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Physiological Behavior of Native Avocado in Nursery

Arellano-Roque L.[1], Damian-Nava A.[1]*, Hernández-Castro E.[1], Palemon-Alberto F.[1], Sarabia Ruiz G.[1], Sotelo-Nava H.[2], Cruz-Lagunas B.[1], Maria de la Cruz Espindola Barquera[3], Vargas Alvarez Dolores[1]

[1]Posgraduate in Agricultural Sciences and Local Management, Autonomous University of Guerrero. Iguala-Tuxpan Highway, Km. 2.5. Iguala, Guerrero, México. C.P. 40101.

[2]Autonomous University of the State of Morelos.

[3]Sanchez Colin Foundation, CICTAMEX.

ABSTRACT: In the State of Guerrero, Mexico, there are several types of native avocados that grow in backyards which die mainly because of adverse environmental conditions and various harmful activities of farmers. This study is one of the pioneers in the area. The aim of this research was to follow the growth in nursery and the physiological behavior of 12 native avocados from the municipality of Ometepec, Guerrero, Mexico, in order to explore their possible use as rootstocks. Thus, 20 fruits per tree of each genotype were harvested, and they were sown on substrate (85% river lama, 5% peat moss and 10% agrolite), at one month of age. A randomized complete block design was used with 12 treatments (each genotype was considered as treatment) and four repetitions. An analysis of variance and Tukey's means tests were performed ($P \leq 0.01$ and 0.05) and LSD ($P \leq 0.05$). The most vigorous rootstock was OME-7, followed by OME-10. The OME-7 showed higher NO_3^- and Na^+ content; while OME-8, was superior in K^+ and Ca^{++}. The length*width of the avocado leaves, multiplied by its conversion factor (0.991), estimated a foliar area very similar to that obtained by the leaf area integrator. It was concluded that the OME-7, OME-3 and OME-10 rootstocks, were the most vigorous and they meet all the characteristics to be used as rootstocks.

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Corresponding Author:
Damian-Nava A.

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INTRODUCTION

Mexico is one of the countries with a very wide variety of avocados, since there are at least 20 different species related to avocado in the country (Barrientos-Priego et al., 2007). The importance of the existing phytogenetic resources in Mexico is invaluable, as they can be used in genetic improvement to solve basic problems of commercial exploitation crops. This diversity of creole avocados allows commercial varieties to be grafted on creole rootstocks, which in certain cases, are tolerant-resistant to salinity and chlorosis (Crowley et al., 2003), as well as to Phytophthora cinnamomi Rands diseases (Teliz, 2000). In addition, they are adapted to different environmental conditions (Bergh, 1992).

This genetic diversity of wild and criollo avocados represent a valuable source of genes that can be used in avocado breeding programs, which they seek, in addition to improving productivity and adaptation, to incorporate high nutritional quality of the fruit and resistance to pathogens. Therefore, it is urgent to rescue the phytogenetic resources of avocado, as they can be lost due to the rapid devastation of forests and jungles (Westeboy, 1989). It is also important to evaluate their potential, based on their fruit size, pulp weight and fruit color (Franco and Hidalgo, 2003).

In the processes of selection, propagation by seed and adaptation of these creole and wild genotypes, different types of avocados have been developed that are known as Mexican, Guatemalan, Antillean and Costa Rican horticultural races (Barrientos-Priego et al., 2007).

The state of Nuevo Leon, Mexico, is considered the center of origin of the creole avocado (Persea americana var. drymifolia), which is very localized in the municipalities of Galeana, Rayones, General Zaragoza and Aramberri. In the creole avocado, local breeds and varieties come from selections, which have been cultivated in many regions and have a strong tendency to be replaced by modern varieties. It is still possible to find creole avocado populations forming part of the orchards or the natural vegetation (Gutierrez-Díez et al., 2009).

It is of major importance to carry out characterization studies and evaluation of these phytogenetic resources, that will allow to determine their potential use as a source of germplasm in the genetic improvement of this species, and that can serve as a basis to help define conservation strategies and use of this species. Therefore, the rescue of avocado germplasm is a priority in order to start the genetic improvement of this fruit. A safe alternative to conservation is through in vitro establishment under normal or limited growth conditions (Vidales-Fernandez et al., 2011).

The seeds are sown in seedbeds for the propagation of these genotypes, and the plants are ready to be transplanted to the nursery at 30 days after germination (dag). In order to choose the best standard, it should be taken into account the ease in getting the seed, the vigorous growth of the seedlings, adaptation, good root development, easy grafting, high degree of compatibility with the variety to be grafted and resistance or tolerance to limiting biotic factors (Bernal and Diaz, 2005).

In the State of Guerrero, Mexico, several municipalities have a wide diversity of genetic resources of avocado (Persea americana Mill.) that represent a huge genetic potential that can be used to obtain various genotypes tolerant or resistant to salinity, pests, diseases and adverse conditions to the environment and to improve productivity and nutritional quality. Unfortunately, these native avocados are being lost by the attack of pests and diseases, or by the rapid devastation of the forests and jungles, or by various destructive actions carried out by local rural producers. For these reasons, it is urgent to rescue and study them in order to know which are their attributes to improve the cultivation of this species. Therefore, the behavior of native avocado rootstocks was studied in this research, with the following objective: to evaluate the behavior in nursery of 12 genotypes of native avocados from Ometepec, Guerrero, in order to verify if they constitute a viable alternative as tropical rootstocks.

MATERIALS AND METHODS

Location of the study area

The study was conducted in Iguala, Guerrero, Mexico, from August to December 2015. The area is located at 757 masl and coordinates: 18°20'39" N and 99°29'53" W (GPS Garmin eTrex 10®). The predominant climate is Awo g(w) (i'), the driest of the warm subhumid, with rain in summer (June to October). The average annual precipitation is 977 mm and the average annual temperature is 25.7°C (García, 1981).

Methodology

This study collected fruits from 12 types of native avocados from Ometepec municipality, Guerrero. In July 2015, the avocado seeds were cut, and 20 seeds of each genotype were planted to obtain the 12 rootstocks of the study. Then they were transplanted 30 days after germination (dag) on substrates (85% river lama, 5% Peat moss and 10% agrolite) under nursery conditions. They were fertilized with ovine and bovine grounded manure (grinder mill, CH620 model, KOHLER® brand) and disinfected (stainless steel steam cooker at 120°C for 30 minutes). Then they were diluted (250 g L-1 of water) and mixed with the commercial mycorrhiza Glumix Irrigation® Biostimulant (5 g L-1 of water). The mixture and dilution of fertilizers and mycorrhizae was carried out with an SSP mixer (angular grinder (230 mm) (9"), 127 V-15 A 50/60 Hz 6 600 rpm, Makita® brand). The dose used for this mixture was 250 mL/ pot, every 30 days, with supplementary water irrigations every other day.

Variables studied

The percentage of seeds germination was registered until 100% of genotypes seeds germination was completed. From August to December 2015, every 30 days, the variables were recorded: plant height (cm), from the neck to the apex of the stem; stem diameter (mm), at 10 cm height with a digital vernier (Digimatic calipper Model: CD-12'CP, Mitutoyo® brand); total number of leaves (NLT), number of young leaves (NLY), well-formed of incomplete size and green-yellow to reddish color; number of mature leaves (NLM), with 100% size and intense green color; number of old leaves (NLO), those with loss of green color, in senescence or close to this stage; leaf length in cm (LL), from the base to the apex of the leaf (without measuring the petiole); leaf width in cm (LW), in the middle part of the limbus, from edge to edge, of mature leaves; chlorophyll content in young (CLY) and mature (CLM) leaves measured with SPAD, 502 plus model, Minolta® brand; leaf growth kinetics (LGK), with size 1 to 1.5 cm in length and 0.4 to 0.7 cm in width, until reaching 100% of its size. Foliar area (FA) and specific weight (SW), by destructible method, the leaf length and width were measured to 100 leaves/ rootstock and numbered from 1 to 100. Then the leaves were scanned (AREA METER SQUARE CENTIMETERS scanner, Model: 3100, LI-COR brand), and dry weight was recorded on an analytical balance (Type: VE-204, Velab brand). Nutrient content was determined by petiole extract from four mature leaves/ repetition/ treatment. The petiole was cut into portions then they were pressed in a garlic press (Kamp® brand) and the extracted sap was deposited in the respective ionometer: NO_3^- (METER, Model B-743), K^+ (METER, Model B-731), Ca^{2+} (METER, Model B-751) and Na^+ (METER, Model B-722).

Design and statistics

A randomized complete block design was used with 12 treatments (in the variance analysis, each genotype was considered as a treatment), with four repetitions. A variance analysis, a Tukey test ($P \leq 0.01$ and 0.05) and LSD ($P \leq 0.05$) and a Pearson correlation between the variables, were carried out with the Statistical Analysis System (SAS) software, version 9.0.

RESULTS AND DISCUSSION

Seeds germination of native avocados

Seed germination was achieved from 11 to 29 days after sowing (das), when 100% germination was obtained. The rootstocks that in 20 das achieved 100% germination, were: OME-5, OME-12, OME-8, OME-4, OME-6 and OME-11. Whereas OME-7, OME-1, OME-9 and OME-10 reached 100% at 26 das (Table 1).

Rootstock behavior in nursery

In the variance analysis of the rootstocks, they showed highly significant differences in: plant height, stem diameter, number of leaves (young and mature), total leaves per plant, length and width of leaves and NO3- content. There was only significant difference in chlorophyll content of young and mature leaves, and in Ca2+ content, while in K+ and Na+ there were no statistical differences (Table 2).

Height and diameter of rootstocks of native avocados from Ometepec, Guerrero

The rootstocks OME-7 (79.3 cm) and OME-8 (79.1 cm), showed higher plant height and statistically were superior to the genotypes OME-3 (77.0 cm), OME-12 (71.58 cm) and OME-2 (74.0 cm). The rootstocks that were significantly lower in height were OME-10 (67.78 cm), OME-11 (65.91 cm), OME-2 (62.92 cm) and OME-1 (56.76 cm). The other rootstocks showed intermediate height: OME-6 (69.62 cm) and OME-9 (68.78 cm) (Figure 1).

As for diameter, the rootstocks OME-7 (7.39 mm), OME-3 (7.37 cm) and OME-10 (7.24 mm) were statistically superior to the genotypes OME-12 (6.59 mm), OME-2 (6.26 mm), OME-11 (6.17 mm) and OME-1 (5.69 mm) (Figure 1).

Number of leaves of rootstocks of native avocados from Ometepec, Guerrero

The total number of leaves in OME-2 (40.4 leaves/ rootstock) was significantly higher than OME-9 (29.2 leaves/ rootstock); in descending order, they were followed by the rootstock OME-10 (35.6 leaves/ rootstock) and the other rootstocks showed intermediate values, between 31 and 34 leaves/ rootstock. As for the number of young leaves, the rootstock OME-7 (7.7 leaves/ plant) showed the highest value and it was statistically superior to the genotypes OME-2 (5.5), OME-4 (5.4), OME-9 (5.0), OME-8 (5.0) and OME-1 (4.5) (Figure 2). In relation to the number of mature leaves, the genotype OME-3 (19.97) was statistically higher than the genotypes OME-4 (17.18), OME-2 (15.51), OME-6 (16.63), OME-8 (17.33), OME-10 (15.90), OME-11 (16.17) and OME-1 (15.08) (Figure 2).

The OME-2 rootstock, showed the highest value of total leaves, because it had a higher number of young leaves; while the OME-10 rootstock, also reached a high value, but due to a higher number of mature leaves.

Kinetics of growth in length and width of leaves of native rootstocks from Ometepec, Guerrero

On the first date there was no significant difference in leaf length among the rootstocks. On date two, the rootstocks that showed the highest values were OME-7 (9.0 cm), OME-11 (9.0 cm), OME-12 (8.7 cm), OME-8 (8.4 cm), OME-6 (8.4 cm), OME-2 (27.0 cm), OME-5 (8.0 cm) and OME-10 (7.8 cm), but they were not statistically different from the other rootstocks. On the third date, the rootstocks that were statistically better were OME-10 (19.75 cm), OME-7 (19.50 cm) and OME-2 (19.5 cm) which exceeded OME-1 (15.0 cm). On the fourth date, the statistically better rootstocks were OME-9 (24.75 cm) and OME-2 (24.25 cm), which statistically exceeded OME-1 (17.75 cm). Lastly, on the fifth date, the rootstocks with the highest values were: OME-10 (25.82 cm) and OME-9 (25.37 cm), but they were not statistically better than the others (Figure 3).

On the first date, the rootstock that had the highest leaf width was OME-1 (0.7 cm) and it was statistically different from the other rootstocks; while on date two, the rootstock that presented the highest value was OME-7 (3.2 cm) and it was statistically better than OME-9 (2.2 cm). On the third date, the rootstock with the highest leaf width was OME-7 (7.3 cm), which statistically exceeded OME-1, OME-3 and OME-11 (all of them with 6.0 cm). As for the fourth date, the best rootstock was OME-4 (9.25 cm) which significantly outperformed OME-1 (6.8 cm), OME-3 (7.3 cm) and OME-11 (6.4 cm). On the fifth date the rootstocks OME-4 (9.3 cm), OME-7 (8.9 cm) OME-2 (9.2 cm) and OME-10 (8.97 cm) were statistically better than OME-11 (6.42 cm) (Figure 4).

When the kinetics of the average leaf growth of the 12 rootstocks was done, it was found that from September 6th-14th, they grew 26.4% width and 37.6% leaf length just in eight days; from September 14th to 22nd, 43.4% and 34.6% width and length respectively; likewise, from September 22nd to 30th, 2015, they reached 20.6% and 16.6% width and length, respectively. That is, taking into account the initial growth (7 days before the sampling started), plus the sum in these three sampling periods, more than 97% and 96% growth in width and length of leaves was achieved at 30 days, respectively. The 100% of both measurements was reached 38 days after the new leaves had sprouted. The maximum growth reached at 30 days was 8.0cm and 29.1cm in width and length, respectively; while at 38 days it reached 8.2 and 30.4cm in width and length of leaves, respectively, which corresponded to a 100% growth of the leaves (Figure 5). The data in Figure 5 were calculated from the values in Figures 3 and 4, on the width and length of leaves of native avocados from Ometepec, Guerrero, Mexico.

The growth kinetic of the leaves of these rootstocks of native avocados, showed a sigmoidal kinetics, which consisted of three stages: stage I (8 days) was slow and they grew 6.5 and 6.9% for width and length of leaves, respectively; the stage II (24 days) was rapid and they grew 70 and 72% width and length of leaves, respectively

Chlorophyll in young and mature leaves in rootstocks of native avocados regarding the content of chlorophyll in young leaves, it was observed that the rootstocks with the highest value were: OME-12, OME-10, OME-9, OME-8 and OME-5 (24.1, 20.8, 20.6, 21.1 and 22.8 SPAD units, respectively), and they were statistically superior to OME-1 (18.8 SPAD units). Whereas the highest chlorophyll values in mature leaves were OME-3, OME-5, OME-8, OME-9 and OME-12 (42.9, 39.8, 41.8, 40.2 and 41.5 SPAD units, respectively) and they statistically exceeded OME-1 and OME-4 (36.6 and 37.3 SPAD units, respectively), the other rootstocks showed intermediate values (Figure 6). As it can be seen in the values obtained, the young leaves only showed half of the chlorophyll content in comparison to mature leaves, because they did not have 100% of the size and they had a yellow/green and reddish/ green coloration, therefore.

The young leaves have low photosynthetic capacity, but this increases progressively with age until the leaf has grown from 70 to 100% of its maximum size.

Foliar area and specific weight in native rootstocks from Ometepec, Guerrero

The rootstocks with the largest leaf area were OME-10 (170.8 cm2 per leaf) and OME-7 (164.0 cm2 per leaf) which were statistically superior to OME-6 (145.81 cm2 per leaf), OME-1 (138.10 cm2 per leaf), OME-12 (136.93 cm2 per leaf), OME-2 (133.81 cm2 per leaf) and OME-5 (132.31 cm2 per leaf), which in turn, they were superior to OME-11 (113.91 cm2 per leaf). The rest showed intermediate values: OME-4 (128.09 cm2 per leaf), OME-3 (127.91 cm2 per leaf), OME-8 (126.71 cm2 per leaf) and OME-9 (121.61 cm2 per leaf) (Figure 7).

As for specific gravity, the rootstock with the highest value was OME-2 (70.114 $mg.cm^{-2}$), which was statistically better than OME-7 (64.06 $mg.cm^{-2}$), OME-6 (63.34 $mg.cm^{-2}$), OME-10 (62.09 $mg.cm^{-2}$), OME-8 (61.75 $mg.cm^{-2}$) and OME-11 (60.96 $mg.cm^{-2}$) (Figure 7).

Nutrient extract per petiole in collected genotypes

The OME-7 (5275 mg L^{-1}) genotype showed the highest value in nitrate and it was statistically superior to OME-9 and OME-10 (4100 mg L^{-1}) genotypes, but not to OME-11 with 5150 mg L^{-1}. The genotypes with the lowest values were OME-1 and OME-3 between 300 and 500 mg L^{-1}; while the intermediates were OME-2 with 3950 mg L^{-1}, OME-12 with 3050 mg L^{-1} and OME-4 and OME-8 with 2625 mg L^{-1} (Figure 8).

The OME-8 (4550 mg L^{-1}) genotype had the highest potassium content and it was statistically superior to all other genotypes that had between 2225 and 3075 mg L^{-1}. Also, the OME-8 (1643 mg L^{-1}) genotype showed the highest calcium value and it was statistically better than genotypes OME-11 (610 mg L^{-1}), OME-9 (546 mg L^{-1}), OME- 1 (533 mg L^{-1}), OME-4 (370 mg L^{-1}), OME-3 (300 mg L^{-1}), OME-6 (293 mg L^{-1}), OME-7 (203 mg L-) ¹) and OME-10 (162 mg L^{-1}).

The OME-7 (4050 mg L-1) genotype had the highest value in sodium, and it was statistically higher than the OME-9 (2625 mg L-1) genotype, but it was not higher than OME-4 (3075 mg L-1). The lowest values were for the rootstocks: OME-8 (160 mg L-1), OME-1 (175 mg L-1) and OME-3 (300 mg L-1); while the intermediates were: OME-12 (800 mg L-1), OME-10 (900 mg L-1), OME-2 (950 mg L-1), OME-6 (1750 mg L-1), OME -5 (1500 mg L-1) and OME-11 (1850 mg L-1) (Figure 13). Castro et al. (2015), reported for sodium in Nabal, Duke 7 and UCV 7 avocado rootstocks very low results of 200 mg L-1, 300 mg L-1 and 400 mg L-1, respectively.

It was found that when using the length*width of the leaf, multiplied by its conversion factor (0.991), a difference of 6.92 cm2 was obtained in the area when calculated by length*width or when calculated by the foliar area integrator. In contrast, when using the leaf length value multiplied by its conversion factor (0.857), the estimated area was 90.36 cm2 higher than the foliar area measured directly with the integrator. Similarly, the area estimated by the product of the width of the leaves by its conversion factor (0.948) was 50.04 cm2 higher than the foliar area by the integrator (Figure 9). As it can be observed, the foliar area is very similar to that obtained by the foliar area integrator. This fact is very important because it allows estimating the foliar area without expensive devices. In studies with diverse plant species, it is mentioned that these methodologies to determine the foliar area are reliable.

Discussion

This results compared with other studies previously reported that germination begins at 20 days and ends at 25 das (Bernal and Diaz, 2005). Also, the total of the germination is reported at 50 das in avocado seeds of the variety Bacon Mexican race (Reyes-Aleman, 2005). Lopez-Palacio (2012) reported 45 das in creole avocados. When cuts are made to cotyledons, the germination percentage improves from 12.2 to 92.2% (Kadman, 1963), as it was done in this research.

These results are lower than those reported for the following rootstocks: Duke 7, 120.0 cm and Toro Canyon, 120 cm (Fassio et al., 2009); Duke 7 with 95.7 cm; Nabal of 91.0 cm and UCV7 with 82.0 cm (Castro et al., 2015); Duke 7 with 138.00 cm and Mexicola of 136.29 cm (Borquez-Lillo et al., 2015) and similar to those cited by: Duke 7 with 50.0 cm, Nabal of 78.5 cm, Mexicola with 57.14 cm, Degania 117 with 64.39 cm and Zutano 42.86 cm (Castro et al., 2009).

The rootstock diameters found in this research are smaller than those found by Fassio et al. (2009) in the Toro Canyon rootstock (14.3 mm) and Duke 7 of 13.9 mm; as well as in Castro et al. (2009) for Mexicola of 21.60 mm, Degania 117 with 18.65 mm, Nabal of 17.61 mm, Zutano 16.75 mm and Duke 7 with 16.73 mm; while in another study, for Duke 7 a diameter of 4.91 cm and for Mexicola 3.39 cm (Borquez-Lillo et al., 2015).

The total number of leaves in the rootstocks used in this investigation were not similar to those reported for rootstock UCV7 of 178.5; Duke 7 with 169.1 and for Nabal with 121.9 leaves/ plant, respectively (Castro et al., 2015). Something similar was reported for the number of young leaves/ plant, in which the rootstocks of Duke 7 avocados were 43.1, for Zutano 42.6, Nabal of 42.6, Degania 117 of 42.5 and Mexicola of 42.3 (Castro et al., 2009). These values exceeded those obtained in this research.

In this regard, it is important to consider that the age of the leaves and the stage of development of the plant are important to determine the morphological and physiological processes in the plants (Chaumont et al., 1994). Normally, photosynthesis is low in young leaves, and it increases until it reaches a maximum when the leaf is fully developed, and then it decreases again with senescence (Gonzalez, 1998).

In mature leaves, net photosynthesis decreases as the age of the plants increases (Horley and Gottschalk, 1993). The leaves of Hass avocados can be exporters of sugar when they reach 80% of their final size, after 40 days. The maximum photosynthetic efficiency is reached around 60 days after the emergency. In this way, when the leaves have reached 100% of their size, they are already photosynthetically active; however, if the leaves are not ready yet and they have a coloration between greenish/yellow or reddish/green, it means that they have not yet reached their maximum net photosynthetic rate (Salisbury and Ross, 1994) and that the chloroplasts are inactive, therefore, they do not produce photo assimilates.

These values found in native avocados from Ometepec, Guerrero, grown in Iguala, Guerrero (tropical climate), had different behavior to the guava leaves grown in the same place, which in order to reach their maximum length and width, they need 70 and 63 days for primary and secondary buds (Damián et al., 2014). However,; and lastly, in the III stage they grew 23.7 and 20.5% width and length, respectively.

They did not reach their maximum net photosynthetic rate (Salisbury and Ross, 1994) due to the immaturity of the stomata (Faust, 1989). Similar values to those found in this research were observed in mature leaves of pinion plants (*Jatropha curcas* L.) (Gonsiorkiewicz et al., 2013). In one-year old Hass and Edrenol avocados on patterns of Allesbeste Nursery, Duiwelkloof, the chlorophyll content in mature leaves was slightly higher than those observed in this work, which oscillated between 48-57 SPAD units (Bekker et al., 2005); which is when it also reaches its maximum rate of net photosynthesis (Díaz, 2002; Salisbury and Ross, 1994). Then, the photosynthesis of the leaf gradually decreases until it becomes senescent, when it is unable to photosynthesize due to the chlorophyll degradation and the loss of functional chloroplasts (Salisbury and Ross, 1994). Additionally, the highest value of the net assimilation rate of CO2 in mature leaves than in the immature ones, confirms that the mature leaves are the most important source of photo assimilates just as it was observed in guava (Damián et al., 2009), and as reported by Acosta (1998) and González et al. (1994) in Carica papaya L., a species with C3 photosynthetic mechanism.

The specific weight represents the accumulation of reserve carbohydrates. The values found in this research were not similar to those in colín V33 avocado, 14 $mg.cm^{-2}$ (Castillo-González et al., 1998); citrus fruits: 11 mg.cm-2 for Citrange Troyer, Tangerino Cleopatra and Citrange Carrizo, and 9 mg.cm-2 for Naranjo Agrio (Nava and Villegas, 1994); varieties of orange: Valencia (11.01 $mg.cm^{-2}$), Marrs (9.96 $mg.cm^{-2}$) and Tangerino Dancy (9.67 $mg.cm^{-2}$) (Reyes et al., 2000)

These results were not similar to those reported for Duke 7 rootstock (44.0 cm^2 per leaf) or Toro Canyon (36.0 cm^2 per leaf) (Fassio et al., 2009). However, it was reported something similar to this study for avocado rootstocks with mycorrhizal treatments (Glomus hoi-like) with a leaf area of 115.50 cm^2 and mycorrhizae (Glomus mosseae) 85.16 cm^2, whereas for the control it was 82.51 cm^2 (Fundora et al., 2011 and Rivera-Espinosa, 2011) (Figure 7).

Therefore, it is suggested that for example, in order to determine the guava foliar area, the length*width of the leaves must be multiplied by its 0.72581647 conversion factor, as this is the one that significantly correlates with the value obtained by the foliar area integrator (Damián et al., 2014). In order to estimate the foliar surface in mango and avocado, the equation: FA = -4.55537 + 0.933938 (length*width) of the leaves, is the one that best explained the relationship with foliar area (Calderón et al, 2009). In red tomato (Lycopersicon esculentum Mill.) a general equation that has proved to be acceptable in the foliar area estimation, independently of the size of the leaves, is: FA = 0.34 (W*L) - 9.31, where FA is the foliar area (cm^2), and W and L are the maximum width and the length of the leaf (cm), respectively (Astegiano et al., 2001). The regression analysis of the area according to the length of the leaf (LL), the maximum width of the leaf (LW) and the product of the length by the width (LL*LW), led to propose models that can be used for the proper estimation of the foliar area of the individual leaf. For all species, the best model was FA = a + b (LL*LW), with coefficients of determination superior than 0.95 (Cabezas-Gutiérrez, et al., 2009). In order to estimate and predict the foliar area (FA) of basil (*Ocimun basilicum* L.) leaves, based on their length (L) and width (W), 500 (n = 500) leaves were randomly sampled of three different ages (young, intermediate and mature). The obtained measurements were correlated in order to generate simple linear regression equations (foliar area as a function of length and width) and multiple linear regression equations (foliar area as a function of length*width). The foliar area was positively correlated with length (r = 0.89) and width of the leaf (r = 0.86) and with width*length (r = 0.97). These results showed the possibility of predicting FA reliably from easily obtainable measurements without destroying the plants (Ruiz-Espinoza et al., 2007).

CONCLUSIONS

100% of seeds of all genotypes germinated before 30 days after sowing; but the most precocious was OME-5, which all its seeds germinated after just 11 days. This was followed by OME-12 (14 days), OME-7 and OME-8 (17 days). Mature leaves had more than double the chlorophyll content than young leaves. The OME-7 rootstock showed the highest values in plant height, stem diameter and number of young leaves. The OME-3 rootstock showed the highest total number of leaves/plant and mature leaves/plant. As well as the highest chlorophyll content in mature leaves. The OME-10 rootstock was the best in leaf length, foliar area and leaf specific weight. The rootstock OME-7 had the highest levels of NO_3^- and Na^+; whereas OME-8 was superior to the other rootstocks in K^+ and Ca^{++} content. The length*width of avocado leaf, multiplied by its conversion factor (0.991), estimates the foliar area very similar to that obtained by the foliar area integrator. This fact is very important because it allows to estimate the leaf area without the use of expensive devices and/or without destroying the leaves.

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Author contribution

An individual contributor may be assigned to multiple roles, and a given role may be assigned to multiple contributors.
Example: Conceptualization, F.Z-P., B.L. Methodology, F.Z-P. Software, F.Z-P. Validation, F.Z-P. Formal analysis, F.Z-P. Investigation, F.Z-P. Resources, B.L. Data curation, B.L. Writing-original draft, F.Z-P. Writing-review & editing, B.L. Visualization, F.Z-P. Supervision, B.L. Project administration, B.L. Funding acquisition B.L. All co-authors reviewed the final version and approved the manuscript before submission.

Conflict of interest

Just include this item if consider pertinent.

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Table 1. Percentage of germination of avocado seeds native to Ometepec, Guerrero, during the period from July 22 to August 9, 2015.

Genotypes	11 days	14 days	17 days	20 days	23 days	26 days	29 days	Total
OME-1	0	31.2	0	31.2	12.5	0	25.0	100
OME-2	0	31.2	43.7	6.2	18.9	0	0	100
OME-3	0	43.7	12.5	31.2	12.5	0	0	100
OME-4	6.2	31.2	43.7	18.7	0	0	0	100
OME-5	100	0	0	0	0	0	0	100
OME-6	0	25.0	37.5	37.5	0	0	0	100
OME-7	0	50.0	25.0	0	6.2	18.7	0	100
OME-8	0	62.5	37.5	0	0	0	0	100
OME-9	0	6.2	18.7	50.0	12.5	0	12.5	100
OME-10	0	0	43.7	18.7	25.0	0	12.5	100
OME-11	0	6.2	31.2	62.5	0	0	0	100
OME-12	0	100	0	0	0	0	0	100

Table 2. Analysis of variance of variables of vegetative growth and nutritional content of rootstocks of avocado in nursery stage, in Iguala, Guerrero, Mexico.

Variables	GEN	REP	ERROR	CV %	Media
ADP	**3724.58 ****	**2920.61**	**114.89**	**15.15**	**70.72**
DDT	**21.82 ****	**10.47**	**0.86**	**13.65**	**6.81**
NHJ	**63.07 ****	**5.18**	**10.95**	**55.56**	**5.95**
NHM	**160.68 ****	**25.64**	**10.76**	**18.96**	**17.3**
TDH/P	**366.84 ****	**51.67**	**21.01**	**19.7**	**23.26**
LDH	**228.05 ****	**27.34**	**7.79**	**12.95**	**21.56**
ADH	**47.73 ****	**5.8**	**5.46**	**29.06**	**8.04**
CEHJ	**35.13 ***	**5.24**	**14.84**	**17.7**	**21.77**
CEHM	**50.71 ***	**61.73**	**22.21**	**11.85**	**39.75**
NO_3^-	**1094532.4 ***	**16473856**	**3355192.1**	**60**	**3051.8**
K^+	**1742317.4 NS**	**1911225**	**1323541.7**	**41.57**	**2767.9**

Ca^{2+}	980304.3 *	11431038	481167.38	101.9	680.1
Na^{+}	6022501.8 NS	1359736.7	3418867.6	122.3	1511.7

ADP (plant height), DDT (stem diameter), NHJ (number of young leaves per plant), NHM (number of mature leaves per plant), TDH (total leaves per plant), LDH (leaf length), ADH (leaf width), CHJ (chlorophyll content in young leaves), CHM (chlorophyll content in mature leaves); CV (coefficient of variation). ** (Highly significant), * (significant), NS (not significant).

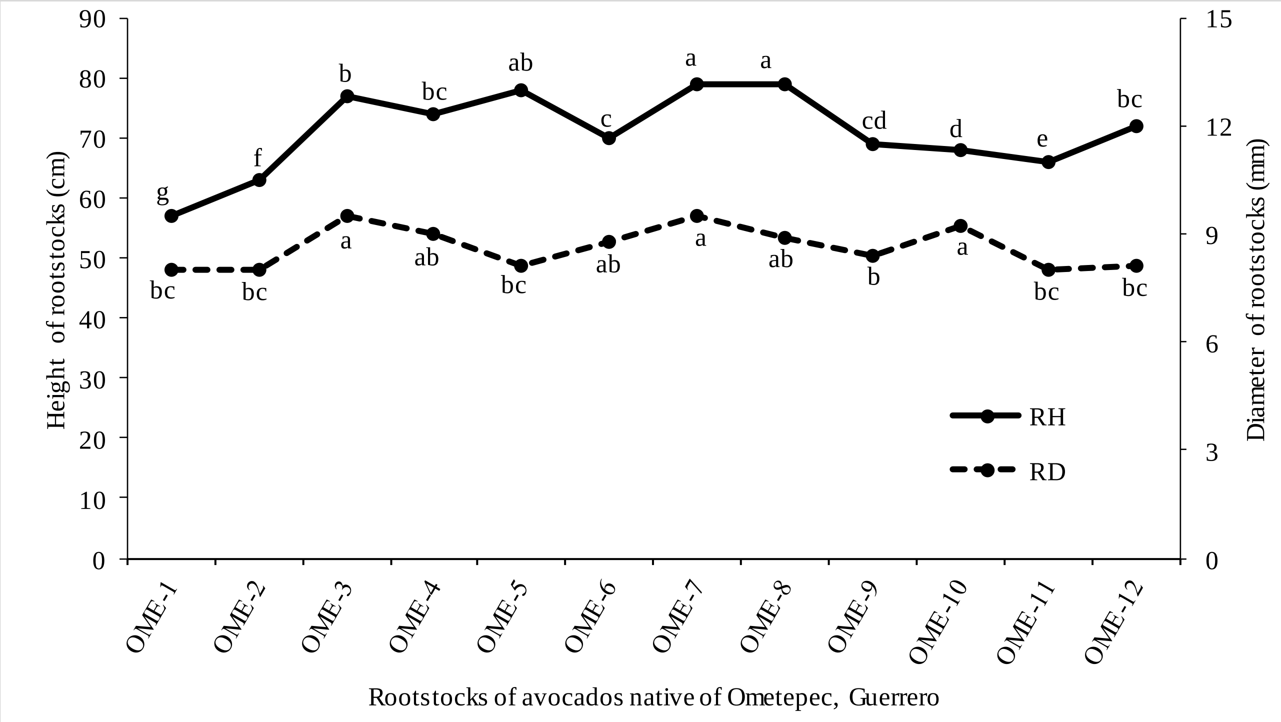

Figure 1. Height and diameter of rootstocks of avocados native to the municipality of Ometepec, Guerrero. From OME-1 to OME-12 (rootstocks collected in the municipality of Ometepec), RH (Rootstocks Height) and RD (Rootstocks diameter), Tukey (P ≤ 0.01).

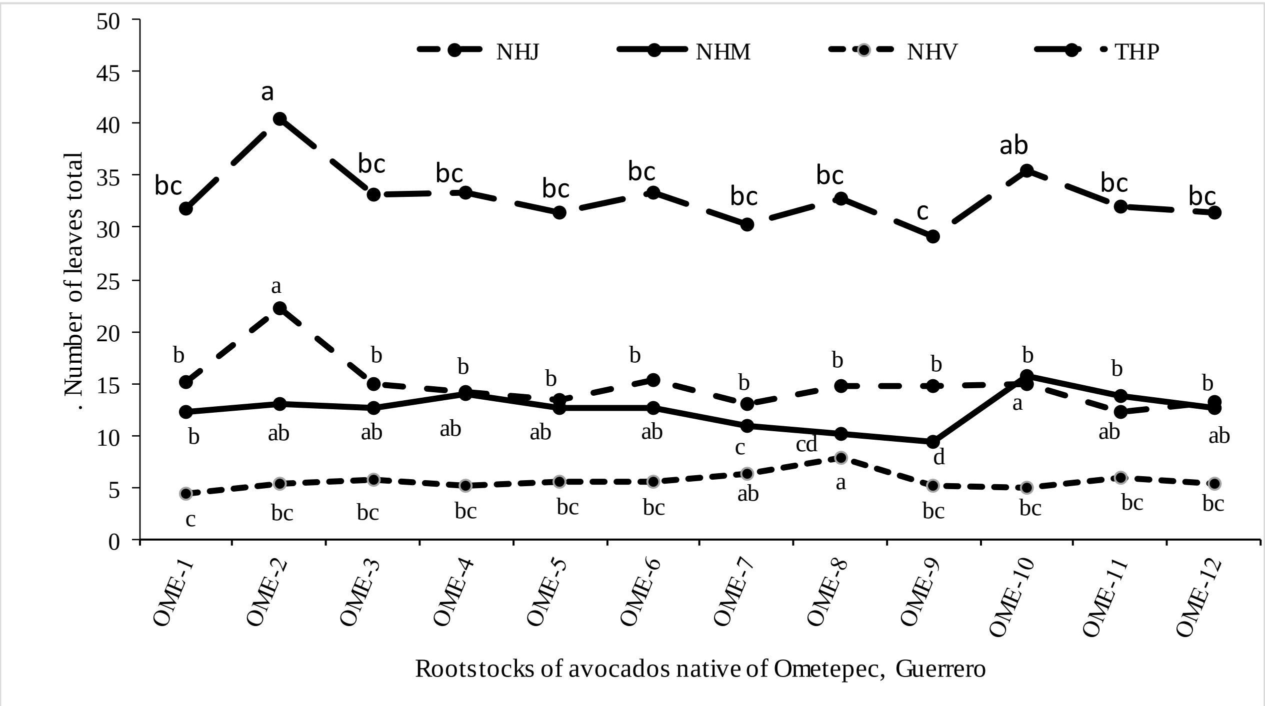

Figure 2. Number of total, young, mature and old leaves of avocado rootstocks; from OME-1 to OME-12 (Avocado Pickups from Ometepec, Guerrero). Tukey (P ≤ 0.01).

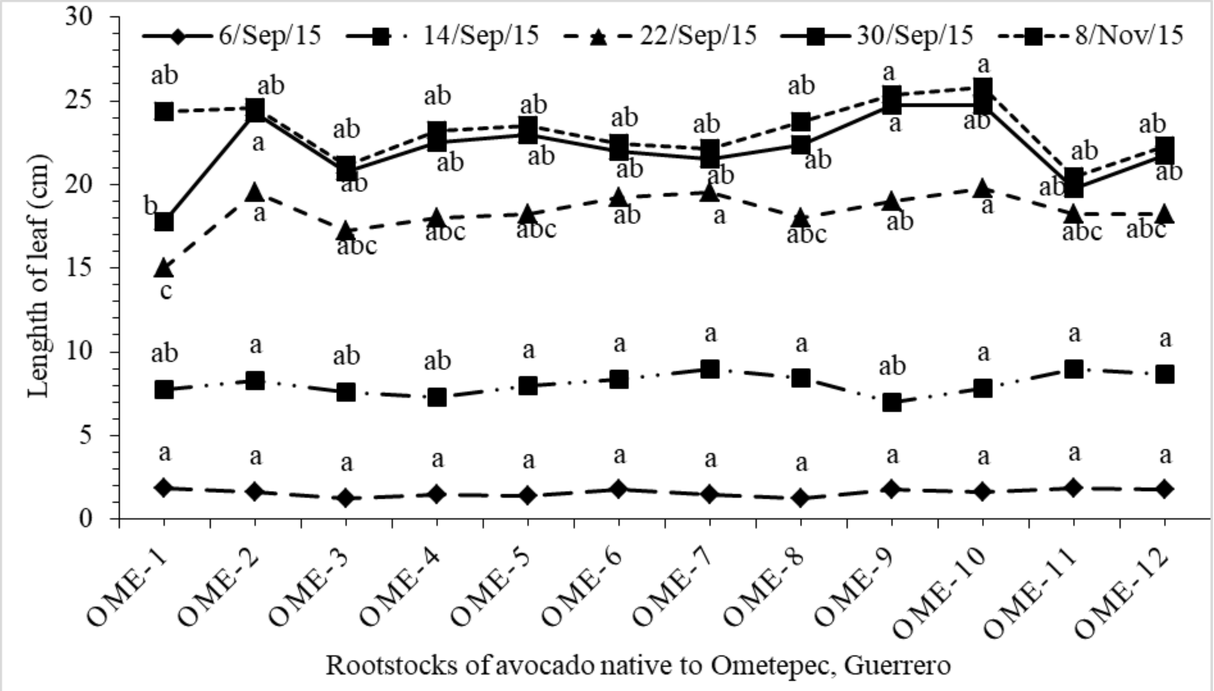

Figure 3. Leaf length growth kinetics in avocado rootstocks native to Ometepec, Guerrero. From OME-1 to OME-12 (avocados collected in Ometepec). Tukey (P ≤ 0.01).

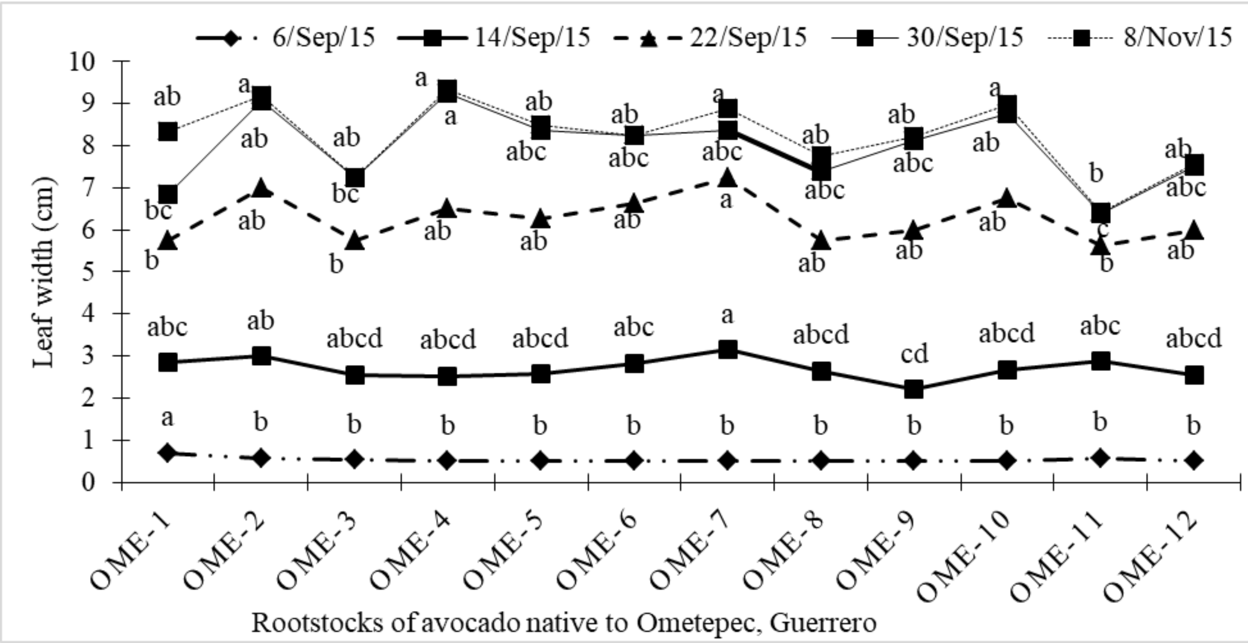

Figure 4. Leaf width growth kinetics in avocado rootstocks native to Ometepec, Guerrero. From OME-1 to OME-12 (avocados collected in Ometepec). Tukey (P ≤ 0.01)

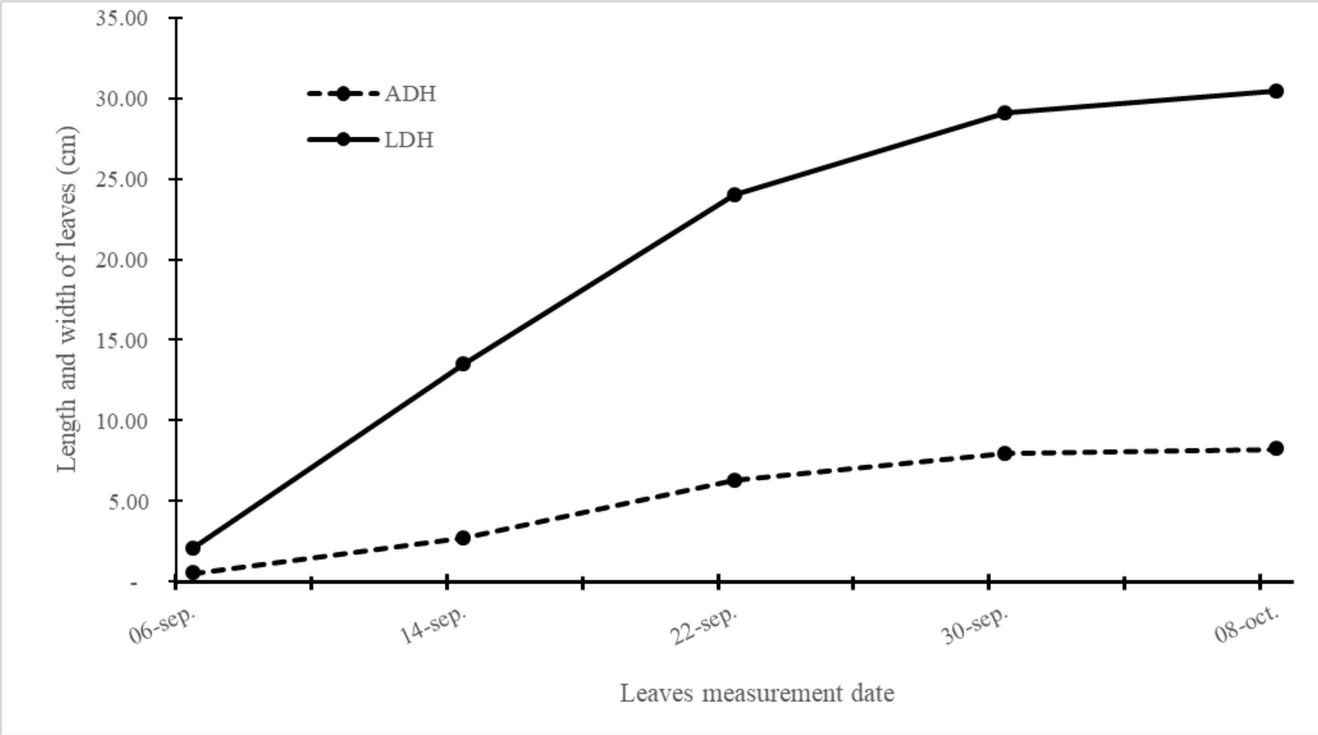

Figure 5. Average length and width behavior of 12 avocado rootstocks native to Ometepec, Guerrero. LDH (Sheet Length) and ADH (Sheet Width).

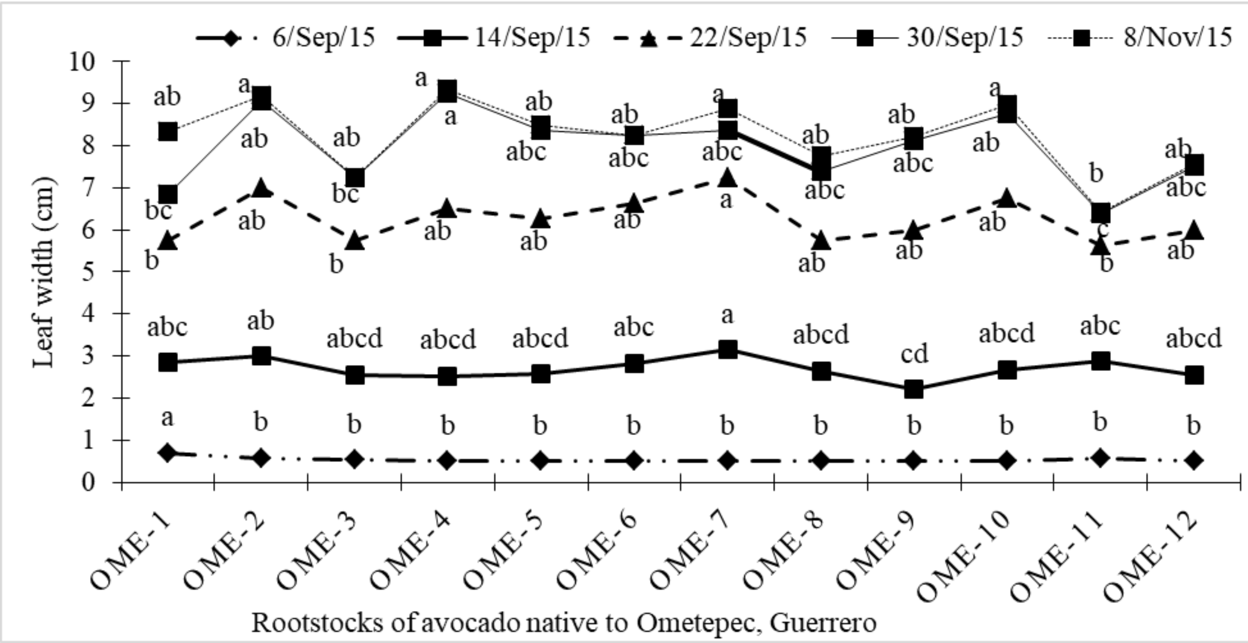

Figure 5. Average length and width behavior of 12 avocado rootstocks native to Ometepec, Guerrero. LDH (Sheet Length) and ADH (Sheet Width).

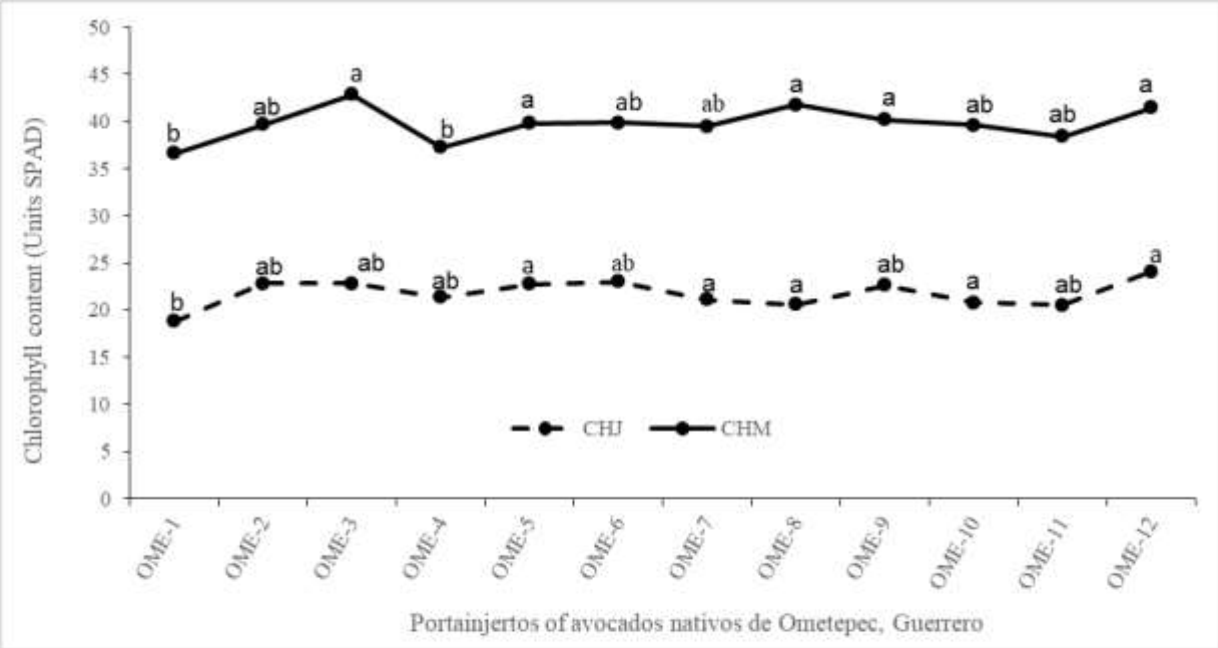

Figure 6. Chlorophyll content in young and mature leaves of rootstocks of avocados native to Ometepec, Guerrero. From OME-1 to OME-12 (avocados collected in Ometepec). CHJ and CHM (Chlorophyll in young and mature leaves). (Tukey (P ≤ 0.01).

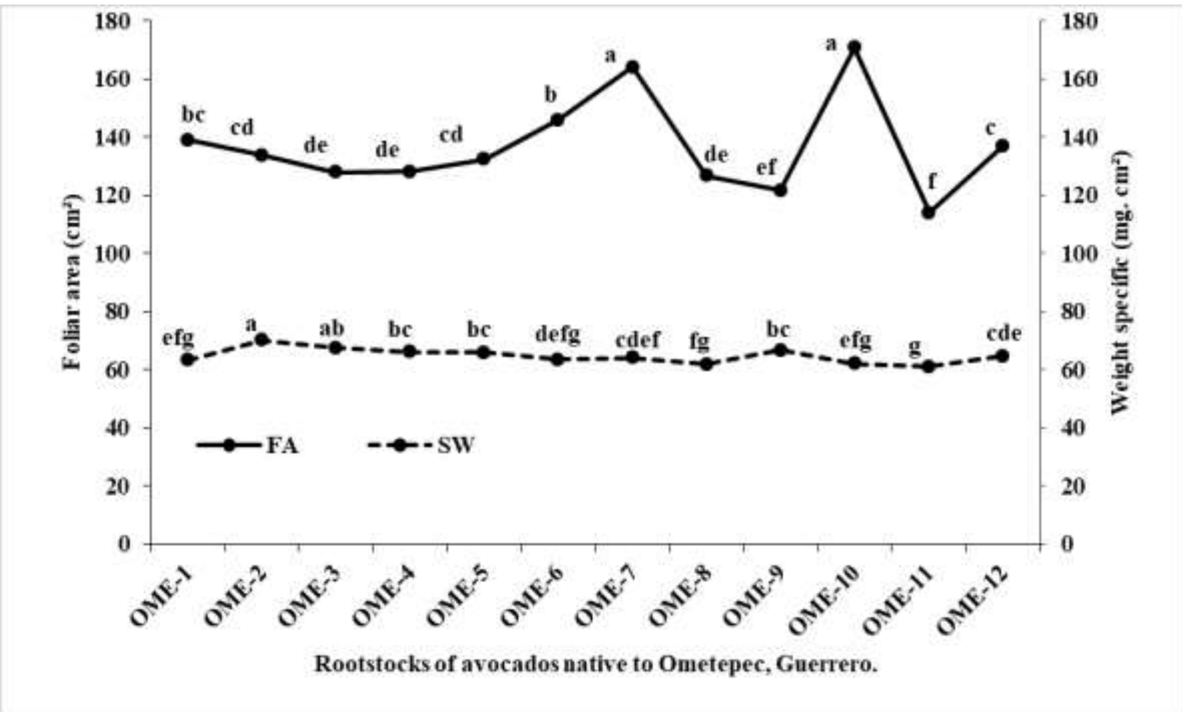

Figure 7. Foliar area and specific weight in rootstocks of avocados native to Ometepec, Guerrero. From OME-1 to OME-12 (avocados collected in Ometepec). FA (Foliar area) and SW (specific weight), Tukey (P ≤ 0.01).

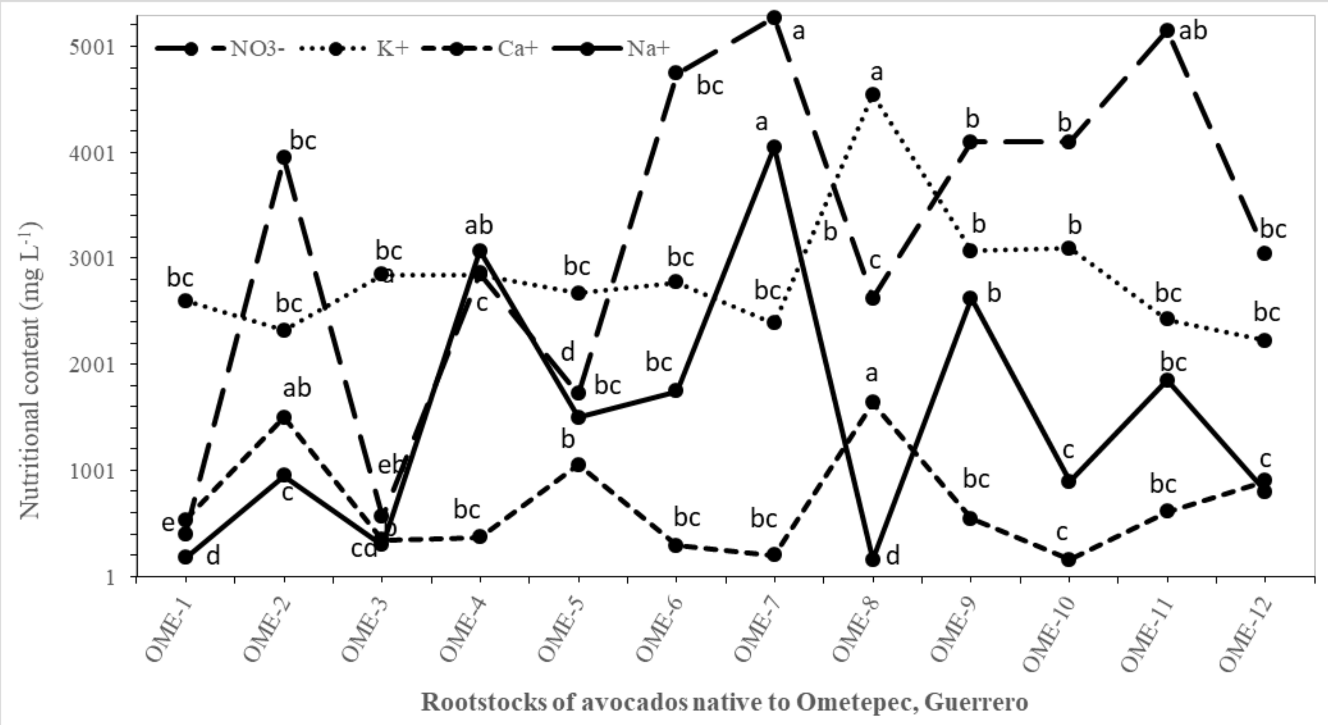

Figure 8. Nutritional content of NO=3, K+, Ca++ y Na+, in avocado native to Ometepec, Guerrero. From OME-1 to OME-12 (avocados collected in Ometepec), LSD (P ≤ 0.05).

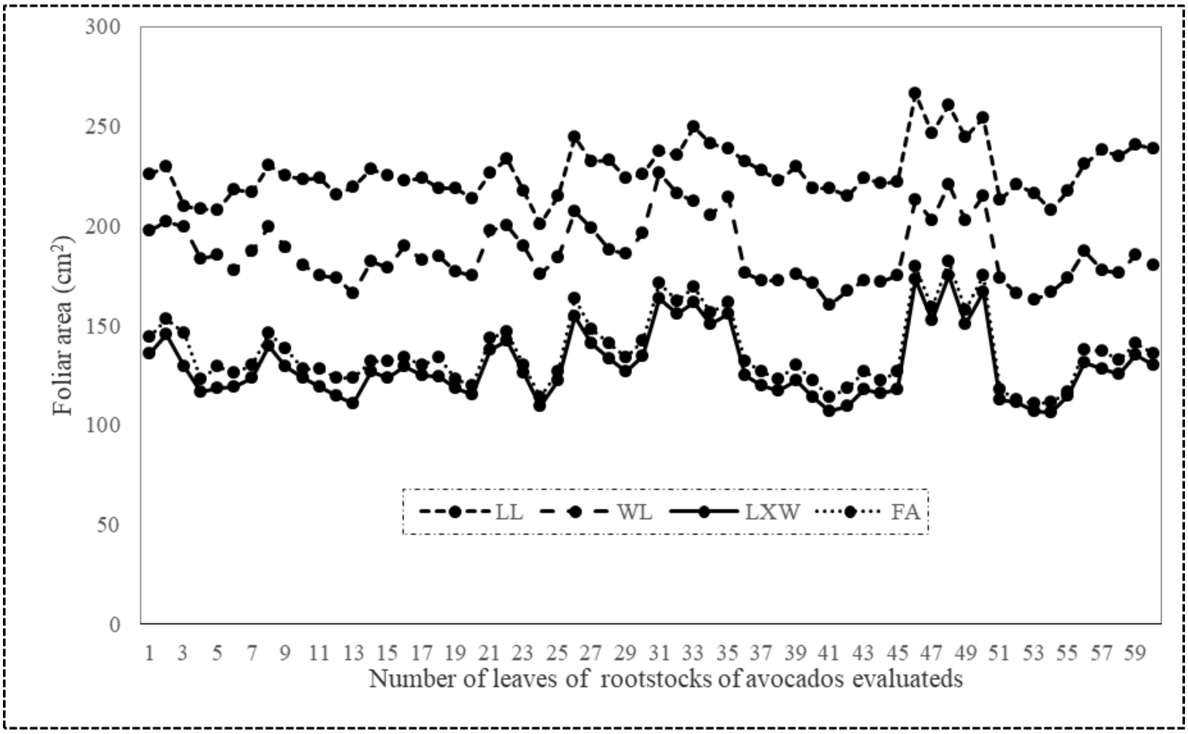

Figure 9. Determination of foliar area through length, width and length x width, multiplied by a correction factor in rootstocks of avocados native to Ometepec, Guerrero. LL (leaves length). WL (leaves width), L X W (length x width) and FA