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Nematology/ Original Article

Management of root-knot nematodes in okra through grafting onto kenaf and false roselle

Abstract – The objective of this work was to evaluate the performance of okra grafted onto *Hibiscus* spp. in the presence and absence of three root-knot nematode species. The experimental design was completely randomized in a 4x4 factorial arrangement with ten replicates. The first factor consisted of the following four rootstocks: kenaf (*Hibiscus cannabinus*), false roselle (*Hibiscus acetosella*), self-grafted, and non-grafted. The second factor was the inoculation of 5,000 eggs and second-stage juveniles of *Meloidogyne incognita*, *Meloidogyne javanica*, and *Meloidogyne enterolobii* in okra plants, plus an uninoculated control. Vegetative development, agronomic performance, and nematode reproduction were evaluated. The grafting of okra onto *Hibiscus* spp. in the presence of root-knot nematodes provided a greater plant height, scion length, fresh shoot mass, number of fruits, and yield, when compared with self-grafted and non-grafted plants. The averages of the root-knot nematode reproduction factor were low in the plants grafted onto *Hibiscus* spp., indicating that grafting in the presence of root-knot nematodes is capable of maintaining okra vegetative development and yield.

Index terms: *Abelmoschus esculentus*, *Hibiscus acetosella*, *Hibiscus cannabinus*, *Meloidogyne*, intergeneric grafting.

Manejo de nematoides-de-galha em quiabeiro por meio de enxertia em vinagreira-roxa e vinagreira-flor-de-veludo

Resumo – O objetivo deste trabalho foi avaliar o desempenho de quiabo enxertado em *Hibiscus* spp. na presença e na ausência de três espécies de nematoides-de-galha. O delineamento experimental foi inteiramente casualizado, em arranjo fatorial 4x4, com dez repetições. O primeiro fator foi constituído pelos quatro seguintes porta-enxertos: vinagreira-roxa (*Hibiscus cannabinus*), vinagreira-flor-de-veludo (*Hibiscus acetosella*), autoenxertia e pé-franco. O segundo fator foi a inoculação de 5.000 ovos e juvenis de segundo estágio de *Meloidogyne incognita*, *Meloidogyne javanica* e *Meloidogyne enterolobii* em plantas de quiabo, além de testemunha não inoculada. Foram avaliados desenvolvimento vegetativo, desempenho agronômico e reprodução do nematoide. A enxertia de quiabo em *Hibiscus* spp. na presença de nematoides proporcionou maiores altura de planta, comprimento de enxerto, massa fresca da parte aérea, número de frutos e produtividade, em comparação às plantas autoenxertadas e pé-franco. As médias do fator de reprodução dos nematoides foram baixas nas plantas enxertadas em *Hibiscus* spp., o que indica que a enxertia na presença de nematoides é capaz de manter o desenvolvimento vegetativo e a produção do quiabo.

Termos para indexação: *Abelmoschus esculentus*, *Hibiscus acetosella*, *Hibiscus cannabinus*, *Meloidogyne*, enxertia intergenérica.

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Introduction

Okra [*Abelmoschus esculentus* (L.) Moench] is a rustic, heat-tolerant, and relatively easy to grow vegetable, with an important socioeconomic role as it is mostly grown by family farmers (Silva et al., 2021). However, crop growth and yield can be negatively affected by a wide range of insects and pathogens (Mukhtar et al., 2013), among which root-knot nematodes (RKN) of *Meloidogyne* spp. stand out as the greatest phytosanitary problem (Hussain et al., 2012; Mukhtar et al., 2013).

In general, okra is an excellent host for RKN (Silva et al., 2019a), especially for *Meloidogyne incognita*, whose occurrence and distribution are high in the Brazilian territory (Oliveira et al., 2007; Hussain et al., 2012; Rosa et al., 2013). *Meloidogyne javanica* and *Meloidogyne enterolobii* are other important species in the country (Silva et al., 2019a).

The control of these nematodes is a challenge, as they multiply quickly under favorable conditions and have a wide host range (Desaeger et al., 2023). Among the various control methods that have been studied, the use of resistant cultivars is indicated as the most efficient and sustainable (Hussain et al., 2014). However, there are still no known reports on genetic resistance in commercial okra cultivars or on genetically close materials that could be used in breeding programs safely and effectively (Mukhtar et al., 2014; Prasanna et al., 2022). Although some slightly tolerant materials have been identified (Hussain et al., 2016; Odeyemi et al., 2016; Mukhtar et al., 2017; Afzal et al., 2019), the successive cultivation of these cultivars, despite their acceptable yield level, increases the population of nematodes, not being viable in the medium and long term (Silva et al., 2019a).

Recently, several authors have found that intergeneric grafting with *Hibiscus* spp. has potential to be used to manage RKN in okra (Marin et al., 2017; Silva et al., 2019b, 2022; Andrade et al., 2020). Marin et al. (2017) highlighted that, although grafting is not yet practiced on a commercial scale, it is expected that the automation of the grafted seedling process would lower production costs and make it possible to use this technique in crops of lesser economic expression, such as okra.

Silva et al. (2019b), when evaluating Malvaceae genotypes for resistance to *M. enterolobii*, *M. javanica*, and *M. incognita* race 3, observed resistance when plants were grafted onto three *Hibiscus* species: roselle (*Hibiscus sabdariffa* L.), false roselle (*Hibiscus acetosella* Welw. ex Ficalho), and kenaf (*Hibiscus cannabinus* L.). Minton & Adamson (1979) and Adegbite et al. (2008) added that the resistance of kenaf and false roselle to *Meloidogyne* spp. is genotype-dependent. In their studies, Silva et al. (2019b) found grafting compatibility between 'Santa Cruz 47' okra and false roselle and kenaf, and Silva et al. (2022), between kenaf and the main Brazilian okra cultivars available to growers. However, in these few available studies on okra grafting, compatibility and agronomic performance were observed in the absence of RKN (Marin et al., 2017; Silva et al., 2019a; Andrade et al., 2020). This shows the need to evaluate whether grafting onto *Hibiscus* spp. would be able to maintain the same yield level in the presence of nematodes, in order to be effectively recommended to okra growers.

The objective of this work was to evaluate the performance of okra grafted onto *Hibiscus* spp. in the presence and absence of three root-knot nematode species.

Materials and Methods

The experiment was conducted at Faculdade de Ciências Agrárias e Veterinárias of Universidade Estadual Paulista Júlio de Mesquita Filho, in the municipality of Jaboticabal, in the state of São Paulo, Brazil (21º14'05"S, 48º17'09"W, at 614 m altitude). According to the classification of Köppen-Geiger, the climate type is Aw, tropical, with a dry winter and excessive rain, in transition to Cwa, subtropical, with a warm and dry winter.

The experimental design was completely randomized, in a 4x4 factorial arrangement with ten replicates. The first factor consisted of the four following grafting treatments: grafting on false roselle and kenaf, self-grafting of okra plants, and non-grafting of okra plants. The second factor was the inoculation or not with root-knot nematodes, as follows: non-inoculated okra plants; and okra plants inoculated with *M. incognita*, *M. javanica*, and *M. enterolobii*. The Santa Cruz 47 okra cultivar was used as a scion for all treatment combinations.

The scion and rootstocks were sown in December 2018 in 128-cell expanded-polystyrene trays filled with the Plantmax commercial substrate (Eucatex

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Agro, Botucatu, SP, Brazil). The trays were placed in a greenhouse, equipped with a sprinkler irrigation system, which was activated four times a day. Crop management was carried out according to Passos et al. (2014).

The seedlings were cleft-grafted 13 days after sowing when presenting the first fully developed true leaf. After grafting, the trays were placed in a humid floating chamber for 19 days to adequately promote the healing of the grafted region.

Subsequently, the grafted seedlings were transplanted into 13 L pots filled with Latossolo Vermelho-Amarelo, according to the Brazilian soil classification system (Santos et al., 2018) i.e., an Oxisol, previously autoclaved at 120°C and 1 atm for 1 hour. Then, the pots with one plant each were placed in an open field, spaced at 1 m from each other.

The subpopulations of each nematode species, previously multiplied on 'Santa Cruz Kada' tomato (*Solanum lycopersicum* L.), were extracted as described in Hussey & Barker (1973). According to the treatments, the okra root systems were inoculated with 5,000 eggs and second-stage juveniles by applying 5 mL of the suspension per pot with the aid of a digital pipette. The nematode was inoculated on the same day the seedling was transplanted. The experiment was drip-irrigated twice a day.

Scion length (in centimeters) was measured for the grafted treatments, whereas total plant height and fresh shoot mass were obtained for all treatments. To evaluate agronomic performance, fruits from 8 to 12 cm were harvested on alternate days from 40 to 120 days after transplanting and weighed to obtain fresh fruit mass (in grams). Afterwards, the samples were placed in a forced-air oven, at 60°C, until reaching a constant mass and, then, weighed to obtain dry mass (also in grams). The number of fruits per plant was counted at harvesting. These data were used to estimate plant yield.

After the last harvest, 120 days after transplanting and nematode inoculation, the shoot part of the plant was cut and weighed to obtain fresh shoot mass. The root systems were removed from the pots, washed, and later processed for nematode extraction according to Hussey & Barker (1973). For the estimation of the final nematode population, the number of eggs and second-stage juveniles was quantified with the aid of a Peters' chamber under the Q7708S-4 photonic microscope (Quimis Aparelhos Científicos Ltda., Diadema, SP, Brazil). The number of eggs per gram of roots was estimated by dividing the final population by root mass. The reproduction factor was obtained through the ratio between the final and initial population of the nematode.

To meet the assumptions of the analysis of variance, the data were transformed to $(x+1)^{0.5}$. Then, the results were subjected to the analysis of variance by the F-test, and means were compared by Tukey's test, at 5% probability, using the AgroEstat software (Barbosa & Maldonado Junior, 2010).

Results and Discussion

The number of eggs per gram of roots was higher in self-grafted and non-grafted okra plants than in those grafted onto *Hibiscus* spp. (Figure 1). This result is an indicative that okra is an excellent host for nematodes and that *Hibiscus* spp. are effective in coping with this pest, as shown by the lower number of eggs per gram of roots in these plants. A high reproduction rate of nematodes was observed in non-grafted and self-grafted okra, as well as the reproduction of *M. javanica* and *M. enterolobii* in okra grafted onto kenaf and false roselle. However, the final population of *M. enterolobii* was lower in okra grafted onto kenaf and false roselle, indicating that these rootstocks are resistant to this RKN species. According to Hussain et al. (2014), resistant or non-host species are able to better suppress conditions favorable to parasitism since juvenile nematodes are unable to penetrate plant roots or die after such penetration due to mechanisms that prevent their full development or female reproduction. This means that if a non-resistant rootstock is adopted, i.e., a genotype in which RKN reproduce over time, nematode populations are likely to increase to levels that make their control unfeasible and culminate in the abandonment of the agricultural activity. Therefore, in this scenario, resistant rootstocks are useful tools to manage the damage caused by nematodes, but RKN management should also consider a variety of strategies to ensure a greater crop efficiency and sustainability (Collange et al., 2011).

There was a significant interaction between grafting combinations and root-knot nematodes for plant height, scion length, and fresh shoot mass (Table 1). Grafting onto kenaf and false roselle resulted in a higher average plant height and, consequently, in a longer scion length, when compared with self-grafted okra in the presence of

M. incognita and *M. javanica*, which can be associated with the nematode effect. Grafting onto *Hibiscus* spp. plants also led to higher averages of fresh shoot mass in the presence or absence of root-knot nematodes, indicating that grafting increased plant vigor.

The lower number of nematodes on the roots of *Hibiscus* spp. indicates less damage to and probably a better vegetative development of the grafted okra. This is explained by the healthier root system of resistant rootstocks, which allow of capturing water and nutrients, normally maintaining plant productive physiology. Daramola et al. (2015) concluded that, in susceptible plants, galls formed from giant cells used by the nematode as a source of nutrients can interfere with the flow of water and nutrients within the plants, resulting in reductions in crop yield.

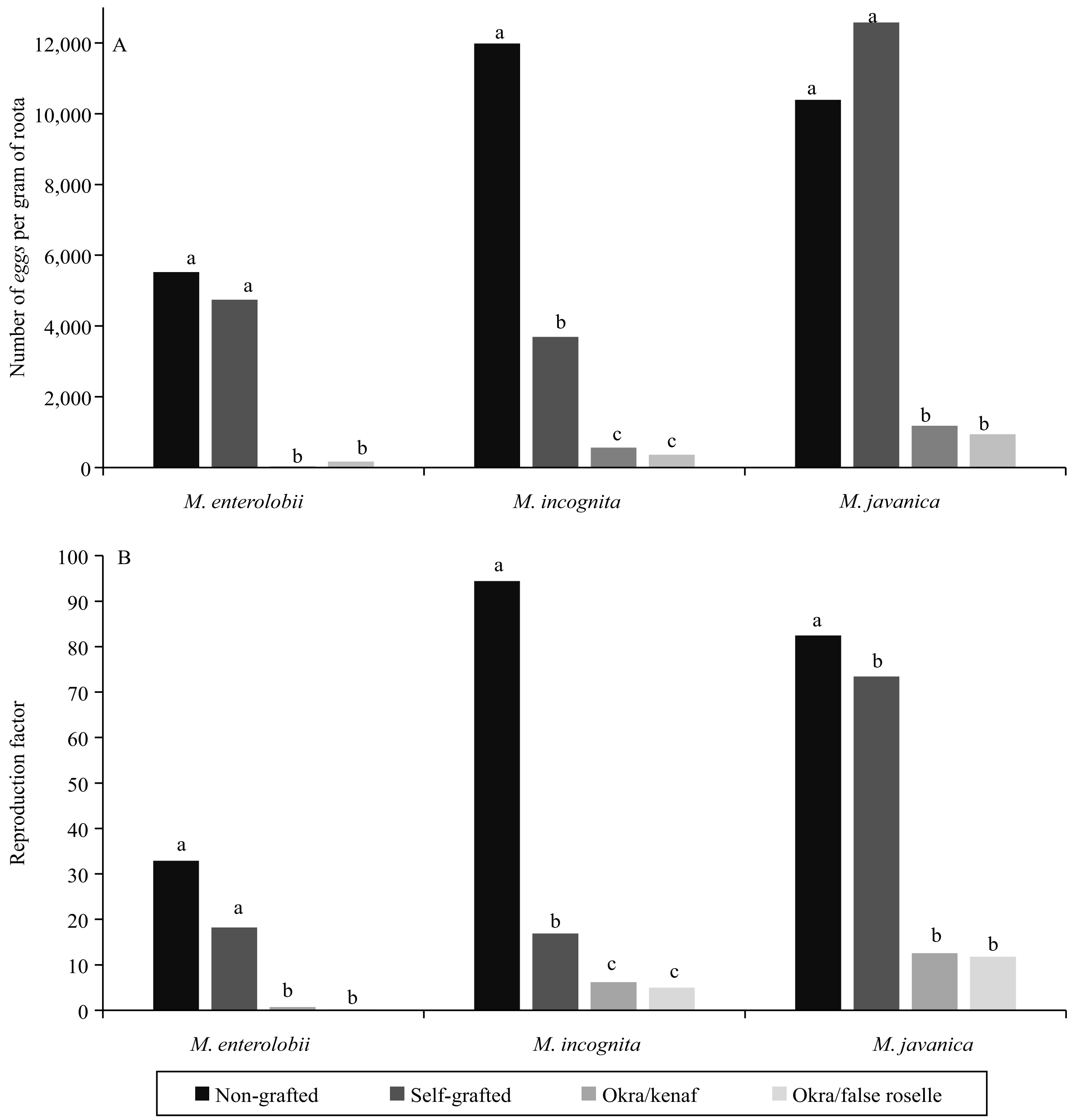

Figure 1. Number of eggs per gram of roots (A) and reproduction factor (B) of three root-knot nematode species (*Meloidogyne incognita*, *Meloidogyne javanica*, and *Meloidogyne enterolobii*) inoculated on grafted and non-grafted okra (*Abelmoschus esculentus*). Non-transformed means with statistics based on data transformed to $(x+1)^{0.5}$.

Self-grafted and non-grafted okra plants showed lower values for plant height, fresh shoot mass, and scion length in the presence of the nematode species, which was not the case in okra grafted onto kenaf or false roselle. Specifically, *M. incognita* and *M. javanica* reduced plant height, fresh shoot mass, and scion length in non-grafted plants and were more aggressive on self-grafted and non-grafted okra than *M. enterolobii.* These results evidence the protective action of grafting in the management of the root-knot nematode, with results similar to those of the non-inoculated control. In the literature, Nacar & Özarslandan (2021) found that *M. incognita* reduces okra plant height and leaf width, length, and N, P, K, and Mg contents, whereas Gálvez et al. (2019) observed several physiological changes caused by RKN in sweet pepper (*Capsicum annuum* L.).

There were no significant differences in the number of fruits, fresh fruit mass production per plant, and dry fruit mass production per plant between the control and inoculation treatments when okra was grafted onto *Hibiscus* spp. (Table 2). This shows that the use of grafting did not harm the reproductive development of the plants, whose levels were similar to those of non-grafted okra in the absence of the phytopathogen, which is a *sine qua non* condition for rootstock positioning (Silva et al., 2019b). Therefore, the present study is the first known report of the agronomic performance of okra when grafted onto kenaf or false roselle in the presence of root-knot nematode species.

For non-grafted and self-grafted okra, the presence of nematodes reduced the average of yield-related traits in comparison with the control, which may be associated with the direct impact RKN has on water and nutrient absorption, and, consequently, on plant development and yield. Although *M. incognita* and *M. javanica* caused more damage to yield-related parameters in okra than *M. enterolobii*, all three nematode species negatively affected self-grafted and non-grafted okra fruit yield.

In the absence of RKN, a similar performance was observed between grafted and non-grafted okra, indicating that the use of grafting would not be justifiable in this case. Likewise, Gálvez et al. (2019) found that grafting is only advantageous for sweet pepper in RKN-

Table 1. Plant height, fresh shoot mass, and scion length of grafted and non-grafted okra (*Abelmoschus esculentus*) in the presence or absence of three root-knot nematode species[(1)].

Treatment	Okra/kenaf	Okra/false roselle	Self-grafted	Non-grafted	F-test	p-value
			Plant height (cm)			
Control	139.43	134.97	134.26A	143.44A	0.40^{ns}	0.7548
Meloidogyne enterolobii	127.42	128.18	111.92B	122.60AB	1.58^{ns}	0.1960
Meloidogyne incognita race 3	129.48a	129.60a	97.74bB	108.88abB	7.13**	0.0002
Meloidogyne javanica	139.55a	124.67ab	101.96cB	106.85bcB	7.94**	<0.0001
F-test	0.95^{ns}	0.50^{ns}	7.42**	7.45**		
p-value	0.4205	0.6843	<0.0001	0.0001		
			Fresh shoot mass (g)			
Control	186.00ab	223.00a	162.00bA	176.00abA	2.72*	0.0467
Meloidogyne enterolobii	203.00a	175.00ab	100.00cB	133.00bcAB	10.75**	<0.0001
Meloidogyne incognita race 3	186.00a	187.00a	53.00bC	87.00bB	29.16**	<0.0001
Meloidogyne javanica	175.00a	180.00a	69.00bBC	93.00bB	19.16**	<0.0001
F-test	0.40^{ns}	1.93^{ns}	15.85**	9.54**		
p-value	0.7534	0.1278	<0.0001	<0.0001		
			Scion length (cm)			
Control	134.20	129.40	127.00A	-	0.24^{ns}	0.7888
Meloidogyne enterolobii	121.70	123.30	104.50AB	-	2.63^{ns}	0.0764
Meloidogyne incognita race 3	123.90a	124.40a	75.10bC	-	23.33**	<0.0001
Meloidogyne javanica	134.90a	119.50a	85.40bBC	-	16.20**	<0.0001
F-test	0.93^{ns}	0.39^{ns}	14.73**	-		
p-value	0.4268	0.7591	<0.0001	-		

[(1)]Means followed by equal letters, lowercase in the rows and uppercase in the columns, do not differ by Tukey's test, at 5% probability. Non-transformed means with statistics based on data transformed to $(x+1)^{0.5}$. Kenaf, *Hibiscus cannabinus*; and false roselle, *Hibiscus acetosella*. ** and *Significant at 1 and 5% probability, respectively. ^{ns}Nonsignificant.

Table 2. Number of fruits, fresh fruit mass production per plant, and dry fruit mass production per plant of grafted and non-grafted okra (*Abelmoschus esculentus*) in the presence or absence of three root-knot nematode species[(1)].

Treatment	Okra/kenaf	Okra/false roselle	Self-grafted	Non-grafted	F-test	p-value
			Number of fruits			
Control	8.00	8.80	7.10A	9.33A	1.35ns	0.2601
Meloidogyne enterolobii	8.80a	7.60ab	5.40bAB	6.50abAB	4.10**	0.0080
Meloidogyne incognita race 3	8.30a	9.20a	2.70bC	3.97bB	19.72**	<0.0001
Meloidogyne javanica	8.80a	7.30a	4.11bBC	4.33bB	10.55**	<0.0001
F-test	0.21ns	0.98ns	7.94*	11.27**		
p-value	0.8880	0.4057	0.0272	<0.0001		
			Fresh fruit mass production per plant (g)			
Control	119.07	160.39	121.91A	169.30A	2.25ns	0.0848
Meloidogyne enterolobii	144.68a	143.07a	92.87bAB	112.02abB	3.68*	0.0137
Meloidogyne incognita race 3	138.08a	168.00a	38.40bC	56.58bC	20.42**	<0.0001
Meloidogyne javanica	150.58a	137.84a	63.78bBC	69.12bBC	11.67**	<0.0001
F-test	0.68ns	0.50ns	8.43**	12.87**		
p-value	0.5676	0.6830	<0.0001	<0.0001		
			Dry fruit mass production per plant (g)			
Control	11.85	14.35	11.38A	15.45A	1.46ns	0.2278
Meloidogyne enterolobii	13.75a	12.93ab	8.97bAB	10.29abB	3.37*	0.0203
Meloidogyne incognita race 3	13.41a	14.77a	3.34bC	4.63bC	20.69**	<0.0001
Meloidogyne javanica	14.17a	12.18a	6.18bBC	5.91bC	11.18**	<0.0001
F-test	0.38ns	0.42ns	8.69**	13.88**		
p-value	0.7655	0.7362	<0.0001	<0.0001		

[(1)]Means followed by equal letters, lowercase in the rows and uppercase in the columns, do not differ by Tukey' test, at 5% probability. Non-transformed means with statistics based on data transformed to $(x+1)^{0.5}$. Kenaf, *Hibiscus cannabinus*; and false roselle, *Hibiscus acetosella*. ** and *Significant at 1 and 5% probability, respectively. nsNonsignificant.

infested soil. Desaeger et al. (2023) highlighted that grafting adds costs to the seedling production process, as more seeds, time, and effort are required to obtain good-quality grafted seedlings, which should also be taken into account for a better use of this technique.

Conclusions

1. Grafting on false roselle (*Hibiscus acetosella*) and kenaf (*Hibiscus cannabinus*) allows of the proper development and yield of okra (*Abelmoschus esculentus*) in the presence of the *Meloidogyne incognita*, *Meloidogyne javanica*, and *Meloidogyne enterolobii* root-knot nematodes.
2. Grafting on false roselle and kenaf does not negatively affect okra yield and performance even in the absence of root-knot nematodes.

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