0 (immune) = no galling; 1 (resistant) = 1-9 galls/ plant; 2 (moderately resistant) = 10-19 galls/ plant; 3 (moderately susceptible) = 20-29 galls/ plant; 4 (susceptible) = 30-39 galls/ plant; and 5 (highly susceptible) = more than 40 galls/ plant.

(3) Resistance grading: I: immune, R: resistant, MR: moderately resistant, MS: moderately susceptible, S: susceptible, HS: highly susceptible.

DISCUSSION

Okra is a highly susceptible crop to root-knot nematodes ‘*Meloidogyne* spp. Above ground symptoms are similar to those described for root rot and wilt diseases but there is appearance of root galls/knots of different sizes, instead of root rotting the infected roots become enlarged and distorted. Among the most damaging root-knot nematode species to okra (*Abelmoschus esculentus* L.) is *M. javanica* and considered of a great importance in vegetable-growing areas in Jordan and other countries of the world. Although nematicides e.g. Rugby–10G (0-ethyl S, S-di-Seobutyl phosporodithioate) expressed best okra growth with minimum disease symptoms and minimal final nematode population, was comparatively expensive (Dubey and Trivedi, 2011). Among the alternatives to nematicides is notably the use of resistant cultivars which are inexpensive and eco-friendly to environment (Mucktar *et al*., 2014).

The thirty-seven okra landraces and accessions tested in this study were responded differentially to the root-knot nematode, *M. javanica*. The degree of susceptibility or resistance ranged from highly susceptible to moderately resistant. Eleven okra landraces and accessions were found moderately resistant to the nematode infection with reproduction factor ranged from moderate to high included international landraces (TOT 581 (from Bangladesh), Egypt Green, TOT 7963 (from Guatemala), TOT 2739 (from Malaysia), Palestine, TOT 7957 (from USA), TOT 7346 (from Vietnam) and USA red and some local landraces (Jordan 12, Jordan 34, and Jordan 169). Sixteen of them were moderately susceptible with reproduction factor ranged from high to very high which exhibit a degree of resistance but less than that moderately resistant landraces and accessions included Egypt Red, Jordan 3, Jordan 8, Jordan 48, Jordan 52, Jordan 84, TOT 1767 (from India), India Prabhani, India Sade, TOT 7159 (from Malaysia), TOT

7164 (from Myanmar), TOT 7101 and 7102 (from Philippines), TOT 7343 and 7345 (from Vietnam), and TOT 7966 (from Yugoslavia). Moderate resistance of Egypt Green was also confirmed under open field conditions. Some okra landraces and accessions were moderately resistant under these conditions included Egypt Red, Jordan 8 and USA lee. The limitation of plant damage despite the presence of potentially damaging levels of plant-parasitic nematodes, which is particularly useful in some crops e.g. okra is usually dependant on the dynamics of nematode population numbers and since many environmental factors influence both plant growth, nematode dynamics and plant-nematode interactions thus some cultural practices can improve crop resistance to nematode such as amending okra with compost (Mcsorley and Gallaher, 1995) and the use of transplanting rather than direct seeding (Mcsorley and Gallaher, 1996). Efficacy of resistant cultivars can integrated with the use of proven effective biological control agents e.g. *Paecilomyces lilacinus* (Thorn) Samson (Zareen *et al*., 2001) or in a crop rotation with poor host. The galling index on okra followed with sweet corn was greatly reduced compared to continuously cropped okra and resulting in an increase in yield within a range of 60-92% (Mweke *et al*., 2008).

The other studied landraces, accessions and the commercial cultivar Fairooz were either susceptible or highly susceptible to the nematode infection and greatly support its reproduction. In a study to evaluation of okra accessions with reported resistance to root-knot nematode, all of the tested accessions were susceptible to *M. incognita* race 3 (Thies *et al*., 2014). Of the twelve cultivars screened for okra resistance to *M. incognita*, none of them was found highly resistant or even moderately resistant. Two cultivars (Selection-31 and Okra Sindha) were susceptible and the cultivar Punjab Selection was found highly susceptible but most of the tested cultivars showed moderate susceptibility towards the nematode and caused reduction in plant growth (Arshad, 2011). A genetic diversity between the different accessions from different regions of okra has been found using AFLP (Salameh, 2014, b), this could be of high interest for breeders to start a successful breeding programs, therefore, more efforts should be directed toward intensive breeding of okra for resistance to root-knot nematodes. Landraces or primitive cultivars of okra are possible source for successful development of improved cultivars.

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تقييم استجابة سلالات الباميا المحلية وخطوطها لنيماتودا تعقد الجذور (*Meloidogyne javanica*)

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ملخص

تم تقييم استجابة سبع وثلاثين سلالة محلية وخط من الباميا تحت ظروف غرفة النمو و تم تقييم استجابة سبعة منهم تحت ظروف الحقل المكشوف لنيماتودا تعقد الجذور (*Meloidogyne javanica*) في الأردن. وقد تراوحت حساسيتها أو مقاومتها من عالي الحساسية إلى متحمل، فوجدت السلالات المحلية الدولية التالية متحملة (فهرس تدرن الجذور، ف ت ج= 2 ومعامل تكاثر للنيماتودا، م ت= 1-5): TOT 581 من بانقلادش، Egypt Green، TOT 7963 من جوتيملا، TOT 2739 من ملايزيا، Palestine، TOT 7957 من الولايات المتحدة الأمريكية، TOT 7346 من فيتنام، USA red، وبعض السلالة المحلية الأردنية (Jordan 12، Jordan 34 ، وJordan 169)، بينما Egypt Red، Jordan 3، Jordan 8، Jordan 48، Jordan 52، Jordan 84، TOT 1767 من الهند، India Prabhani، India Sade، TOT 7159 من ماليزيا، TOT 7164 من مينمار، TOT 7101 من الفلبين، TOT 7343 وTOT 7345 من فيتنام، وTOT 7966 من يوغسلافيا السابقة وجدت متوسطة الحساسية للنيماتودا (ف ت ج= 3 و م ت= 3 إلى أكثر من 5). تم تأكيد تحمل Egypt Green، Egypt Red، Jordan 8، وUSA lee تحت ظروف الحقل المكشوف. لذلك لا بد من تضافر الجهود باتجاه تحسين الباميا من خلال برامج التهجين للحصول على مقاومة عالية للنيماتودا تعقد الجذور.

الكلمات الدالة:*Abelmoschus esculentus* ، تفاعلات النيماتودا مع العائل،المقاومة النباتية.

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