

## Behavior of grafted orange varieties in different rootstocks in Xalostoc, Morelos

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### Abstract

Citrus groves in Morelos are recent and expanding; however, present technologies are scarce. There are problems due to pathogen attacks and therefore insufficient productivity, so it is necessary to improve the production and profitability of the crop using varieties with better behavior, that have an optimal vegetative development, production and quality of the fruit. The research was carried out during 2016, with the aim of evaluating the behavior and development of four varieties of orange grafted in different patterns, under the edaphoclimatic conditions of East of Morelos. Sixteen rootstock-variety combinations, planted at a planting distance of 7 x 4 m, were compared. Agronomic variables were evaluated: stem diameter and plant height, flowering and fruiting, relative chlorophyll content, height and crown diameter. The Volkamerian rootstock combined with the Mars variety reached the largest stem diameter without differences with the other varieties. When the Jaffa, Hamlin and Mars varieties were used on this pattern, the trees with the highest growth as well as the highest height and diameter of the crown were obtained. The Jaffa variety on Volkamerian had the highest number of flowers and fruits, as well as the highest leaf chlorophyll content. The smallest, least leafy trees with thin trunks and the least number of flowers and fruits were those achieved on the Naranja Agrio pattern.

**Keywords:** citrus, pattern, variety.

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## Introduction

Citrus farming is an important economic activity in national fruit growing. The main citrus fruits that are produced are: orange (*Citrus sinensis* (L.) Osbeck), Mexican lemon (*Citrus aurantifolia* (Christm) Swingle), Persian lime (*Citrus latifolia* Tanaka) and mandarin (*Citrus reticulata* Blanco). In 2016, the established area with citrus was 555 330.82 ha, which produced 4 586 245 t of lemon, orange and grapefruit fruit SIAP (2018). Mexico is in fifth place in citrus production worldwide and in the case of limes and lemons, it remains the second producer (FAOSTAT, 2016).

Citrus grows in the tropical and subtropical regions of the world. In our country, the citrus agribusiness represents one of the most important, generating an economic spillover of more than 375 million dollars (SIAP, 2018). Genetic improvement of this crop constitutes a routine activity that is carried out in several countries with the aim of improving the quality of the fruit or achieving tolerance to biotic and abiotic stress (Tozlu *et al.*, 1999; Mendoza *et al.*, 2001; Machado *et al.*, 2011; Omura and Shimada, 2016).

The state of Morelos registered 620.8 ha of citrus in 2016, of which Persian lime and Valencia orange occupied 60 and 30% of the total area, respectively, while the remaining 10% was occupied by Mexican lemon, tangerine, grapefruit and lime. The orange and Persian lime producing areas in the state are located in the municipalities of Coatlan del Río, Tlaquiltenango, Jojutla, Zacatepec, Jantetelco, Jonacatepec, Puente de Ixtla, Tepalcingo, Tlaltizapan and Ayala (SIAP, 2018).

In citrus farming, rootstocks are used for their ability to modify fruit quality (Dubey and Sharma, 2016) because they can increase fruit yield (Georgiou and Gregoriou, 1999), crown height (Mademba *et al.*, 2012) the nutrient content in leaves (Ahmed and Al, 1984) and can provide tolerance to pests and diseases (Castle *et al.*, 1993). Most of these rootstocks are apomictic, so if it is required to maintain genetic homogeneity, this condition may be advantageous as a clonal multiplication process.

In this way, uniform plants can be produced from seeds at low cost (Khan and Kender, 2007). Until 2005, the main rootstock used was the Naranja Agrío, because it is susceptible to the citrus sadness virus, the use of rootstocks tolerant to this disease was promoted. The most commonly used are Lemon Volkamerian, Citrumelo Swingle and Citrange Carrizo, among others (Cruz, 2006).

The grafting pattern can confer tolerance or resistance to fungal attacks or other aspects that can compromise free standing crops (Loussert, 1992). The correct choice of a pattern is of utmost importance, since it affects the good development of the crop, also having direct influence on the adaptability to the soil and the climate, vigor, fruit quality, production and behavior to different diseases (Gardiazabal and Rosenberg, 1991).

In addition, the use of rootstocks gives cultivars important characteristics such as internal and external quality of the fruits and anticipation or delay in ripening, which is why, in the different citrus regions, the constant search for appropriate rootstocks is required (Palacios, 2005). There

are some reports made by Garza *et al.* (2003), on the evaluation of orange cultivars on different rootstocks in Colima, Veracruz by Curti *et al.* (1998) and in Persian lemon by (Curti *et al.*, 2000, 2012).

Al *et al.* (2005) pointed out that the quality of the fruit depends on the rootstock used, obtaining as a result that the Volkamerian and Naranjo Agrio rootstocks registered the highest fruit weight from 149 to 185 g, fruit diameter from 6.30 to 6.67 cm and thickness of shell from 4.28 to 4.93 mm. In studies by Arbeu *et al.* (2016) on the quality of Persian lime fruit in different rootstocks in the state of Veracruz, reported that the rootstock/variety interaction modified the physical characteristics of the fruit in the variables fruit mass, fruit diameter, peel thickness and firmness of fruit, finding the least solidity of the same when the Naranjo Agrio was used.

They also reported that the percentage of export fruit on Volkamerian was over 80%, recommending that producers have three or more rootstocks on their land and that they gradually replace the Naranjo Agrio. On the other hand, Ahmed *et al.* (2018) obtained on Volkamerian good height and extension of the crown of the grafted plants. Regarding the quality and performance of the fruit, the results were exceptionally good, reaching an average fruit weight of 163.33 g.

In Morelos, basic information has been generated on the phenology of Persian lemon and Valencia orange (Alia *et al.*, 2011), but not from other rootstocks and citrus varieties such as: Amblycarpa, Citrange C-35, Volkamerian and Naranjo Agrio. And the orange varieties: Campbell, Hamlin, Jaffa and Mars. Most of the citrus fruits in the state are established on Volkamerian Lemon (*C. volkameriana* Ten. & Pasq), and some on sour Orange (*Citrus aurantium* L.) and Cleopatra mandarin (Lugo *et al.*, 2009).

It is assumed that there is a poor diversity of rootstock-varieties, so there is great concern on the part of researchers. The objective of the work consisted in evaluating the behavior and development of four varieties of orange grafted in different combinations of rootstocks in the edaphoclimatic conditions of East of Morelos.

## Materials and methods

### Geographic location

The study was carried out in the Experimental Field of the School of Higher Studies of Xalostoc (EESuX) of the Autonomous University of the State of Morelos (UAEM), Parque Industrial Cuautla, Cd. De Ayala, Morelos, during the year 2016. 6 000 m<sup>2</sup>, planted on a sandy-clay crumb soil, with a pH of 7.03, located at the geographical coordinates of 18° 49' north latitude and 99° 01' west longitude, at an altitude of 1 330 meters above sea level, with a warm climate prevailing subhumid (INEGI, 2009).

## Vegetal material

The rootstocks and varieties were obtained from the certified nursery ‘Cazones’, located in Cazones de Herrera, Veracruz. 149 one-year-old plants were purchased after grafting. The rootstocks used were: Amblycarpa (*Citrus amblycarpa* Ochse), Naranja Agrio (*Citrus aurantium* L.), Limon Volkamerian (*Citrus volkameriana* Pasq) and Citrange C-35 (*Poncirus trifoliata* [L.] Raf. X *Citrus sinensis* L.), with the four varieties of orange ‘Campbell’, ‘Hamlin’, ‘Mars’ and ‘Jaffa’. Sixteen rootstock-variety combinations were made (Table 1).

**Table 1. Relationship of the rootstock-variety of citrus fruits and their combinations.**

No. of combinations	Rootstock	Variety	No. of plants
1	Amblycarpa	Campbell	10
2		Hamlin	10
3		Jaffa	10
4		Mars	7
5	Volkamerian	Campbell	10
6		Hamlin	10
7		Jaffa	10
8		Mars	9
9	Citrange C-35	Campbell	10
10		Hamlin	10
11		Jaffa	9
12		Mars	9
13	Naranja Agrio	Campbell	9
14		Hamlin	9
15		Jaffa	8
16		Mars	9
Total			149

The plants were certified by the Xalapa High Technology Laboratory, SC (LATEX) registered with SENASICA in Xalapa, Veracruz, they were free of the citrus sadness virus (VTC). They were transferred to the experiment area and kept in a greenhouse to be acclimatized until the transplant.

## Plantation density and topological arrangement

Trees were planted in a rectangular spatial arrangement with a completely random design, where the experimental unit was a tree, with seven to ten replications (Table 1). The planting distance was 7 m between rows and 4 m between plants, giving a planting density of 357 plants per ha. The transplant was carried out in April 2015, in strains 35 to 50 cm deep, 3 kg of vermicompost was added to the bottom of each strain and the plant was subsequently placed. Irrigation was applied to avoid stress and eliminate porous space.

## **Agronomic management of the plantation**

The agronomic management of the plantation was according to that recommended by Lugo *et al.* (2009); Ariza *et al.* (2009). Drip irrigation was performed every other day. It was fertilized with the 150-60-60 dose (N-P-K) according to the soil analysis and nutritional requirements. Triple fertilizer was applied 17 to 20 cm from the plant in two lateral furrows, 100 g were deposited on each side. Foliar fertilization was carried out with Bayfolan Forte<sup>®</sup> using an Echo<sup>®</sup> brand motorized backpack sprayer at 4 ml L<sup>-1</sup> of water each month. Formation pruning was performed every two months, eliminating suckers developed on the main stem of the rootstock, the cuts were covered with vinyl paint.

## **Agronomic variables evaluated**

Stem diameter (mm) and plant height (cm). They were evaluated monthly with a Caliper<sup>®</sup> brand digital vernier and a tape measure in all the plants. The stem diameter was taken at 20 cm above ground level.

Flowering-fruiting. The number of flowers in the selected plants was evaluated when the first flower buds appeared every 15 days. The number of fruits in each plant was also evaluated.

Chlorophyll content. It was determined quickly, directly and non-destructively. The readings were performed with a portable chlorophyllometer (Minolta Chlorophyll Meter SPAD-502<sup>®</sup>; Markwell *et al.*, 1995). Two leaf readings were taken from the top and two leaf readings from the bottom, making a total of 3 evaluations every 4 months in each of the combinations.

Cup diameter and height. They were evaluated one year after transplant, when the crown was fully formed. The diameter was measured using a tape measure. The height of the tree canopy was determined with the support of two completely straight thin iron rods, one rod was placed horizontally at the base of the canopy and another at the top of the tree, taking measurements from the bottom up.

## **Statistical analysis**

The data were processed with the statistical program SAS<sup>®</sup> version 9 SAS (1996), applying a simple analysis of variance (Anova) and comparison test of means according to Tukey, after checking the parametric assumptions of Normality.

## **Results and discussion**

The agronomic variables evaluated are shown in Table 2.

**Table 2. Comparison of means of the agronomic variables evaluated in the rootstock-variety of citrus in Xalostoc, Ayala, Morelos.**

Treat	Var	DT (mm)	Height (cm)	Number flowers	Number fruits	Chlorophyll content US			AC (cm)	DC (cm)
						Jun-01	Oct-02	Feb-03		
I	Camp	20.59 cd	60.45 cf	0.87 fg	0.7 f	75.08 ab	83.87 a	83.89 ab	109.1 c	113 de
	Ham	18.81 ce	63.9 be	0.7 g	0.7 f	78.15 ab	82.16 ab	81.2 abc	102.7 cd	114.2 d
	Jaf	16.26 dg	60.55 cf	0.7 g	0.7 f	79.37 ab	80.68 ab	79.24 abc	83.4 f	80.6 i
	Mar	19.43 ce	59.28 cf	1.46 c	1.49 b	82.17 ab	77.42 ab	79.11 abc	94.2 e	107.2 e
II	Camp	26.39 ab	64.2 bd	2.07 b	1.27 c	81.99 ab	83.26 ab	84.02 ab	120.8 b	143.6 b
	Ham	26.33 ab	73 ac	0.7 g	0.7 f	81.06 ab	83.51 a	84.17 ab	139.1 a	135.5 c
	Jaf	27.37 ab	82.6 a	2.54 a	1.89 a	84.04 a	82.62 ab	83.38 ab	127.3 b	172 a
	Mar	30.83 a	67.77 ad	1.1 de	0.96 d	86.35 a	85.15 a	85.62 a	135 a	175.5 a
III	Camp	20.69 cd	79.9 ab	1.17 d	0.9 de	81.36 ab	82.79 ab	83.69 ab	86.6 f	113.5 de
	Ham	17.9 cf	44.5 fg	1.13 d	0.79 f	70.2 ab	80.48 ab	79.99 abc	85.6 f	100.9 f
	Jaf	19.68 cd	51.55 dg	0.83 fg	0.7 f	83.47 ab	79.08 ab	77.66 bc	98.5 de	116.8 d
	Mar	19.59 cd	46.05 eg	1.01 def	0.91 d	80.15 ab	82.31 ab	84.03 ab	80 fg	112.7 de
IV	Camp	14.42 eh	43 fg	0.99 def	0.88 de	82.3 ab	75.65 b	75.66 c	87.2 f	94.1 g
	Ham	11.36 gh	37.77 g	0.93 ef	0.7 f	80.43 ab	82.97 ab	83.21 ab	74.8 g	95.5 fg
	Jaf	10.45 h	34.61 g	1.14 d	0.94 d	66.09 b	81.96 ab	80.64 abc	67.7 h	86.8 h
	Mar	12.88 fg	35.81 g	1.13 d	1.23 c	80.67 ab	79.73 ab	81.98 abc	62.6 h	97.2 fg

Treatments= rootstock: I) Amblycarpa; II) Volkamerian; III) Citrange C -35 and IV) Naranja-Agrio. Grafted varieties: Campbell (Camp); Hamlin (Ham); Jaffa (Jaf); Mars (Mar). Means with same letters in columns are not statistically different (Tukey, 0.05). DT= stem diameter; A = crown height; DC= cup diameter.

### Stem diameter and plant height

The stem diameter (mm) and the plant height (cm) evaluated in the different combinations showed significant statistical differences ( $p < 0.05$ ; Table 2). The combinations: Volkamerian-Mars, Volkamerian-Jaffa, Volkamerian-Campbell and Volkamerian-Hamlin were the ones that threw the greatest thickness with 30.83, 27.37, 26.39 and 26.33 mm respectively, without statistical differences between them ( $p > 0.05$ ), while cultivars that were grafted on Naranja Agrio showed the lowest values ( $p < 0.05$ ), with the Naranja Agrio-Jaffa combination showing the smallest diameter of the tree trunk (10.45 mm).

The height of orange trees was greater when the Volkamerian rootstock was used ( $p < 0.05$ , Table 2), the Volkamerian-Jaffa combination was the one that presented the greatest growth with 82.6 cm, without significant difference with Volkamerian-Hamlin, Volkamerian-Mars and the Citrange C-35-Campbell combination ( $p > 0.05$ ), coinciding with that reported by Anderson (2012).

Who pointed out that Volkamerian induces vigorous and large plants, as with the results reported by Milla *et al.* (2009) in Tahiti lime tree, where plants grafted on Volkamerian presented larger sizes than those grafted on mandarin Cleopatra and Naranjo Agrio. Arrieta *et al.* (2010) in studies carried out on different rootstocks, they reached greater height and diameter with the Volkamerian and Amblycarpa patterns.

This coincides with the results obtained by Girardi *et al.* (2007), reporting that lemon-type rootstocks were more vigorous and developed faster from nursery than tangerines. Zamora *et al.* (2003) obtained similar results when carrying out a study on the selection of rootstocks for 'Valencia' orange in calcimorphic soils, they found that the grafts made on Volkamerian, produced the largest and leafiest trees.

The rapid growth of the stem of Volkamerian Lemon has also been pointed out by Girardi *et al.* (2007), who stated that it is a vigorous rootstock in the nursery. This has a larger stem diameter, indicating that both variables are related. The stem diameter is very important in the nursery stage, because the grafting is done when the stem is greater than 0.55 cm. This reaches this thickness faster, which represents less time in the acclimatization phase, lower production costs and an excellent alternative for nurserymen.

The smallest trees were obtained with the Naranjo Agrio pattern ( $p < 0.05$ ; less than 43 cm). Del Valle *et al.* (1981) showed that the orange trees grafted on Amblycarpa and Volkamerian showed greater height and thickness than those grafted on Naranjo Agrio ( $p < 0.05$ ), which agrees with what was obtained in this work and with what was reported by Pérez *et al.* (2003) and Navejas *et al.* (2010).

From which it follows that the choice of a pattern for conventional use constitutes an important decision, which must be based on the characteristics and qualities that it presents, both in the juvenile phase and in field conditions, given that the behavior of the patterns are variable in relation to their adaptability to climatic conditions, soils, tolerance to diseases and pests, also having a marked influence on the behavior of the graft and therefore on vigor, earliness, production level and the quality of the fruits of the different varieties of orange.

### **Flowering-fruiting**

When comparing the different rootstock-cultivar combinations in relation to flowering, statistical differences were observed between them ( $p < 0.05$ ; Table 2), detecting that the Jaffa variety grafted on the Volkamerian pattern had the highest emission of flower buds per tree ( $p < 0.05$ ) with an average of 2.54 flowers every 15 days, during the evaluated period and differs significantly with the rest of the combinations.

The lowest number of flowers was observed in the Hamlin, Jaffa and Campbell varieties grafted on Amblycarpa and Hamlin on Volkamerian with 0.7 flowers/tree, without statistical differences between them ( $p > 0.05$ ). The highest number of fruits achieved was in the Volkamerian-Jaffa combination (1.89;  $p > 0.05$ ). The Amblycarpa-Hamlin, Amblycarpa-Campbell, Amblycarpa-Jaffa, Volkamerian-Hamlin, Citrange C-35-Jaffa and Naranjo Agrio-Hamlin combinations reported the lowest number of fruits, with no differences between them ( $p > 0.05$ ).

In Mexico, the species of *C. macrophylla* and *C. volkameriana* are commonly used as rootstocks for the propagation and establishment of Mexican lemon trees (Bermúdez *et al.*, 2017). Three lemon species are cultivated: sour (Mexican) lemon *Citrus aurantifolia*, Persian lemon *Citrus latifolia* and Italian lemon *Citrus limon* (SIAP, 2018).

Most of the citrus fruits in the state of Morelos are established on Lemon Volkamerian (*C. volkameriana*) and some on Naranja Agrio (*C. aurantium*; Lugo *et al.*, 2009). Confirming the acceptance of this lemon hybrid as a good producer of large, vigorous and productive plants.

### **Chlorophyll content**

The chlorophyll content (SPAD units), indicator of the green color of the leaves, was a parameter that did not show great variation during the study. In the first evaluation, the Volkamerian-Mars and Volkamerian-Jaffa combination presented the highest values, with 86.35 and 84.04 (SPAD units) respectively, with no statistical differences between them or with the rest of the combinations ( $p > 0.05$ ), only with the combination Naranja Agrio-Jaffa that obtained the lowest value ( $p < 0.05$ ; 66.09 SPAD units). During the second evaluation, the Hamlin, Jaffa and Mars varieties grafted onto Volkamerian with 83.51, 82.62, 85.15 (SPAD units) and the Amblycarpa-Campbell combination (83.87 SPAD units) were those that showed the highest chlorophyll content, without statistical differences between them nor with the rest of the combinations ( $p > 0.05$ ). In this period the Naranja Agrio-Campbell combination yielded the lowest value (75.65 SPAD units).

In the third evaluation, the combinations had a similar behavior to the second, highlighting again the combinations with Volkamerian which reached the highest chlorophyll units per leaves evaluated without difference with the others ( $p < 0.05$ ), only with the Naranja Agrio-Campbell combination that yielded the lowest chlorophyll content per leaf ( $p < 0.05$ ; 75.66 SPAD units).

Achor *et al.* (2010) cited that the SPAD readings are only taken from a partial part of the leaf, so as the destruction of chloroplast is not uniform, the sensor can capture areas with different concentrations of chlorophyll in the same leaf. In this case, the rootstock cannot be directly related to the chlorophyll content in the varieties evaluated, since this may be influenced by other factors such as fertilization and the agronomic management offered to the plantation.

Some authors, such as Lizana and Errazuriz (1980) have inferred that low temperatures, especially at night, could induce a disappearance of chlorophyll and an increase in the synthesis of carotenes, before the fruit is ripe. Other trials justified that after 15 days; from the application of chelates to the soil, the SPAD readings, which indicate the foliar content of chlorophyll, increased significantly with the doses of 50 and 100 g of Fe EDDHA per tree to values of almost 47 (SPAD index), corresponding to the green color. Common of healthy leaves and that indicates a normal content of chlorophyll in them (Sánchez *et al.*, 2015).



### Cup height (AC)

The Hamlin and Mars varieties on the Volkamerian pattern presented the highest crown height ( $p < 0.05$ ) with 139.1 cm and 135 cm respectively, without statistical differences between them ( $p > 0.05$ ) in contrast to the Jaffa and Mars varieties grafted on Naranjo Agrio exhibited the lowest crown height ( $p < 0.05$ ) with 67.7 cm and 62.6 cm, with no differences between them ( $p > 0.05$ ).

This is in agreement with what several researchers have pointed out about the ability of the Volkamerian rootstock to induce large size (Stenzel and Neves, 2004). However, it differs with that reported by Curti *et al.* (2012) in Persian lemon grafts made on Volkamerian, as those with the lowest height and volume of the glass.

Other researchers such as Ledo *et al.* (2008) pointed out that the rootstocks should form small crowns, with high productive efficiency in relation to the volume of the same, in order to increase the density of plantation and the production of the orchard. The Jaffa and Mars varieties grafted onto Naranjo Agrario were those with the lowest crown height at 67.7 cm and 62.6 cm, with no differences between them.

The results show that there are rootstocks different from the Naranjo Agrio, which is the one that has traditionally been used in citrus farming throughout the years, which can be used as an alternative to achieve high productions and more leafy trees.

### Cup diameter (DC)

The Jaffa and Mars cultivars grafted on Volkamerian presented the highest crown diameter ( $p < 0.05$ ) with 172 cm and 175.5 cm respectively, with no differences between them ( $p > 0.05$ ). On the other hand, the Jaffa cultivar grafted on Amblycarpa presented the smallest crown diameter ( $p < 0.05$ ) with 80.6 cm. Figueredo *et al.* (2002) and Quijano *et al.* (2002) indicated that the Persian lime plants grafted on Volkamerian presented the highest height and diameter of the crown than those grafted on 'Cleopatra' and 'Carrizo'.

The graft/pattern relationship is important since the pattern constitutes the base where the crown will develop (graft) and the future production of the tree depends on it. Reyes and Ruiz (1983) rated the most leafy and grafted grafts as those that were made on Volkamerian, Rugoso lemon, Cravo lemon, which coincides with the results obtained, however it differs from that reported by Pérez *et al.* (2003) who cataloged Amblycarpa as the pattern that offered the highest height, rootstock and graft diameters, crown and crown volume.

According to Orozco (1995) the grafts made on Volkamerian constitute an alternative since they provide leafy trees and tolerant to the conditions of calcareous soils, but they have the disadvantage that they are sensitive to the sadness of citrus fruits, an aspect that must be taken into account time to plant them. Avilan (1993) pointed out that the knowledge of a reference pattern is important because its genetic characteristics can induce plants different development capacities in the aerial part.

From which it follows that it is significant to know the behavior of each combination in the plant production phase since their interactions affect the development of the graft, accelerating or retarding it, when presenting differences in compatibility according to the grafted varieties. The differences between rootstocks may be related to their response to limiting water conditions, as in the case of deficit irrigation or drought stress (Pérez *et al.*, 2008).

Greater aerial part/root ratio implies more leaf area and therefore, greater transpiratory surface, which results in greater absorption and transport of water and nutrients. The incorporation of new patterns is invaluable both for the good development of the graft and for its behavior against different pathogens, giving quality to the fruits and accelerating their production.

## Conclusions

The Volkamerian rootstock combined with the Mars variety had the largest stem diameter. When the Jaffa, Hamlin and Mars varieties were used on this pattern, the trees with the highest growth as well as the highest height and diameter of the crown were obtained. The Jaffa variety on Volkamerian had the highest number of flowers and fruits, as well as the highest leaf chlorophyll content. The smallest, least leafy trees with thin trunks and the least number of flowers and fruits were those achieved on the Naranja Agrio pattern.

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