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cunhajunior.l.c@ufg.br

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Laurimar do Nascimento Andrade, Francisco; Ferreira Monteiro, Silvia Marcela;  
Santana Muniz, Carla Caroline; Fazzi Gomes, Rafaelle; da Silva Santos, Lucas  
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# Compatibility and yield of ‘Santa Cruz 47’ okra onto rootstocks of the Malvaceae family<sup>1</sup>

Francisco Laurimar do Nascimento Andrade<sup>2</sup>, Silvia Marcela Ferreira Monteiro<sup>2</sup>,  
Carla Caroline Santana Muniz<sup>2</sup>, Rafaelle Fazzi Gomes<sup>2</sup>, Lucas da Silva Santos<sup>3</sup>

## ABSTRACT

The little-known use of okra grafting, mainly to incorporate nematode resistance, requires evaluation of interspecific compatibilities. This study aimed to determine the compatibility of the okra ‘Santa Cruz 47’ cultivar grafted onto different rootstocks of the Malvaceae family. The research was divided into two experimental stages, with the treatments consisting of non-grafted, self-grafted or grafted okra plants onto rootstocks of mallow, roselle and pima cotton. In the first stage, a completely randomized design was used, with four replications and seedlings grown in a humidity chamber. In the second stage, the seedlings were transplanted to a greenhouse and a randomized block design was used, with five replications. The results of the first stage demonstrated that the self-grafting provided the best results for the growth characteristics assessed, except for the diameter of the grafted region. There was no difference among the treatments for the graft survival rate. In the second stage, the use of roselle as a rootstock enabled an adequate vegetative development, but did not differ from the treatments of self-grafting and mallow rootstock for the graft survival rate. The treatments did not differ for fruit diameter, length and average mass. Grafts onto roselle were the most precocious ones, with estimated means for production per plant (478.75 g), commercial yield (10.07 t ha<sup>-1</sup>) and total yield (10.64 t ha<sup>-1</sup>) similar to those observed for self-grafted and non-grafted plants. Hence, among the assessed rootstocks, roselle was identified as the most promising one for grafting with okra.

KEYWORDS: *Abelmoschus esculentus*, grafting, heterografts.

## RESUMO

Compatibilidade e produção de quiabeiro ‘Santa Cruz 47’ sobre porta-enxertos da família Malvaceae

O uso pouco difundido da enxertia em quiabeiro, principalmente para incorporar resistência a nematoides, requer avaliação de compatibilidades interespecíficas. Objetivou-se determinar a compatibilidade do quiabeiro ‘Santa Cruz 47’ em diferentes porta-enxertos da família Malvaceae. A pesquisa foi dividida em duas etapas experimentais, sendo os tratamentos constituídos por plantas de quiabeiro pé-franco, autoenxertadas ou enxertadas sobre porta-enxertos de malva, vinagreira e algodoeiro arbóreo. Para a primeira etapa, adotou-se o delineamento inteiramente casualizado, com quatro repetições e mudas conduzidas em câmara úmida. Na segunda etapa, as mudas foram transplantadas para casa-de-vegetação, empregando-se o delineamento em blocos ao acaso, com cinco repetições. Os resultados da primeira etapa mostraram que a autoenxertia proporcionou melhor desempenho nas características de crescimento avaliadas, exceto para o diâmetro da região enxertada. Não houve diferença entre os tratamentos para a porcentagem de enxertos vivos. Na segunda etapa, o uso da vinagreira como porta-enxerto possibilitou adequado desenvolvimento vegetativo, mas não diferiu dos tratamentos autoenxertia e porta-enxerto de malva para a porcentagem de enxertos vivos. Os tratamentos não diferiram em diâmetro, comprimento e massa média de frutos. As enxertias em vinagreira foram as mais precoces, com médias estimadas de produção por planta (478,75 g), produtividade comercial (10,07 t ha<sup>-1</sup>) e produtividade total (10,64 t ha<sup>-1</sup>) similares às obtidas para autoenxertia e pé-franco. Logo, entre os porta-enxertos avaliados, a vinagreira foi identificada como o mais promissor para enxertia com quiabeiro.

PALAVRAS-CHAVE: *Abelmoschus esculentus*, enxertia, heteroenxertos.

## INTRODUCTION

Okra [*Abelmoschus esculentus* (L.) Moench] is an annual vegetable that can be grown in tropical and subtropical regions (Oliveira et al.

2007). Nematode species in these regions have a high reproductive rate, damaging susceptible okra crops and causing yield losses (Mukhtar et al. 2014). The okra susceptibility to nematodes allows soil populations to increase, precluding the

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2. Universidade Federal Rural da Amazônia, Capanema, PA, Brasil. E-mail/ORCID: franlaurimar@gmail.com/0000-0003-2709-4302, maar Monteiro@gmail.com/0000-0002-5087-0560, carolinesantana23@yahoo.com.br/0000-0001-9668-8986, rafaelle.fazzi@yahoo.com.br/0000-0001-8242-8104.

3. Fundação Universidade Federal de Rondônia, Departamento de Agronomia, Rolim de Moura, RO, Brasil. E-mail/ORCID: lucasmelhorista@gmail.com/0000-0002-2261-3962.

cultivation of this crop in infected areas (Oliveira et al. 2007).

In Brazil, the low availability of okra cultivars due to poor investment in breeding programs for the species (Silva 2016) restricts the crop production to certain regions, because of its limited adaptability to soil and climate conditions and/or susceptibility of the few varieties to *Meloidogyne* spp. (Filgueira 2008).

*Meloidogyne* is a major problem in okra, causing the emergence of root hyperplasia, commonly known as galls, which damages the vascular tissue responsible for transporting sap, leading to delayed growth and yield losses (Daramola et al. 2015). In order to overcome problems related to the root-knot nematode attack in okra, grafting may be an alternative to incorporate resistance via rootstock with affinity, such as with roselle (Silva et al. 2019).

According to Fallik & Ilic (2014), the rootstock-scion compatibility of genotypes is important, because an inadequate graft union between the plants may affect the final production and compromise the fruit quality. The perfect healing of the graft union is closely linked to compatibility, which, as reported by Silva et al. (2019), is related to taxonomic proximity, whereby the more closely related the plants, the greater the chance of a successful graft.

According to Mudge et al. (2009), intraspecific grafts exhibit a higher degree of affinity between rootstock and scion than interspecific grafts. However, Lee et al. (2010) reported that using rootstocks from different botanical species is sometimes preferable, because it allows the genetic diversity within a family to be exploited by associating species with desirable characteristics.

Grafting has a great potential for okra plants, but research in the area is scarce, because okra is less economically important to Brazil than other fruiting vegetables. Ito et al. (2009a) found that the ‘Colhe Bem’ and ‘Santa Cruz 47’ okra cultivars are susceptible to *Meloidogyne incognita*, whereas representatives of the Malvaceae family are resistant to the pathogen, making them suitable as rootstock. In studies conducted by Marin et al. (2017) and Silva et al. (2019), the roselle rootstock provided a greater protection for okra against nematodes, as well as a good plant development, indicating graft compatibility.

Given the difficulties involved in growing okra, particularly concerning the low yield and

incidence of root-knot nematodes, this study aimed to assess the compatibility of the ‘Santa Cruz 47’ okra cultivar grafted onto different rootstocks of the Malvaceae family.

## MATERIAL AND METHODS

The study was carried out in two experimental stages, the first involving seedlings and the second in the field, at the Igarapé-Açu farm school (01°07’48.47”S, 47°36’45.31”W and altitude of 54 m) of the Universidade Federal Rural da Amazônia (Pará state, Brazil), from September 2018 to February 2019. The climate in the region is humid megathermal (Ami), according to the Köppen’s classification (Alvares et al. 2013).

The first experimental stage involved assessing the compatibility of ‘Santa Cruz 47’ okra scions (Feltrin®) grafted onto four Malvaceae rootstocks: roselle (*Hibiscus sabdariffa* L.), cotton (*Gossypium barbadensis* L.), mallow (*Urena lobata* L.), self-grafting (okra grafted onto okra) and control (non-grafted okra plants). The choice of the okra cultivar was due to its wide use in Brazil and its susceptibility to root-knot nematodes. A completely randomized design was used, with four replications and each plot consisting of ten plants.

The scion, rootstock and non-grafted okra seedlings were grown in 200-cell expanded polystyrene trays filled with commercial substrate (Tropstrato®; two seeds per cell) and subsequently thinned.

The scions were planted at 16 days after the mallow and roselle rootstocks and four days after the cotton rootstocks, to ensure similar diameters at the moment of grafting. Seeds for the non-grafted okra treatment were planted at the same time as the scions. After planting, the trays were placed in a greenhouse covered with 50 % shade cloth and watered twice a day.

For grafting purposes, the seedlings were transplanted into 100 mL plastic cups filled with Tropstrato® commercial substrate. Mallow and roselle seedlings were transplanted about 25 days after sowing and cotton seedlings at nine days after sowing. Grafting was carried out at three days after transplanting.

The cleft grafting technique was used, whereby the stem of the rootstock is cut straight across (cross-section) and a split or “cleft” is then cut into the center of the stock. The base of the scion is cut in a wedge

shape and then inserted into the cleft with the graft fixed in place using silicone clips. A standard stem length of 3.0 cm was used for both the scion and rootstock, with 60 grafts performed per treatment.

After grafting, the seedlings were kept in a humidity chamber (2.0 m long, 1.0 m wide and 1.0 m high) for ten days, until the grafts had taken. One centimeter of water was placed in the humidity chamber, which was then covered with transparent plastic film. The plants were assessed at ten days after grafting by removing ten samples per plot for both the grafted and non-grafted plants.

The temperature inside the humidity chamber was measured twice a day (9 a.m. and 4 p.m.), using an LM35 temperature sensor linked to the Arduino software. The chamber was opened once a day to allow the seedlings to acclimatize to the external environment.

The variables assessed in the first experimental stage were plant height (cm), number of leaves, rootstock stem diameter (mm), scion stem diameter (mm), diameter of the graft region (mm) and graft survival rate (%). Only the first three characteristics were evaluated in non-grafted plants.

After healing and acclimatization, which occurred up to 10 days after grafting, the seedlings of the respective treatments were transferred to an arched roof greenhouse (51.0 m long, 14.0 m wide and 3.5 m high), covered with 150 micra transparent polyethylene film and 50 % shade cloth on the sides, for the second stage of the experiment.

To that end, a randomized block design was used, with five treatments, five replications and four plants in each plot. The treatments consisted of grafting the 'Santa Cruz 47' okra cultivar (scion) onto roselle, mallow and cotton rootstocks, as well as self- and non-grafted plants.

The plants were grown in a greenhouse, in a sandy-textured Yellow Dystrophic Argisol. The topsoil (0-20 cm) analysis identified the following characteristics: pH ( $H_2O$ ) = 3.95; P = 4.0 mg  $dm^{-3}$ ;  $K^+$  = 0.55 mmol  $dm^{-3}$ ;  $Ca^{+2}$  = 5.8 mmol  $dm^{-3}$ ;  $Mg^{+2}$  = 3.0 mmol  $dm^{-3}$ ;  $Al^+$  = 5.4 mmol  $dm^{-3}$ ; H + Al = 46.5 mmol  $dm^{-3}$ ; SB = 9.35 mmol  $dm^{-3}$ ; CEC = 56.3 mmol  $dm^{-3}$ ; V (%) = 17.31.

Liming was carried out at six weeks before transplanting, to raise the base saturation to 70 %. Liming, fertilization at planting and topdressing were performed in line with the recommendations of Passos et al. (2014). Plants were spaced 0.45 m

apart, with 1.0 m between rows. Drip irrigation was applied via self-compensating hoses equipped with emitters spaced 15 cm apart, at a flow rate of 2 L  $h^{-1}$ .

Harvest began at 41 days after transplanting, with fruits being harvested between 9 cm and 12 cm in length (CEAGESP 2018), due to their low fiber content. A total of 27 harvests were performed, with around three days between them, to enable the standardization of the collected fruits.

The growth characteristics assessed in the second stage were plant height (cm), rootstock stem diameter (mm), scion stem diameter (mm), diameter of the graft region (mm) and graft survival rate (%). Only plant height and stem diameter were evaluated in non-grafted plants. The assessments were conducted at 15 and 30 days after transplanting.

The yield traits evaluated were precocity (days), fruit diameter (mm), fruit length (cm), average fruit weight (g fruit $^{-1}$ ), production per plant (g plant $^{-1}$ ), estimated commercial yield (t ha $^{-1}$ ) and estimated total yield (t ha $^{-1}$ ). Statistical analysis consisted of analysis of variance and means were compared by the Tukey test at 5 % of probability, using the AgroEstat statistical software (Barbosa & Maldonado Júnior 2015).

## RESULTS AND DISCUSSION

During the graft-take period, there were no sudden variations in temperature inside the humidity chamber (Figure 1) that might compromise the grafting success. The average temperature ranged from 29.38 °C at 9 a.m. to 31.17 °C at 4 p.m., higher than the ideal temperatures recommended by Goto et

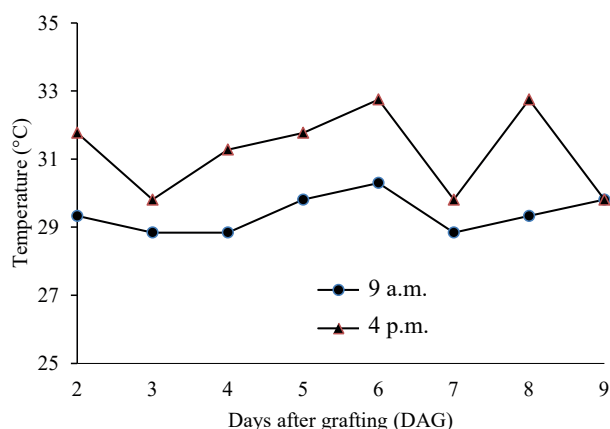


Figure 1. Air temperature at 9 a.m. and 4 p.m. inside the humidity chamber, during the graft-take period.

al. (2003) to keep vegetable grafts alive. Successful grafting may be due to okra being a tropical climate species.

Self-grafting resulted in taller plants (Table 1). This rapid response in vegetative development is related to the healing process, due to the similarity between the vascular tissues.

Thus, the initial positive results in seedling growth may indicate that the energy expenditure occurred primarily during the wound healing, since, in some cases, hormonal signals may be involved in tissue regeneration (Goldschmidt 2014). As such, self-grafted plants may be more predisposed to vascular reconnection, resulting in less energy expenditure, because the metabolic and taxonomic similarities between the grafted parts facilitate the cellular interaction (Pina et al. 2012).

In addition to indicating the potential failure of vascular reconnection, a decline in seedling size at the initial days after grafting may be associated with the stress caused by wounding. About this, Simões et al. (2014) reported that recently grafted seedlings may initially exhibit a delayed growth due to the rupture of xylem vessels, exposing them to stress until a successful reconnection is achieved, in the event of plant compatibility.

For the number of leaves, the highest results per seedling were obtained in the roselle and self-grafting treatments, but did not differ from the non-grafted plants (Table 1). As such, this characteristic directly influences the healing process, since leaves are vital to photosynthesis, which provides the energy needed for tissue regeneration.

According to Martinez-Balesta et al. (2010), the rootstock-scion interaction is directly related to bud differentiation into new leaves, whereby healing

difficulties disrupt the plant physiology and limit the normal growth, preventing the emergence of functional leaves.

Regarding the rootstock stem diameter, there was no significant difference between the means for self-grafting and the cotton treatment (Table 1). The higher values recorded for these rootstocks are related to the period before grafting, when their stems were naturally thicker than the others.

For the scion stem diameter, self-grafting produced the largest diameters (Table 1), likely due to a better translocation of water and nutrients (Martinez-Balesta et al. 2010). The same authors also reported that the connection formed between the scion and rootstock allows the establishment of a hydraulic architecture and successful water transport to the shoot, controlling processes that are vital to scion growth and development, such as mineral nutrition, photosynthesis and respiration.

Regarding the diameter of the graft region, only the cotton rootstock treatment displayed large diameters (Table 1). According to Pina & Errea (2005), the increased diameter of the graft region, also known as graft callus, occurs in both compatible and incompatible plants, as a result of the mechanical damage inflicted at the moment of grafting.

There was no significant difference between treatments for the graft survival rate (Table 1), with similar means for all treatments (93.75 %). Comparable results were obtained by Marin et al. (2017), who found no difference between self-grafting and cotton or roselle genotypes for this variable, in the initial days after grafting onto okra.

Table 2 presents the results obtained in the field phase. For plant height, self- and non-grafting resulted in a better plant development in both the

Table 1. Means<sup>1</sup> for plant height, number of leaves, rootstock stem diameter, scion stem diameter, diameter of the graft region and graft survival rate in okra (*Abelmoschus esculentus*) grafted onto different rootstocks, in the seedling phase.

Treatment	Plant height (cm)	Number of leaves	Rootstock stem diameter (mm)	Scion stem diameter (mm)	Diameter of the graft region (mm)	Graft survival (%)
Mallow	6.34 c	2.47 c	1.09 d	1.69 b	2.38 d	85.00 a
Roselle	7.58 b	3.49 a	1.37 c	1.70 b	2.85 c	95.00 a
Cotton	6.51 c	3.08 b	1.96 a	1.62 b	3.79 a	97.50 a
Self-grafting	9.61 a	3.53 a	2.00 a	1.86 a	3.14 b	97.50 a
Non-grafted plants	6.87 c	3.23 ab	1.81 b	-	-	-
F Test	114.24**	23.40**	439.19**	13.98**	101.38**	3.09 <sup>ns</sup>
CV (%)	3.37	5.61	2.31	3.06	3.83	7.22

<sup>1</sup> Means followed by the same letter in the columns do not differ by the Tukey test at 5 % of probability; \*\* significant values by the F-test at 1 % of probability; <sup>ns</sup> not significant value at 5 % of probability; CV: coefficient of variation (%).



Table 2. Means<sup>1</sup> for plant height and rootstock stem diameter, at 15 and 30 days after transplanting (DAT), in okra (*Abelmoschus esculentus*) grafted onto different rootstocks and grown under greenhouse conditions.

Treatment	Plant height (cm)	Rootstock stem diameter (mm)	Plant height (cm)	Rootstock stem diameter (mm)
	15 DAT		30 DAT	
Mallow	8.79 c	1.99 c	15.43 c	4.66 c
Roselle	15.05 b	3.42 b	30.62 b	8.69 b
Cotton	8.02 c	2.06 c	-	-
Self-grafting	17.62 a	3.95 ab	37.59 a	12.12 a
Non-grafted plants	18.06 a	4.15 a	41.05 a	12.69 a
F Test	214.94**	46.13**	97.32**	64.83**
CV (%)	5.42	10.88	8.26	10.79

<sup>1</sup> Means followed by the same letter in the columns do not differ by the Tukey test at 5 %; \*\* significant values by the F-test at 1 % of probability; CV: coefficient of variation (%).

assessments. The satisfactory results observed for self-grafting may be attributed to the rapid graft-take of seedlings while still in the humidity chamber, which favored the development in the greenhouse due to the adequate root-shoot ratio.

The variables rootstock stem diameter and plant height behaved similarly, with statistically superior results for self- and non-grafted plants, in relation to the other treatments, at both the assessments (Table 2). This is due to the greater vigor of okra plants, when compared to the other genotypes used, since it is a genetically improved cultivar.

By contrast, there was no difference for scion stem diameter between the roselle and self-grafting treatments at either assessment (Table 3), indicating a proper translocation in the graft region. This is likely due to a satisfactory cell rearrangement, since a poor vascular reconnection disrupts the upward and downward plant transfer routes (Goldschmidt 2014).

The initial decline in the rootstock stem diameter for mallow during healing, combined with

its different genus from okra, may have contributed to slow the scion development in the greenhouse (Table 3). Peil (2003) reports that a complete vascular reconnection requires similar stem diameters, what suggests an adequate morphological and anatomical affinity.

The best results for the graft region diameter were recorded for the roselle rootstock (Table 3) at both times (15 and 30 days after transplanting). According to Rodrigues et al. (2004), this variable directly influences the graft union, and a significant increase in diameter at the graft junction may represent a correct lignification. Ito et al. (2009b) also studied different rootstocks for net melon and found that plants with a high graft region diameter exhibit a better graft union, due to intense cell proliferation.

With respect to the graft survival rate, the roselle rootstock performed better (100 %), but did not differ from the mallow or self-grafting treatments (Table 3), what supports the notion that okra is compatible with the rootstocks used, except for cotton.

Table 3. Means<sup>1</sup> for scion stem diameter, diameter of the graft region and graft survival rate, at 15 and 30 days after transplanting (DAT), in okra (*Abelmoschus esculentus*) grafted onto different rootstocks and grown under greenhouse conditions.

Treatment	Scion stem diameter (mm)	Diameter of the graft region (mm)	Graft survival rate (%)	Scion stem diameter (mm)	Diameter of the graft region (mm)	Graft survival rate (%)
	15 DAT			30 DAT		
Mallow	2.68 b	4.48 b	95.00 ab	5.75 b	8.51 c	95.00 a
Roselle	4.17 a	6.20 a	100.00 a	12.07 a	15.83 a	100.00 a
Cotton	1.65 c	4.73 b	75.00 b	-	-	-
Self-grafting	3.69 a	4.88 b	95.00 ab	11.32 a	12.61 b	95.00 a
F Test	61.65**	11.24**	3.58*	34.83**	31.22**	0.44 <sup>ns</sup>
CV (%)	10.47	10.12	14.37	13.45	11.91	10.02

<sup>1</sup> Means followed by the same letter in the columns do not differ by the Tukey test at 5 % of probability; \*\* significant values by the F-test at 1 % of probability; <sup>ns</sup> not significant value at 5 % of probability; CV: coefficient of variation (%).

An important result observed is that, after the first assessment (15 days after transplanting), all the plants grafted onto cotton began to wilt and ultimately died (Table 3). Thus, although cotton and okra belong to the same botanic family, and they had similar stem diameters, the tissue regeneration was not achieved, suggesting incompatibility. Similar findings were reported by Silva et al. (2019), who observed all the cotton genotypes grafted on okra exhibiting a complete incompatibility at five days after transplanting. Marin et al. (2017) also noticed signs of incompatibility between cotton rootstocks and okra, with low vegetation indices.

According to Martinez-Balesta et al. (2010), the morphology of the vascular system changes after grafting and during the healing process. Gainza et al. (2015) found that a successful graft union requires species to be closely related; otherwise, characteristic symptoms of incompatibility will emerge. Thus, the results may be associated with the greater relationship between okra, formerly classified as *Hibiscus esculentus* (Bazán 2006), and roselle, that belongs to the *Hibiscus* genus; what is not the case for *Gossypium* (cotton) and *Urena* (mallow). To some extent, the taxonomic proximity confirms the results obtained for roselle, corroborating Peil (2003), who related it to possible morphophysiological similarities between plants.

Concerning the final agronomic traits, significant differences ( $p < 0.05$ ) were observed for precocity, production per plant and for estimated commercial and total yields (Table 4). The roselle treatment resulted in the most precocious plants, although not differing statistically ( $p > 0.05$ ) of non-grafted plants. This is associated with the fast healing of the roselle-okra graft in the seedling phase. According to Gisbert et al. (2011), the rootstock-scion compatibility enables

a fast development of the grafted plants, definitively influencing precocity and providing a short-term return on production investments.

There were no differences ( $p > 0.05$ ) among the treatments for fruit diameter, length and average weight (Table 4), demonstrating that the fruit development traits behaved similarly, regardless of grafting; i.e., the different rootstocks did not affect these variables. These findings confirm that the rootstocks used enabled a similar translocation of photoassimilates by the plants.

Costa et al. (2014) studied the effect of different rootstocks on yield, compatibility and phenology of chili pepper and found no difference for fruit diameter, length or average weight, when compared to non-grafted plants.

Regarding the production per plant, higher mean values were recorded for roselle, self- and non-grafted plants (Table 4). The similar production means for roselle (almost 500 g plant<sup>-1</sup>), in relation to the self- and non-grafted treatments, indicate its significant potential as a rootstock for farmers, enabling a continued production throughout the growth cycle.

For the estimated commercial and total yield, again, roselle, self-grafting and non-grafted treatments did not differ ( $p > 0.05$ ) from each other and resulted in the highest means. Similar results for total yield were reported by Gaion et al. (2017), assessing net melon grafted onto Cucurbitaceae rootstocks, who recorded higher values for the 'Caipira' cucumber cultivar, self-grafted and non-grafted plants.

The results confirm that grafting was successful for okra, when compared to the non-grafting treatment. Additionally, the fact that roselle displayed no signs of incompatibility or stress due to wounding

Table 4. Means<sup>1</sup> for fruit precocity, diameter, length and weight, production per plant and estimated commercial yield and total yield of okra (*Abelmoschus esculentus*) grafted onto different rootstocks and grown under greenhouse conditions.

Treatment	Precocity (days)	Fruit diameter (mm)	Fruit length (cm)	Fruit weight (g)	Production per plant (g)	Commercial yield (t ha <sup>-1</sup> )	Total yield (t ha <sup>-1</sup> )
Mallow	88.08 a	14.67 a	11.44 a	13.56 a	294.92 b	6.14 b	6.55 b
Roselle	69.80 b	14.39 a	11.02 a	13.78 a	478.75 ab	10.07 ab	10.64 ab
Self-grafting	83.35 a	14.35 a	10.78 a	12.69 a	618.75 a	13.01 a	13.75 a
Non-grafted plants	80.20 ab	14.55 a	10.91 a	12.83 a	676.50 a	14.07 a	15.03 a
F Test	6.06**	0.42 <sup>ns</sup>	2.68 <sup>ns</sup>	1.95 <sup>ns</sup>	8.26**	7.28**	8.25**
CV (%)	8.76	3.56	3.51	6.48	25.55	27.22	25.56

<sup>1</sup> Means followed by the same letter in the columns do not differ by the Tukey test at 5 % of probability; \*\* significant values by the F-test at 1 % of probability; <sup>ns</sup> not significant values at 5 % of probability; CV: coefficient of variation (%).

at grafting enabled satisfactory gains throughout the growth cycle. Thus, it can be inferred that roselle does not affect the okra fruit production, when used as a rootstock.

## CONCLUSIONS

1. Roselle is compatible as a rootstock for 'Santa Cruz 47' okra, exhibiting similar vegetative and production characteristics to self- and non-grafted plants;
2. Mallow is compatible for grafting onto 'Santa Cruz 47' okra, but results in a slow vegetative development, which negatively affects precocity, making it unsuitable as a rootstock, in terms of obtaining short-term financial returns;
3. Cotton plants of *Gossypium barbadensis* showed to be incompatible rootstocks for grafting with okra.

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