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Histological Variations in Cucumber Grafted Plants and Their Effect on Yield

Valentín Robledo-Torres [1], Areli González-Cortés [1,2,*], Laura Raquel Luna-García [1], Rosalinda Mendoza-Villarreal [1], Miguel Ángel Pérez-Rodríguez [3] and Neymar Camposeco-Montejo [4]

[1] Departamento de Horticultura, Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro 1923, Saltillo 25315, Coahuila, Mexico; robledo3031@gmail.com (V.R.-T.); lauraluna_1985@hotmail.com (L.R.L.-G.); rosalindamendoza@hotmail.com (R.M.-V.)
[2] Instituto de Ciencias y Humanidades, Universidad Autónoma de Coahuila, Avenida Cuquita Cepeda de Dávila s/n, Adolfo López Mateos, Saltillo 25125, Coahuila, Mexico
[3] Departamento de Botánica, Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro 1923, Saltillo 25315, Coahuila, Mexico; miguel_cbg@hotmail.com
[4] Centro de Capacitación y Desarrollo en Tecnología de Semillas, Departamento de Fitomejoramiento, Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro 1923, Saltillo 25315, Coahuila, Mexico; neym_33k@hotmail.com
* Correspondence: areli.gonzalez@uadec.edu.mx; Tel.: +52-844-111-2553

Abstract: Cucumber is a vegetable crop of economic importance in Mexico. In 2023, fresh or frozen cucumber and pickle international sales reached USD 783M, placing Mexico in second rank among countries with the highest currency inflow in the world. This crop is mostly grown under protected environments with new production technologies, including rootstocks that are tolerant to biotic and abiotic factors that limit the yield and quality of the harvest. Therefore, the goal of this work was to assess three cucurbits as rootstocks (*Cucurbita maxima*, *Cucurbita moschata* and *Citrullus lanatus*), as well as studying the response of cucumber crop to the tested rootstocks. The following treatments were assessed: T1:PSP = cucumber Cv. Paraizo F1 without rootstock; T2:P/MA = cucumber grafted over *Cucurbita maxima*; T3:P/MO = cucumber grafted over *Cucurbita moschata*; T4:P/CL = cucumber grafted over *Citrullus lanatus* Citroides Var. The assessed variables included the number of fruits per plant (NFPP), fruit weight per plant (PFPP), average fruit weight (PPF), plant dry weight (PSP), xylem vessel area (AVX), number of xylem vessels (NVX), vascular bundle area (AHV) and number of vascular bundles (NHV) in roots (R), basal stalk (TB) and apical stalk (AT). Significant differences among treatments were found in the results for variables: NFPP, highlighting that T2:P/MA was superior by 46.15% versus T1:PSP and by 67.69% versus T3:P/MO. Regarding PFPP, T2:P/MA achieved the highest weight (5445 g), as it proved superior by 99.45% than the cucumber without rootstock (T1:PSP). Regarding PSP, T2:P/MA achieved the highest weight (196.65 g), significantly exceeding T1 by 137.5%. Regarding AVXR, T4:P/CL had the highest value (30,063 μm^2). Regarding NHVR, watermelon had (T4:P/CL) 39.33% more vascular bundles than the root system of cucumber (T1:PSP). Treatments T2:P/MA and T4:P/CL had the highest values at AVXTB, while for variable NVXTB, treatments T3:P/MO and T2:P/MA were almost equal, surpassing non-grafted cucumber by 175.47%. However, T2:P/MA showed the greatest AHVTB, and T1:PSP was significantly superior to T3:P/MO, by 34.93%, for NHVTB. Furthermore, positive and significant correlations were found among PFPP, AHVR, and AVXTB, as well as among PSP, AVXTB, and AHVTB. Therefore, we may say that having a larger area of vascular vessels in the roots and a larger area of xylem vessels in the basal stalk leads to higher fruit yields per plant. In the same vein, the use of rootstocks is a sustainable alternative to increase fruit yield.

Keywords: rootstock; anatomy; histology; vascular bundles; xylem; yield

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Citation: Robledo-Torres, V.; González-Cortés, A.; Luna-García, L.R.; Mendoza-Villarreal, R.; Pérez-Rodríguez, M.Á.; Camposeco-Montejo, N. Histological Variations in Cucumber Grafted Plants and Their Effect on Yield. *Agronomy* **2024**, *14*, 1377. https://doi.org/10.3390/agronomy14071377

Academic Editor: Vincenzo Candido

Received: 4 June 2024
Revised: 18 June 2024
Accepted: 25 June 2024
Published: 26 June 2024

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1. Introduction

Cucumber is a vegetable crop of high economic impact, considering that it is an export crop that is grown and consumed in many regions of the world. In 2023, Mexico reached USD 783M in international sales of fresh and frozen cucumbers, ranking second among the countries with the highest currency inflow in the world—Sinaloa (USD 783M), Sonora (USD 124M), Michoacan (USD 79.5M), Baja California (USD 74M) and Jalisco (USD 58.8M) being the main exporting Mexican States [1]. However, Queretaro state has achieved the best yields in the country, with an average of 268.67 t.ha^{-1} [2]. The current use of technology in agriculture has modified food production and it undergoes constant changes in order to deal with the emerging challenges, while increasing fruit yields [3]. This is the case of protected agriculture, which has experienced a significant increase in recent years, reaching 47,795 ha in 2021 [4]. Cucumber cropping encompasses approximately 9.52% of the surface area under protected agriculture in Mexico [5]. Growing vegetable crops like cucumber under protected environments produces 2- to 9-fold higher yields than conventional production systems [6]. On the other hand, the most vulnerable aspects of traditional Mexican agriculture are water stress and adverse weather conditions, excessive use of fertilizers and aggressive or hazardous chemical applications. Furthermore, in open-field agriculture, rain washes out fertilizers, making them less effective and causing damage to other ecological systems. It has been estimated that medium-and high-technology protected agricultural facilities produce much lower Greenhouse Gas fingerprints and make more efficient use of energy, water and chemicals [7].

Grafting is an additional solution to these issues, leading to sustainable agricultural production and can also be practiced in protected agriculture. Grafting has been defined as the method used to splice two sections of different plants, promoting their development as a single plant. [8]. An important point in using grafting as a quick plant breeding technique is to use rootstocks with special qualities (disease resistance, high water and nutrient uptake capacity, salinity tolerance, etc.) that can benefit the grafted plants [9,10]. The use of grafted plants provides ecological benefits and reduces pesticide application costs, fostering a more efficient use of resources and greater environmental sustainability [3].

Likewise, the vascular system in plants is linked to cell differentiation and the vascular cambium activity, which can be correlated to plant growth and the hormonal balance of auxins, cytokinins and brassinosteroids. However, the rootstock–scion physical interactions can cause alterations in the xylem vessels and in the vascularization pattern [11]. The larger size of vascular bundles and xylem vessels is crucial to transport water and nutrients in grafted plants, because the yield increases and the plants' adaptation to adverse conditions will depend on such transportation [12]. The rootstock–scion interaction modifies the rootstock's epigenome, providing traits that are not expressed by non-grafted plants [13], leading to potential changes in the anatomic and morphological plant structures. These changes can have negative or positive impact on fruit bearing [11].

Therefore, this work proposes the use of grafting in order to analyze the changes occurring in the vascular system of grafted cucumber plants that may have an impact on the yield and quality of cucumber crop.

2. Materials and Methods

The research work was conducted in a tunnel-shaped greenhouse with polyethylene cover (600 gauge; 80% light transmission) and overhead ventilation, located within the premises of "Universidad Autónoma Agraria Antonio Narro" in Saltillo, Coahuila, Mexico (25°21′22″ N, 101°02′9″ W, at an altitude of 1760 m asl), from August to December 2022.

Plant Material: The scion was a cucumber commercial hybrid (*Cucumis sativus* L.) Cv. Paraizo F1 (American slicer fruit) by Enza Saden (Spain) and the rootstocks were landrace pumpkin cultivars (*Cucurbita maxima* Duch. and *Cucurbita moschata* Duch.) and watermelon (*Citrullus lanatus* Thunb. Citroides Var.). The cucumber hybrid and the rootstocks were planted in polystyrene trays of 128 cavities, in a germination substrate of Sphagnum Premier Peat (Angeles Millwork & Lumber Co, Port Angeles, WA, USA) and

perlite, at a ratio of 70:30, respectively. Cucumber seeds were planted on 24 August 2022 and the rootstock seeds were planted on 29 August in order to achieve the right synchronization of the stalk diameter in the planting material. After nine days, the spike grafting technique was performed [14]. The grafted plants were placed inside a healing chamber (4 m long × 3 m wide × 2 m high, 600 gauge polyethylene) for five days, with temperatures ranging between 23 and 26 °C and 70 to 85% RH. The plants were then placed under the greenhouse and shade mesh to be conditioned during two days, with 50% lower solar radiation, before being transplanted into the greenhouse on 15 September 2022.

Crop Establishment and management: The experiment was established under a fully randomized block design with four treatments, ten plants per treatment and three replicates. The treatments included T1:PSP = cucumber Cv. Paraizo F1 without rootstock; T2:P/MA = cucumber grafted over *Cucurbita maxima*; T3:P/MO = cucumber grafted over *Cucúrbita moschata* (P/MO); T4:P/CL = cucumber grafted over *Citrullus lanatus* Citroides Var. The plants from each treatment were established in greenhouse soil, on 1.5 m-wide, 30 cm-long beds, leaving 40 cm between plants. Each bed had black plastic mulch and 6000-gauged sub-surface drip irrigation lines (Irritec Tape®, Capo d'Orlando, Italy), with drippers at 30.5 cm, at 1-LPH flow rate. A Steiner Nutrient Solution was used to fertilize the plants [15]. The first application was performed at 50% after transplanting, with some modifications based on the results from the irrigation water test. The plants were pruned to promote the development of two stalks, removing the lateral shoots. Each stalk had a clamping ring (HORTOCLIPS®, Buenos Aires, Argentina) attached to a raffia cord, allowing their vertical movement. The cord was supported by galvanized steel wire installed 3.5 m-high along the greenhouse.

For the purpose of this essay, the plants were harvested six times, picking fruits of at least 17 cm in length.

Assessed variables:

Agronomic variables: Weight of fruits per plant (PFP) measured in g, for which three plants per treatment were harvested and obtained from the sum of all the fruits of six cuts using an electronic scale (SARTORIUS-TS 1352Q37®, Goettingen, Germany). The number of harvested fruits per plant (NFPP) was calculated at the end of the harvest, counting all fruits obtained throughout the six harvests. Average fruit weight (PPF) in g, estimated based on the total fruit weight/number of harvested fruits. Plant dry weight (PSP) in g, which was obtained after cutting the plants, following the sixth harvest. The cutting was made at the basal stalk, 8 cm below the splitting of the two productive stems. The plants were immediately bagged in brown paper and were placed inside a drier oven (Yamato®, Miyagi, Japon) for 96 h at 50 °C. The dry matter was weighed later using a digital scale (Valtox®, Lima, Peru).

Histological Analysis: The samples were taken two days after the sixth harvest. Three plants per every repeated treatment were processed in the laboratory using the paraffine histological technique described herein: In order to preserve the tissues and minimize potential alterations, the samples were placed inside 15 mL-glass flasks with a fixing agent FAA (Formaldehyde (36–40%) at 5 cc; ethylic alcohol (70%) at 90 cc and glacial acetic acid at 5 cc). The samples were dehydrated in ethylic alcohol concentrations of 50, 60, 70, 85 and 96%, plus eosin, followed by absolute ethylic alcohol I, absolute ethylic alcohol II, absolute ethylic alcohol plus xylol at a volume-based ratio of 3:1, absolute ethylic alcohol plus xylol at a ratio of 1:1, absolute ethylic alcohol plus xylol at a ratio of 1:3, and lastly, the tissues were placed in pure xylol and remained in every solution for 2 h, imbibed in paraffine with a melting point of 58 °C. Alcohol was later replaced by xylol [16–18]. Once the necessary samples were obtained, the paraffine was introduced in the following way: 20-micron cross-cuts were made in the tissues with a rotary microtome (LEICA®, Wetzlar, Alemania). The cross-cuts were placed in slides and dyed with the Perez and Tomasi method [19]. A double safranine-fast green dyeing staining was applied, passing through different alcohol and xylol solutions until the preparations were taken out from the last xylol solution to be drained. They were sealed with a drop of Canada's balsam

and a coverslip of the same size than the tissue, before being dried in the oven (THELCO GCA-PRECISION CAT. No 31468. USA) at 30 °C for a week. The tissues were analyzed under a CARL ZEISS® compound microscope (White Plains, NY, USA)with an integrated Pixera Wiender Pro digital camera (Wallern, Austria). The best samples were selected and their stalk tissue was photographed with a 2.5× zooming target, as well as the root tissue with a target of 5× [20]. The measurements were made on digital images with Axion Vision Rel. 4.8 measuring software.

Micro-anatomic variables:

(a) The average xylem vessel area (AVXR), the number of xylem vessels per vascular bundle (NVXR), the vascular bundle area in the root (AHVR) and the number of vascular bundles in the root (NHVR) were calculated using roots of 3 mm in diameter and 10 cm in depth.
(b) The same variables than in the previous case were estimated, including the xylem vessel area (AVXTB), number of xylem vessels per vascular bundle (NVXTB), vascular bundle area (AHVTB), number of vascular bundles (NHVTB) at the basal stalk, 8 cm below the splitting of the two productive stems, as well as below the grafting point, in all treatments.
(c) At the section between the 5th and 6th leaves, below the plant apex the following variables were estimated: xylem vessel area (AVXTP), number of xylem vessels per vascular bundle (NVXTP), finally the vascular bundle area (AHVTP) and the number of vascular bundles (NHVTP).

Statistical analysis: Variance analysis ($p \leq 0.05$) and Tukey's mean test were applied to those cases were significant differences among treatments were found. Pearson's (r) correlation coefficient was calculated among the test variables. Statistical data package "Statistics Analysis System [21] 9.0" was used to analyze the data.

3. Results and Discussion

Agronomic variables: Significant differences ($p \leq 0.05$) were found among the treatments in NFPP, with T2:P/MA being almost equal to T4:P/CL, highlighting that T2:P/MA was superior by 46.15% versus T1:PSP and by 67.69% versus T3:P/MO. Regarding PFPP, significant differences were also found ($p \leq 0.05$) among treatments. T2:P/MA achieved the highest weight (5445 g), being inside the same statistical group to treatments T4:P/CL and T3:P/MO; however, it proved superior by 99.45% than the cucumber without rootstock (T1:PSP) (Table 1). Regarding NFPP and PFPP, a major increase was found in grafted plants versus non-grafted plants, matching the observations of other research works with grafted versus non-grafted plants of bell pepper and watermelon [22–24]. However, other research works have reported low yields of plants that were grafted in different rootstocks, as compared to non-grafted cucumber plants [25]. One reason is the lack of compatibility between the scion and the rootstock leads to poor yields; and on the other hand, some authors point out that grafting creates a barrier that impairs water movement, resulting in lower hydraulic conductance of the stem [26].

Table 1. Effect of the treatments on agronomic variables: Number of fruits per plant (NFPP), fruit weight per plant (PFPP), average fruit weight (PPF) and plant dry weight (PSP).

Treatment	NFPP	PFPP (g)	PPF (g)	PSP (g)
T1:PSP = cucumber without rootstock	13.00 B	2730.0 B	208.42 A	82.80 C
T2:P/MA = cucumber/*C. maxima*	19.00 A	5445.0 A	286.55 A	196.65 A
T3:P/MO = cucumber/*C. moschata*	11.33 B	3503.9 AB	305.44 A	153.18 B
T4:P/CL = cucumber/*C. lanatus*	14.00 AB	4270.0 AB	304.92 A	159.39 B
CV (%)	14.6	22.0	14.6	3.80
DMS	5.94	2484.1	114.54	15.93

Significance levels at $p \leq 0.05$. Values with different letters per column indicate statistical differences by Tukey's mean test $p \leq 0.05$.

Regarding PPF, no significant differences were found among treatments, coinciding with the remarks of Cushman and Huan, [27], who stated that in order to obtain high yields, it is important to select grafts capable of the increasing number of fruits per plant, which is an essential factor to forecast high yields in grafted plants, highlighting the fact that total yield depends on the number of fruits per plant, instead of depending on the size of individual fruits. Regarding PSP, T2:P/MA achieved the highest weight (196.65 g), significantly exceeding by 137.5% the dry weight of T1:PSP, stressing the fact that grafted treatments showed greater accumulation of dry matter than the non-grafted treatment, coinciding with the results of Godoy et al. [28], who, on assessing the effect of grafting and nutrition in tomato, noticed that grafted plants showed more vigor, leading to an increase of 9% in the accumulation of dry matter.

As with the yield, PSP was strongly impacted by the rootstocks, since in this variable, we found up to 237% more dry matter in grafted versus non-grafted plants, coinciding with the results of Godoy et al. [28] and Velasco et al. [8] in *Solanum lycopersicum* and Calatayud et al. [29] in *Capsicum annuum*, who reported a higher dry weight in grafted plants, compared with the same cultivars without grafting. The highest accumulation of dry matter in grafted plants is the result of higher nutrient uptake, since the number of roots in grafted plants was twice the number of roots in non-grafted cucumber plants, resulting in 30% more nitrogen and potassium uptake, as well as 80% more phosphorous uptake in grafted plants with access to phosphorous reserves at deeper soil horizons as in *Solanum lycopersicum* [30].

Micro-anatomic variables

Root: Significant differences were found for variables AVXR, AHVR and NHVR among treatments, except for variable NVXR.

T4:P/CL had the highest value (30,063 μm^2) in AVXR, although it was almost equal to the areas found in treatments T2:P/MA and T3:P/MO. These three rootstocks had significantly ($p \leq 0.05$) larger xylem vessel areas, as compared to T1:PSP (Figure 1). T4:P/CL was superior by 175.12%, over the non-grafted cucumber. On the other hand, at NVXR, treatment T4:P/CL with the watermelon rootstock had 41.13% more xylem vessels than T3:P/MO, although no significant differences were found.

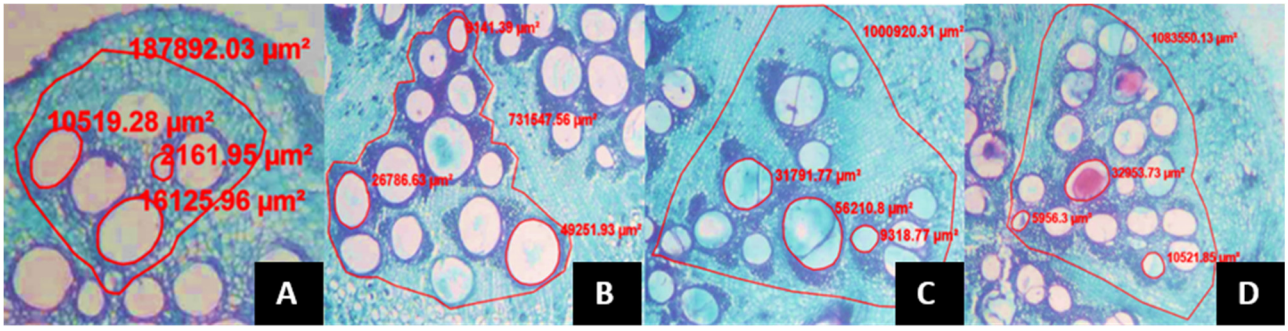


Figure 1. Xylem vessel area and vascular bundle area in the roots of cucumber rootstocks; (**A**) T1:PSP = cucumber without rootstock, (**B**) T2:P/MA = cucumber/*C. máxima*, (**C**) T3:P/MO = cucumber/*C. moschata*, and (**D**) T4:P/CL = cucumber/*C. lanatus*.

Treatment T2:P/MA had the highest AHVR (1,621,251 μm^2), exceeding by 498.48% the area found in T1:PSP (Figure 1). It must be noted that there were also significant differences among the rootstocks—T2:P/MA was superior by 185.59% than T4:P/CL and by 61.47% than T3:P/MO. Regarding NHVR, the three rootstocks had significantly similar numbers, but they were significantly superior to the numbers found in non-grafted cucumber. Watermelon had (T4:P/CL) 39.33% more vascular bundles than the root system of cucumber (T1:PSP) (Table 2). The previous results show the effect of tested genotypes and their interaction with the environment where they evolved, in order to adapt themselves to the

conditions of their place of origin. These adaptations involve the diameter of xylem vessels, as well as the number of xylem vessels or the number of vascular bundles. Paul et al. [31] concluded that genotypes with smaller xylem vessels are the most susceptible to blossom end rot, due to a reduction in their calcium transportation capacity. Furthermore, rootstocks with larger vessels have greater capacity to carry water and nutrients to the stalks, as has been observed in tomato rootstocks [12].

Table 2. Effect of the treatments over the root micro-anatomic variables: average xylem vessel area (AVXR), number of xylem vessels per vascular bundle (NVXR), vascular bundle area (AHVR) and number of vascular bundles (NHVR).

Treatments	Mean Values from the Root			
	AVXR (μm^2)	NVXR	AHVR (μm^2)	NHVR
T1:PSP = cucumber without rootstock	10,927 B	12.557 A	270,894 C	3.11 B
T2:P/MA = cucumber/*C. maxima*	27,988 A	13.223 A	1,621,251 A	3.89 A
T3:P/MO = cucumber/*C. moschata*	25,006 A	10.000 A	1,004,008 C	4.22 A
T4:P/CL = cucumber/*C. lanatus*	30,063 A	14.113 A	567,684 B	4.33 A
CV (%)	14.81	22.34	113.25	5.87
DMS	9838.1	7.87	324,311	0.64

Significance levels at $p \leq 0.05$. Values with different letters per column indicate statistical differences with Tukey's mean test $p \leq 0.05$.

Basal stalk: Significant differences were found in AVXTB, NVXTB, AHVTB and NHVTB ($p \leq 0.05$) among treatments (Table 3).

Table 3. Effect of the treatments over the micro-anatomic variables at the basal stalk: xylem vessel average area (AVXTB), number of xylem vessels by vascular bundle (NVXTB), vascular bundle area (AHVTB) and number of vascular bundles (NHVTB).

Treatments	Mean Values at Basal Stalk			
	AVXTB (μm^2)	NVXTB	AHVTB (μm^2)	NHVTB
T1:PSP = cucumber without rootstock	8360 C	13.25 C	471,927 C	9.00 A
T2:P/MA = cucumber/*C. maxima*	22,838 A	29.00 AB	1,863,489 A	7.00 AB
T3:P/MO = cucumber/*C. moschata*	14,147 BC	36.50 A	1,179,155 B	6.67 B
T4:P/CL = cucumber/*C. lanatus*	19,338 AB	20.25 BC	1,070,061 B	8.50 AB
CV (%)	12.80	22.32	10.45	9.26
DMS	5852.6	15.61	338,541	2.03

Significance levels $p \leq 0.05$. Values with different letters per column indicate statistical differences with Tukey's mean test $p \leq 0.05$.

Mean comparison results indicate that treatments T2:P/MA and T4:P/CL had the highest values at AVXTB and were significantly superior to T1:PSP. Treatment T2:P/MA significantly surpassed the non-grafted cucumber by 173.18%, which showed the lowest value (Table 3, Figure 2). It is important to assess the diameter or the area of xylem, since in *Solanum lycopersicum* cultivation, it shows that the vessels' diameter is more important than the vessels' number, for hydraulic conductance [26]. It should also be noted that fruit-tree rootstocks can supply larger quantities of mineral nutrients to the shoots, due to an increase in the concentration of these mineral elements inside the xylem vessels and/or due to an increase in the transpiration rate [32].

While for variable NVXTB, treatments T3:P/MO and T2:P/MA were almost equal, the first one was significantly greater than T4:P/CL and T1:PSP, surpassing non-grafted cucumber by 175.47%. At the basal stalk, T2:P/MA showed the greatest AHVTB, being significantly superior to C. moschata and watermelon (T3:P/MO and T4:P/CL), which in turn were significantly superior, in terms of the vascular bundle area of T1:PSP. Therefore, the highest value was recorded in treatment T2:P/MA, exceeding by 294.86% the value

found in the cucumber without rootstock (Figure 2). For NHVTB, T1:PSP was significantly superior to T3:P/MO by 34.93% and was statistically similar to the rest of the treatments (Table 3). The vascular system in plants is very important for the transportation of water and mineral salts. In this regard, the vascular bundles and in particular the xylem vessels are crucial to maintain hydraulic conductivity and provide adequate supply to the foliar area of each plant. Furthermore the rootstocks are capable of making a difference before contrasting environmental conditions, due to their genetic plasticity. In this regard, Xiaohong et al. [33] mentioned that the grafting mechanisms impacting mineral nutrient uptake and transportation in vegetable crops are controlled by the rootstock genotype or by the rootstock–scion interaction, which can increase the tolerance of grafted vegetable plants to high/low levels of mineral elements.

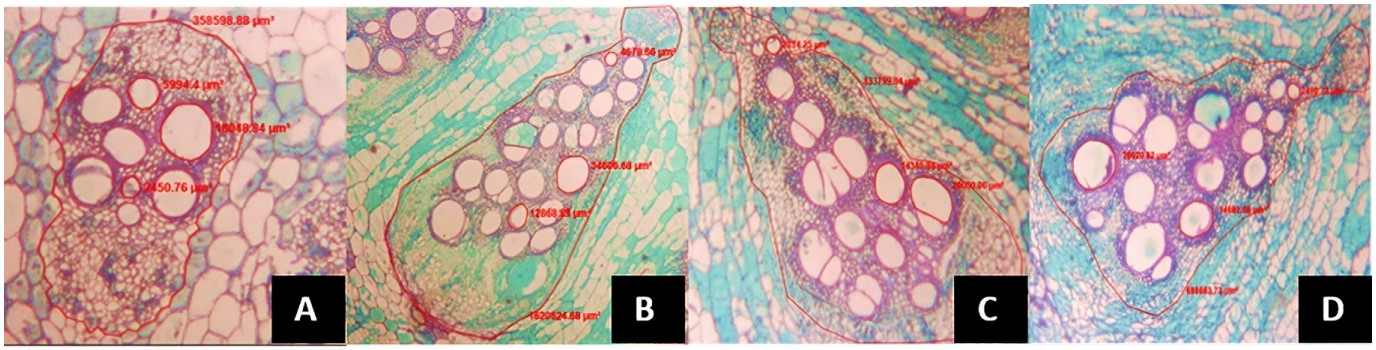


Figure 2. Area of xylem vessels and vascular bundles at the basal stalk of cucumber rootstocks; (**A**) T1:PSP = cucumber without rootstock, (**B**) T2:P/MA = cucumber/*C. maxima*, (**C**) T3:P/MO = cucumber/*C. moschata*, and (**D**) T4:P/CL = cucumber/*C. lanatus*.

Apical Stalk: No significant difference was found for variables AVXTP, NVXTP, AHVTP, NHVTP ($p \leq 0.05$) among treatments (Table 4, Figure 3). Its well known that different compounds are transferred between the rootstock and the scion, and vice versa, including plant growth regulators, which might influence the diameter of the xylem vessels or the vascular bundles when gibberellins mobilize; however, Osugi et al. [34], mentioned that in *Arabidopsis*, some regulators such as cytokinins and gibberellins are transported to the shoots through the xylem, promoting branching and elongation of the internodes, rather than the elongation of the xylem vessels.

Table 4. Effect of the treatments over the micro-anatomic variables at the apical stalk: Xylem vessel average area (AVXAT), number of xylem vessels per vascular bundle (NVXAT), vascular bundle area (AHVAT) and number of vascular bundles (NHVAT).

Treatments	Mean Values in Cucumber Grafting			
	AVXTP (μm²)	NVXTP	AHVTP (μm²)	NHVTP
T1:PSP = cucumber without rootstock	3747 A	14.917 A	263,516 A	9.833 A
T2:P/MA = cucumber/*C. maxima*	4749 A	13.250 A	316,058 A	10.333 A
T3:P/MO = cucumber/*C. moschata*	3644 A	16.667 A	266,838 A	9.667 A
T4:P/CL = cucumber/*C. lanatus*	5008 A	14.250 A	261,098 A	9.000 A
CV (%)	32.30	16.61	19.62	12.76
DMH	3914.8	6.93	153,607	3.50

Significance levels at $p \leq 0.05$. Values with different letters per column indicate statistical differences with Tukey's mean test $p \leq 0.05$.

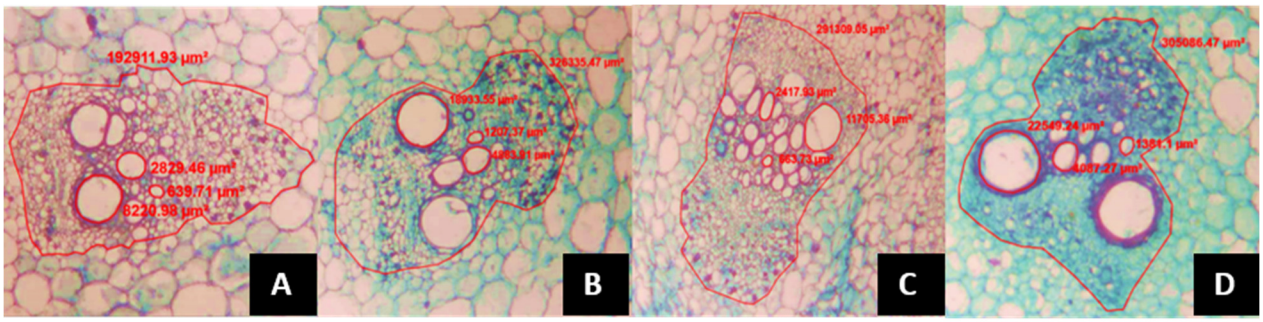


Figure 3. Area of xylem vessels and vascular bundles estimated at the section between the 5th and 6th leaves, below the apex of a cucumber plant with different rootstocks (**A**) T1:PSP = cucumber without rootstock, (**B**) T2:P/MA = cucumber/*C. máxima*, (**C**) T3:P/MO = cucumber/*C. moschata* and (**D**) T4:P/CL = cucumber/*C. lanatus*.

Correlation among variables:

The correlation analysis conducted among the test variables showed that at higher AHVR, there is also higher AVXTB. These results were obtained from the tests performed in the different rootstocks and highlight the importance of the vascular system in these plants, as well as their connection with mineral salts and water transportation. Nevertheless, the correlation analysis also showed that at higher NHVTB, there was a lower NVXTB per vascular bundle (Table 5).

The AHVR and AHVTB values were positively and significantly correlated with PFPP, having a positive impact on the total plant yield. It is important to stress that AVXR and NHVR showed a positive and significant correlation with PPF, contributing as well to an increase on biomass production.

The AVXTB and AHVTB values were positively and significantly correlated with PSPP. Thereof, it can be affirmed that the area of xylem vessels and the number of vascular bundles have a positive impact on fruit yield.

The highest numbers of vascular bundles and xylem vessels were obtained from the roots and stems of the rootstocks. Thus, the highest number of fruits and the highest fruit weight were obtained from grafted plants versus non-grafted plants. Hereinabove mentioned micro-anatomic traits, along with the high rootstock–scion compatibility of this essay were key to attain high yields. Tamilselvi and Pugalendhi mention that in the cultivation *Momordica charantia* ofgraft compatibility is defined as successful splicing between two plant sections [35], and this process depends on the hormonal quality of both sections, in particular from the auxin hormone as it happens in *Vitis vinifera* plants [36]. Later on, the behavior of the grafted plant will depend on the dynamic relation between the spliced sections [37]. Although, in this work, the rootstock did not have any effect on the apical stalk, and no significant differences were found among the treatments, it is important considering that a rootstock with a suitable root system, such as watermelon or pumpkin, may have a positive influence in other important variables, as has been pointed out by Milenkovi'C et al. [30], who indicated that the number of roots in grafted plants was twice the number of roots in non-grafted plants, resulting in 30% higher nitrogen and potassium uptake, and approximately 80% more phosphorus uptake by grafted plants, contributing to better nutrition and to understand the strong existing correlation between yield variables and the vascular system of plants. Therefore, rootstocks significantly improved nitrate content inside the xylem vessels and the latter are positively and significantly correlated with plant growth rates [32], which in turn might lead to higher fruit yields like in this research work.

Table 5. Pearson's correlation coefficients (r): Positive and significant correlations among variables estimated at the roots and basal stalks of three rootstocks and a cucumber crop, in Saltillo, Coahuila.

Studied Variables	AVXR	NVXR	AHVR	NHVR	AVXTB	NVXTB	AHVTB	NHVTB	AVXTA	NVXTA	AHVTA	NHVTA	NFPP	PFPP	PPF	PSP
AVXR	1.000															
NVXR	0.188	1.000														
AHVR	0.745	0.463	1.000													
NHVR	0.932	−0.066	0.461	1.000												
AVXTB	0.892	0.420	0.965	0.670	1.000											
NVXTB	0.578	−0.648	0.331	0.660	0.416	1.000										
AHVTB	0.761	0.076	0.919	0.557	0.901	0.664	1.000									
NHVTB	−0.541	0.578	−0.452	−0.551	−0.482	−0.974	−0.766	1.000								
AVXTA	0.704	0.828	0.789	0.466	0.829	−0.124	0.522	0.083	1.000							
NVXTA	−0.227	−0.873	−0.727	0.124	−0.599	0.392	−0.426	−0.232	−0.785	1.000						
AHVTA	0.331	0.187	0.831	0.031	0.676	0.341	0.846	−0.536	0.374	−0.634	1.000					
NHVTA	−0.227	−0.243	0.315	−0.412	0.098	0.278	0.454	−0.471	−0.253	−0.235	0.788	1.000				
NFPP	0.361	0.582	0.888	0.004	0.743	0.009	0.736	−0.200	0.667	−0.897	0.907	0.545	1.000			
PFPP	0.783	0.417	0.998	0.515	0.977	0.388	0.937	−0.499	0.776	−0.679	0.815	0.296	0.857	1.000		
PPF	0.950	−0.109	0.534	0.999	0.721	0.739	0.657	−0.654	0.451	0.095	0.155	−0.279	0.088	0.588	1.000	
PSP	0.910	0.130	0.903	0.751	0.955	0.668	0.961	−0.717	0.633	−0.370	0.681	0.192	0.624	0.930	0.821	1.000

Note: AVXR: average xylem vessel area (μm^2); NVXR: number of xylem vessels per vascular bundle; AHVR: vascular bundle area (μm^2); NHVR: number of vascular bundles; AVXTB: xylem vessel average area (μm^2); NVXTB: number of xylem vessels by vascular bundle; AHVTB: vascular bundle area (μm^2); NHVTB: number of vascular bundles; AVXTA: Xylem vessel average area (μm^2); NVXTA: number of xylem vessels per vascular bundle; AHVTA: vascular bundle area (μm^2); NHVTA: number of vascular bundles; NFPP: number of fruits per plant; PFPP: fruit weight per plant (g); PPF: average fruit weight (g); PSP: plant dry weight (g).

The better performance of the grafted plants is a consequence of the tolerance of the rootstocks to some pathogens or the greater tolerance to abiotic stress conditions. However, there are no studies that indicate the causes of the greater vigor that the rootstocks provide to the grafts. Anatomically, the water Flow can be affected by the number of xylem vessels, their diameter, and the capacity of plants to modulate stem water potential along the water path, as occurred in *Solanum lycopersicum* where it was observed that the rootstock has a vascular system with larger vessels in a higher density than non-grafted plants, but the stem hydraulic conductance is lower [32]. Tsaballa et al. [38] when doing a review on the Involvement of Epigenetics in Rootstock-Scion Interactions, pointed out the amelioration of the grafting technique requires further research on the molecular mechanisms that govern grafted plants performance and significantly impact the phenotype. In this research, it was possible to observe that the differences in the cross sections of the rootstocks influenced the phenotypic behavior of the grafts, which was confirmed. Therefore, it opens the possibility of using this knowledge for the development of superior rootstocks. Comprehensive knowledge about the grafted plants would greatly assist in the development of some desirable rootstocks to serve sustainable agriculture [39].

There is still limited knowledge about the physiological and genetic factors determining the interactions between the rootstock and the scion and vice versa [40]. In a word, there is an urgent need for studies on rootstock breeding and the rootstock–scion interaction in grafted vegetables [33]. In this research, the interaction of three genotypes with the same cucumber genotype was studied, and it was demonstrated that the genotype with the highest AHVR and AVXTB had the phenotype with the highest fruit yield per plant. Paul et al. [31] worked on the potato crop and concluded that those genotypes with smaller xylem vessels are more susceptible to blossom-end rot because of a reduction in transport capacity, highlighting the importance of xylem vessels in other important aspects of crop production.

4. Conclusions

The cucumber grafted on *Cucurbita maxima* (T2) generated positive results, since there was a greater number of fruits per plant, a greater weight of fruits per plant, more dry weight, a greater area and number of xylem vessels in the basal stem, as well as a greater area of vascular bundles in the basal stem. While the cucumber without rootstock (T1:PSP) generated a lower number and weight of fruits per plant, and lower dry weight, the same occurred with the anatomical variables area of xylem vessels in the root and there was lower number of areas of vascular bundles in the root.

In the present research, it was possible to confirm that the highest fruit weight per plant is highly correlated with the highest values of AHVR and AVXTB; therefore, if you want to develop rootstocks to achieve high PFPP, in the genetic improvement strategies, it will be necessary to consider the genotypes that present high values of AHVR and AVXTB. The aforementioned can be considered before releasing new rootstocks to the seed market.

Having a larger area of vascular vessels in the roots and a larger area of xylem vessels in the basal stalk leads to higher fruit yields per plant. In the same vein, the use of rootstocks is a sustainable alternative to increase fruit yield.

The results obtained in the present work indicate that the selection of appropriate rootstocks can be an alternative to achieve a high yield in cucumber cultivation, since the effects on yield are strongly correlated with the characteristics of the xylem vessel and bundles and vasculature of the stem and root of the rootstocks, indicating that the functions of these tissues influence the growth of the cucumber crop. Therefore, the number and area of vascular bundles and vessels of the stem and root xylem of the rootstock can be considered predictive variables of graft growth. The information obtained represents an advance in the generation of knowledge to reinforce decisions in the establishment of cucumber plantations.

Fruit yield is improved by larger areas of the xylem vessels in basal stalks, or by larger areas of the vascular bundles in the roots.

Author Contributions: Conceptualization, V.R.-T. and A.G.-C.; methodology, A.G.-C. and L.R.L.-G.; software, R.M.-V. and N.C.-M.; validation, V.R.-T. and M.Á.P.-R.; formal analysis, V.R.-T. and M.Á.P.-R.; writing—original draft preparation, A.G.-C.; writing—review and editing, V.R.-T., N.C.-M. and L.R.L.-G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Acknowledgments: To the National Council of Humanities Science and Technology (CONAHCYT, Mexico) and the Antonio Narro Autonomous Agrarian University, under the Postdoctoral Stays scheme in Mexico 2022 (3).

Conflicts of Interest: The authors declare no conflict of interest.

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