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Short note

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Grafting compatibility between Okra cultivars and root-knot nematode resistant Kenaf

E.H.C. Silva (*), J. Versuti, L.T. Braz
Department of Agricultural Production Sciences, São Paulo State University, Via de Acesso Prof. Paulo Donato Castellane s/n, CEP 14884-900, Jaboticabal-SP, Brazil.

Key words: *Abelmoschus esculentus* L. Moench, *Hibiscus cannabinus* L., intergeneric grafting, *Meloidogyne* spp.

Abstract: The use of intergeneric grafting has been reported as an alternative to manage root-knot nematodes in okra, but the compatibility for grafting has only been tested in a few okra (*Abelmoschus esculentus* L. Moench) cultivars. The kenaf (*Hibiscus cannabinus* L.) is resistant to root-knot nematode species and is a potential rootstock for okra. The objective was to study the compatibility of kenaf as rootstock with okra cultivars. It was used a completely randomized design, in factorial scheme 3x10, with five repetitions. The compatibility was assessed by measuring several vegetative characteristics. All cultivars are compatible for grafting with kenaf as rootstock. Grafting onto kenaf may be an option to control root-knot nematodes.

(*) **Corresponding author:**
edgarddragde@gmail.com

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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1. Introduction

The okra (*Abelmoschus esculentus* L. Moench) is mainly cultivated for its immature fruits but presents several industrial applications (Dantas *et al*., 2021). In Brazil, okra is widely cultivated, mainly by family farmers, as it is considered a low production cost crop that adapts to tropical and subtropical climates. Although it is rustic, a limiting factor for the crop has been the root-knot nematodes (*Meloidogyne* spp.) (Silva *et al*., 2019 a), which are favored by high temperature and humidity, which are also necessary for the development of the crop.

To control the root-knot nematode, it is necessary to integrate several management practices, from the choice of the planting area to pre-sowing, such as prevention, crop rotation, fallow, and the use of antagonistic plants (Collange *et al*., 2011; Nascimento *et al*., 2020). Other approaches, such as organic fertilization, biological control, and heat-based methods are also a possibility (Mahalik and Sahoo, 2019). However, the use of resistant cultivars is considered the most efficient method, but, to date, there are no reports of genetic resistance effectively incorporated in commercial cultivars.

Recent studies have been exploring promising alternatives, such as intergeneric grafting with species resistant to root-knot nematodes. Among the possible rootstocks, some *Hibiscus* spp. have been shown to be interesting for being resistant and compatible for grafting with okra (Marin *et al*., 2017; Silva *et al*., 2019 b; Andrade *et al*., 2020). In addition to genetic resistance, rootstocks recommended to manage some type of pathosystem must be compatible with the scion, ensuring normal vegetative and reproductive development. It is noteworthy that, by obtaining resistant and compatible rootstock, crop yield can be enhanced by minimizing negative effects generated by the nematodes on the plant.

Research has tended to focus on only two open-pollinated cultivars, namely 'Santa Cruz 47' and 'Colhe Bem', with the need to verify whether compatibility also occurs in other cultivars, as the compatibility may vary according to rootstock-scion combinations (Reig *et al*., 2018). Given the above, the objective of the present study was to assess the compatibility of the kenaf (*Hibiscus cannabinus* L.) with commercial okra cultivars.

2. Materials and Methods

The experiment was carried out at the Sector of Vegetables and Aromatic-Medicinal Plants, School of Agriculture and Veterinary Sciences, Unesp, Campus of Jaboticabal, SP (21°14'05'' S, 48°17'09" W, 614 m of altitude), from March to May 2019. The climate type is Aw, described as a tropical winter dry season, which occurs from April to September and rains are concentrated from October to March, with transition to Cwa. The average temperature and relative humidity during the period of the experiment is presented in figure 1.

A completely randomized design in a factorial scheme 3x10 with five replications was used. The first factor was seedling production modalities, being non-grafted, self-grafted, and grafted onto kenaf. The second factor consisted of ten okra cultivars, being 6 hybrids and 4 open-pollinated cultivars (Table 1).

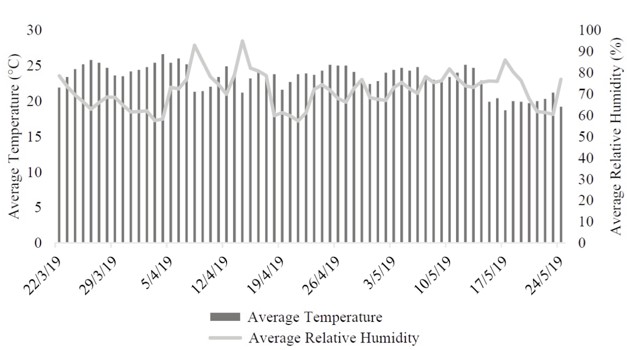

Fig. 1 - Changes in temperature and relative humidity during the experiment.

The okra seeds were immersed in acetone for 30 minutes to break dormancy, whilst kenaf seeds were immersed in water for five hours. Then, the genotypes were sown in 128-cell expanded polystyrene trays, filled with commercial substrate coconut-fiber based Bioplant® (Nova Ponte, MG, Brazil) to produce vegetable seedlings. The trays were placed in a greenhouse equipped with a sprinkler irrigation system.

Table 1 - Identification and origin of the okra and *Hibiscus* genotypes used in the experiment

Cultivar	Origin	Pollination
Brutus	Feltrin Sementes	Hybrid
Canindé	Isla Sementes Ltda.	Hybrid
Clemson	Isla Sementes Ltd.	Open
Colhe Bem	Sakata Seed Sudamérica	Open
Esmeralda	Agristar do Brasil Ltd.	Hybrid
Guará	Isla Sementes Ltd.	Hybrid
Santa Cruz 47	Feltrin Sementes	Open
V8	Agristar do Brasil Ltd.	Hybrid
Valença	Feltrin Sementes	Open
Xingó	Eagle Flores, Frutas e Hortaliças Ltd.	Open
Kenaf	Embrapa Hortaliças	Open

The seedlings were cleft-grafted as described by Silva *et al*. (2019 b) when the seedlings presented the cotyledonary leaves fully exposed. The scion was inserted in a cut of approximately 1 cm between the cotyledonary leaves of the rootstock. The scions were standardized to 3 cm in length. The scion was fixed so that it did not shade the cotyledonary leaves. After positioning the scion, a clip was placed to ensure the connection with the rootstock, until complete healing of the grafting site. The grafting was carried out on the seedling production trays.

After the grafting was carried out, the plants were placed in a humid floating chamber to secure high temperature and humidity, which is adequate for okra grafting healing. The grafted seedlings remained in the humid chamber for 11 days, and then transplanted to pots containing autoclaved clayey red latosol (oxisol) soil. The pots were placed in a greenhouse.

The percentage of grafting success was assessed for the self-grafted and grafted onto kenaf treatments. The number of leaves (NL), the number of internodes (NI), and the number of internodes up to the first flower (NIF) were counted. The plant height (PH) and first flower height (FFH) were evaluated with the aid of a ruler graduated in centimeters. The diameter of the rootstock (DR) was measured with the aid of a digital caliper. The plants were cut close to the ground when the plants started flowering, which occurred 49 days after grafting, followed by weighing the fresh shoot mass (FM), and the dry shoot mass (DM) was weighed after drying in a forced air circulation oven, set at 60°C for 72 hours.

To meet the assumptions of ANOVA, the data was transformed using the Box-Cox method (Box and Cox, 1964), except for first flower height, number of internodes up to the first flower and diameter of the rootstock. Then, the data was subjected to two-way ANOVA and post-test. The means were compared using the Scott-Knott test at 5% significance, using the AgroEstat statistical software (Barbosa and Maldonado, 2015).

3. Results and Discussion

The percentage of grafting success was evaluated, however, it was not statistically analyzed, since the self-grafted and grafted onto kenaf treatments reached 100% of success for all cultivars, so there was no variance. Okra and kenaf are both malvaceous species but belonging to different genera, which theoretically hamper grafting healing process as physiological, morphological, and botanical discrepancies are expected to occur (Silva *et al*., 2019 b). However, despite the distinguishing characteristics, adequate development was verified for grafted okra seedlings of all cultivars. Thus, all cultivars are compatible with the kenaf rootstock as they present vegetative development similar to the self-grafted and non-grafted seedlings, except for number of leaves, plant height, and first flower height (Table 2). According to Belmonte-Ureña *et al.* (2020), there is compatibility when the plant formed by scion and rootstock has the capacity to develop as a single plant. These results confirm the compatibility with

Table 2 - Analysis of variance and test of comparison of means of vegetative variables of okra cultivars and seedling production modalities

	NL	NI	NIF (cm)	PH (cm)	FFH (mm)	DR (mm)	FM (g)	DM (g)
Cultivar (C)								
Brutus F1	8.13 a	8.40 a	6.13 b	42.47 c	28.53 b	14.40	49.70	9.61 a
Canindé F1	8.67 a	6.66 c	4.33 d	47.93 b	18.40 d	14.35	40.44	7.50 b
Clemson Americano 80	6.00 b	7.06 c	4.86 d	40.40 c	21.80 c	14.46	38.08	7.03 b
Colhe Bem	7.33 a	8.53 a	7.80 a	37.53 d	34.26 a	14.44	45.39	7.96 b
Esmeralda F1	6.40 b	7.60 b	5.40 c	42.27 c	25.33 c	14.44	41.21	8.25 a
Guará F1	6.33 b	6.53 c	4.33 d	72.80 a	28.13 b	14.30	37.23	9.79 a
Santa Cruz 47	6.33 b	8.33 a	7.80 a	35.00 d	33.47 a	14.43	40.63	6.65 b
V8 F1	5.87 b	7.80 b	6.20 b	37.97 d	27.87 b	14.35	41.87	6.82 b
Valença	6.73 b	7.26 c	4.86 d	41.47 c	22.60 c	14.41	45.05	8.75 a
Xingó F1	6.33 b	7.53 b	5.40 c	44.80 b	28.13 b	14.49	51.29	9.37 a
Test F	4.56 **	8.93 **	26.18 **	40.70 **	19.59 **	1.07 NS	0.95 NS	1.98 *
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.3960	0.4873	0.0471
Seedling production (S)								
Non-grafted	8.58 a	7.68	5.80	47.64 a	29.44 a	-	44.27	8744
Self-grafted	5.94 b	7.62	5.82	46.78 a	28.52 a	14.44	43.64	8145
Grafted onto kenaf	5.92 b	7.42	5.52	38.37 b	22.60 b	14.37	41.35	7625
Test F	37.77 **	1.34 NS	1.55 NS	35.62 **	36.27 **	3.81 NS	0.86 NS	1.74 NS
P-value	<0.0001	0.2669	0.2169	<0.0001	<0.0001	0.0543	0.4262	0.1808
Interaction C × S	2.40 **	1.57 NS	1.48 NS	2.53 **	1.58 NS	1.41 NS	1.18 NS	1.46 NS
P-value	0.0027	0.0799	0.1105	0.0015	0.0749	0.1975	0.2884	0.1159
CV (%)	9.47	6.19	16.84	11.20	16.23	1.25	17.21	33.97

NS, **, * not significant or significant at 1 or 5% probability. Data transformed according to the Box-Cox method.
Means followed by the sameletter do not differ by the Scott-Knott test, P<0.005.
NL = number of leaves, NI = number of internodes, NIF = number of internodes up to the first flower, PH = plant height, FFH = first flower height, DR = diameter of the rootstock, FM = fresh shoot mass, DM = dry shoot mass.

okra that, until then, had been demonstrated only for the open-pollinated cultivars ‘Colhe Bem’ and ‘Santa Cruz 47’ (Marin *et al*., 2017; Silva *et al*., 2019 b).

The interaction between cultivar and seedling production modality was significant only for the number of leaves and plant height (Table 2). Thus, the seedling production modality influenced the cultivars equally for most traits, even though they are genetically different. The grafting influenced the number of leaves of the cultivars Brutus, Clemson Americano 80, Esmeralda, Valença, Xingó, and V8, as the non-grafted showed higher average than the other seedling production modalities (self-grafting and grafting onto kenaf) (Table 3). It should be considered that grafted plants are subjected to additional stress that involves the grafting and healing process (Melnyk, 2017). After grafting, to heal the wound, there is callus formation followed by tissue differentiation to reestablish the vascular connections (Xie *et al*., 2019). Thus, it is expected that the non-grafted plants have a faster initial development, which does not necessarily indicate grafting incompatibility.

The cultivars differed as to the number of leaves (Fig. 2). For non-grafted plants, ‘Brutus’and ‘Valença’ had the highest number of leaves. ‘Canindé’ presented the highest number of leaves self-grafted and grafted onto kenaf (Fig. 2). The grafting, either self-grafting or onto kenaf, reduced the number of leaves and plant height of five cultivars (Brutus, Esmeralda, V8, Valença, and Xingó), which may indicate slower initial development due to the stress caused by the grafting procedure.

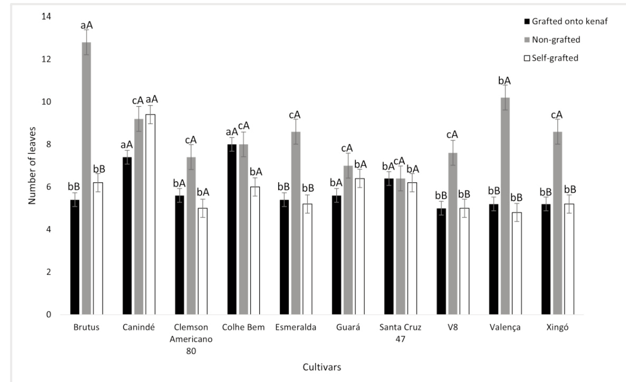

Fig. 2 - Interaction between cultivars and seedling production modality for the number of leaves. Means followed by the same uppercase (between seedling production modalities for each cultivar) and lowercase (between cultivars for each seedling production modality) letter do not differ by the Scott-Knott test (P<0.005).

Higher non-grafted cultivars tended to result in higher plants when grafted, either self-grafted or onto kenaf (Fig. 3). In this sense, ‘Guará’ presented the highest value of plant height in all seedling production modality. ‘Brutus’, ‘Canindé’, ‘Clemson Americano 80’, ‘Guará’, ‘Valença’, and ‘Xingó’ presented shorter plants when grafted onto kenaf (Fig. 3). The plant height should be considered concomitantly with the number and length of internodes (Sandeep *et al*., 2022), as fruit production is directly correlated with number of internodes. Higher plants

Table 3 - Post-analysis of the interaction between cultivars and grafting for the number of leaves of okra cultivars and seedling production

Cultivar	Number of leaves			Test F	P-value
	Non-grafted	Self-grafted	Grafted onto kenaf		
Brutus F1	12.80 aA	6.20 bB	5.40 bB	23.53 **	<0.0001
Canindé F1	9.20 b	9.40 a	7.40 a	2.29 NS	0.1059
Clemson Americano 80	7.40 bA	5.00 bB	5.60 bB	2.92 NS	0.0579
Colhe Bem	8.00 b	6.00 b	8.00 a	1.90 NS	0.1534
Esmeralda F1	8.60 bA	5.20 bB	5.40 bB	6.42 **	0.0022
Guará F1	7.00 b	6.40 b	5.60 b	1.00 NS	0.3708
Santa Cruz 47	6.40 b	6.20 b	6.40 a	0.03 NS	0.9702
V8 F1	7.60 bA	5.00 bB	5.00 bB	4.30 *	0.0158
Valença	10.20 aA	4.80 bB	5.20 bB	15.40 **	<0.0001
Xingó F1	8.60 bA	5.20 bB	5.20 bB	7.31 **	<0.0001
Test F	4.69**	3.34**	2.09*		
P-value	<0.0001	0.0011	0.0353		

The data presented is original, but for statistical analysis, the data were transformed into $(x+0.5)^{1/2}$.

Means followed by the same uppercase (row) and lowercase (column) letter do not differ by the Scott-Knott test (P<0.005).

NS = Not significant; ** Significant at 1% probability; * Significant at 5% probability.

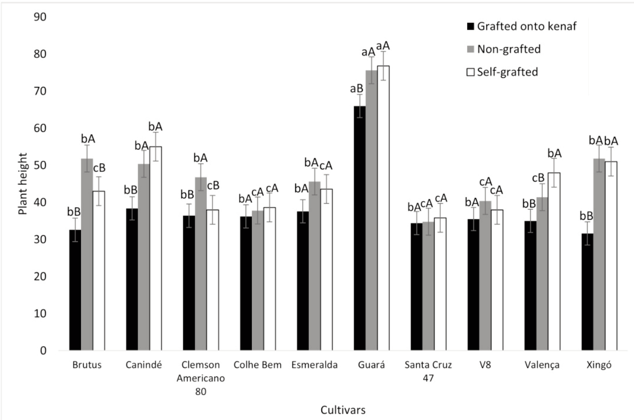

Fig. 3 - Interaction between cultivars and seedling production modality for plant height. Means followed by the same uppercase (between seedling production modalities for each cultivar) and lowercase (between cultivars for each seedling production modality) letter do not differ by the Scott-Knott test (P<0.005).

with long internodes are not ideal, as the plant will probably reach a stature that will hamper harvesting the fruits sooner.

The genotypic variability and the differential behavior as for the seedling production modalities can be confirmed from the analysis of variance and the mean test (Table 2). Significant differences between cultivars were detected for the variables number of internodes, number of internodes to first flower, first flower height, and scion diameter. Furthermore, the seedling production modalities differed as to first flower height and scion diameter.

The cultivars were grouped into two groups for the variables scion diameter and dry shoot mass, into three groups for the variable number of internodes, and into four groups for the variables number of internodes up to the first flower and first flower height. There were no significant differences for the variables diameter of the middle portion of the plant, rootstock diameter and fresh shoot mass.

The cultivars Colhe Bem, Brutus, and Santa Cruz 47 had higher number of internodes. The cultivars Colhe Bem and Santa Cruz 47 had higher NIF; The cultivars Colhe Bem and Santa Cruz 47 had the highest first flower height; the cultivars Xingó, Colhe Bem, Esmeralda, V8, Valença, Guará, and Brutus had higher scion diameter and the cultivars Guará, Brutus, Xingó, Valença, and Esmeralda had higher dry shoot mass.

As for the factor seedling production modality, besides number of leaves and plant height, differences were detected for the first flower height and scion diameter, with smaller and larger means for grafting onto kenaf, respectively. This indicates that most variables are not affected by grafting. No differences were detected for number of internodes to first flower, which suggests that the first flower insertion height differed due to internode length of plants grafted onto kenaf. The yield potential of okra depends considerably on the number of nodes per plant as the inflorescence consists of a single flower (Bhatt and Rao, 2009), so the smallest first flower height insertion can be advantageous as production starts lower.

The scion diameter was greater in plants grafted onto kenaf than self-grafted plants. This differs from the findings reported by Andrade *et al*. (2020), who verified largest diameters for self-grafted 'Santa Cruz 47' plants compared to roselle (*Hibiscus sabdariffa* L.) and other malvaceous rootstocks. The scion diameter depends greatly on the water and nutrients translocation, which can be influenced by the compatibility between rootstock and scion. Silva *et al.* (2019 b) previously reported that kenaf is a better rootstock for okra as it presents greater compatibility compared to other malvaceous genotypes. This result may be associated to the large root volume of kenaf plants (Alexopoulou *et al*., 2013), which improve water and nutrient absorption (Gaion *et al*., 2017).

Okra grafting is a potential tool to cope with root-knot nematodes, which are one of the main phytosanitary issues in okra cropping. Although most okra farmers employ little technology, we anticipate that grafted seedlings will be a feasible option as grafting will become cheaper when this process is automatized (Silva *et al*., 2019 b). Furthermore, grafting onto resistant rootstocks is a sustainable strategy that contributes to lessen pesticides use, which are potentially damaging to the environment and human's health (Thies, 2021).

The cultivars Brutus, Canindé, Clemson Americano 80, Colhe Bem, Esmeralda, Guará, Santa Cruz 47, V8, Valença, and Xingó are compatible with the kenaf rootstock, therefore the grafting can be used in okra as a strategy to control root-knot nematodes in all analyzed cultivars. Although it is an intergeneric grafting combination, the grafted seedlings showed no sign of incompatibility until flowering. However, further studies are necessary to confirm compatibility throughout the cycle. Furthermore, the agronomic performance should be evaluated under field conditions to validate the use of this technique in the okra crop.

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