



Article Effect of Pepper Rootstocks as a Sustainable Alternative to **Improve Yield and Fruit Quality**

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Abstract: Sustainable agriculture is a good alternative for the healthy production of food in the present, without the risk to global agriculture in the future. Therefore, feasible production techniques have been sought, such as the use of rootstocks, which provide tolerance to biotic and/or abiotic stresses, to avoid an excessive use of agrochemicals and damage from pathogens, without affecting crop production. The objective of this study was to analyze the morphological and productive adaptation of three commercial hybrids (Avante, DiCaprio and Ucumari) grafted onto three rootstocks (Fundación, Yaocali and UAN), in order to evaluate and determine their effect on the quality and yield of bell pepper fruit, which has a high demand and great economic potential. Significant differences were found between the rootstocks and hybrids for the commercial classification of the fruits, with Yaocali producing larger fruits, which have the highest value in the export market, surpassing the UAN with 63.9%. The DiCaprio graft outperformed the Ucumari interactions by 231.5%, and the Avante interactions by 142.8%, for the extra-large (XL) fruit production. Based on the results obtained, it is possible to conclude that the grafted plants improved the quantity of the fruits of higher classification. However, they did not have a significant influence on the yield and quality of the fruits, which could be attributed to the lack of compatibility between them; therefore, the vigor of the plant was not expressed. This study showed that the rootstock did not influence the yield and fruit quality components of the grafts.

Keywords: Capsicum annuum; abiotic stress; greenhouse; root system

1. Introduction

Currently, global agriculture faces significant challenges that put it at risk [1-4], compromising the healthy production of food and leading the population to a state of food insecurity [5]. As an example of this, at the beginning of 20th century, losses in horticultural production increased due to soil-borne pathogens [6], a problem that prevails today. Additionally, conventional agronomic practices use synthetic chemical products for their control, which bring about strong environmental problems such as affecting the biodiversity, degrading soils, destroying the ozone layer and causing significant risks for those who manipulate them [7,8]. Therefore, it is imperative to search for more sustainable alternatives. One of most viable options is the use of rootstocks, which increase crop production [9] and improve fruit quality to counteract the uncertainty of food shortages, in addition to being more resistant to soil pathogens [10], avoiding an indiscriminate use of biocides that harm the environment and promoting the rational use of agrochemicals.



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Grafting is a process of plant propagation that involves joining two plants with different characteristics, which are forced to develop a vascular connection forming a single plant [11,12]. This process consists of a rootstock and a graft or variety/hybrid, with the first playing a crucial role as it contains the genes for resistance or tolerance to biotic or abiotic stress [13]. The main advantage of grafting is that it does not contaminate the environment [6], favoring the elimination of biocides. Currently, studies on grafts have reported significant advances in the field of vegetables [14]. For instance, the potential to increase important properties for human consumption, such as certain amino acids [15], product safety, nutraceutical content [16] and some antioxidants [17,18]. They also provide greater root mass, which allows a high exploration capacity, favoring greater absorption of water and nutrients, improving vigor, development, flowering, fruiting and longevity of the plant [19,20]. Consequently, this could enhance the utilization of fertilizers applied during the production cycle, resulting in fruits of higher commercial quality, in addition to generating resistance to salinity [21] and other factors. Rootstocks are essential for increasing plant vigor, enhancing fruit yield and improving nutrient absorption. They also help reduce fungal infections in soil and the need for agrochemicals, as well as improving tolerance to adverse temperatures, salinity and drought [22].

Around the world, millions of hectares of chili peppers are cultivated, mainly of the species *Capsicum annuum* L. In Mexico, chili is the second most important horticultural crop after tomato. In 2021, green chili pepper production reached MXN 34,012 million, and the value of fresh bell pepper exports amounted to USD 1366 million; with a per capita consumption of 17.2 kg, it ranks as one of the main foods of the population. In recent years, Mexico has become the leading exporter of fresh peppers with a world share of 29 percent [23]. The inclusion in internal and external markets that are increasingly specialized and demanding requires products of higher quality and safety. These characteristics are essential for food to nourish consumers without causing harm to their health due to contaminants [24]. Moreover, these products should also contribute to environmental preservation. In Mexico, there are several research studies that have demonstrated the benefits of using grafting and rootstocks in Capsicum annuum. One example is Gisbert-Mullor et al. [25], who subjected grafted and non-grafted peppers to water stress and found higher dry weight and root volume in the grafted plants. This is useful for reducing water usage in the plant without reducing the yield and fruit quality. Other relevant studies, as cited by Sanchez et al. [20], Navarrete-Mapen et al. [26], Penella et al. [27] and Camposeco-Montejo et al. [28], have used grafting and rootstocks to improve the safety, tolerance and/or resistance to stresses and the yield and nutraceutical quality of the bell pepper, with excellent results. However, new materials continuously emerge in the market. Therefore, the objective of this research was to analyze the morphological and productive adaptation of three commercial bell pepper hybrids grafted onto three rootstocks (two commercially available first generation and one created by the authors), to evaluate and determine the effect of these grafting combinations on the quality and yield of the bell pepper fruits, which have a high demand and great economic potential. The study aimed to demonstrate that grafting is a viable option to address current agricultural challenges.

2. Materials and Methods

2.1. Location of Experimental Site

The experiment was conducted in a greenhouse located in the Department of Horticulture, Universidad Autonoma Agraria Antonio Narro (UAAAN) in Saltillo, Coahuila, Mexico (located at 25°21′24″ N and 101°02′05″ W, at an altitude of 1762 masl); temperature conditions during the development of crop were from 16 to 32 °C; relative humidity was from 60 to 90% and photosynthetically active radiation was from 800 to 1300 W m⁻² s⁻¹.

2.2. Genetic Material

Three rootstocks were used, which are described as follows:

- Foundation (F1 from *Rijk Zwaan*, Culiacan, Sin. Mx [29]): A first generation rootstock that produces compact, highly productive and early-maturing plants, with strong root system and tolerance to cold soils and environments, as well as resistance to *Tobacco Mosaic Virus* (TM) and *Potato Virus* (PVY) and good resistance to nematodes.
- Yaocali (F1 from *Enza Zaden*, Culiacan, Sin, Mx [30]): A rootstock known for its high production, fruit quality and early crop maturity. Its strong root development promotes plant vigor and structure. It is recommended for crops exposed to root asphyxia, soil pathogens and high salinity. It also exhibits good resistance to TM.
- UAN (developed material at UAAAN-Saltillo, Coah. Mx): A vigorous plant with an excellent root system and high resistance to *Fusarium oxysporum* (Fo). It is an early and productive rootstock option.

The grafts consisted of three commercial bell pepper hybrids, described as follows:

- Avante (F1 from *Rijk Zwaan*, Culiacan, Sin. Mx [29]): High quality fruits (L and XL) with 3 and 4 locules. They have a bright red color with thick walls, providing a long shelf life. The plant exhibits excellent fruit set and high yield. It shows high resistance to *Phytophtora capsici* (Pc) and soil nematodes.
- DiCaprio (F1 from *Enza Zaden*, Culiacan, Sin, Mx [30]): A vigorous and early-maturing plant with good foliage coverage. It produces high quality fruits with great uniformity, predominantly extra-large (XL) and large (L) fruits with 4 locules. The fruits have thick walls, good firmness and a yellow color.
- Ucumari (F1 from *Enza Zaden*, Culiacan, Sin, Mx [30]): A highly productive plant with superior fruit quality. It exhibits quick and uniform fruit ripening, firm locules, a long shelf life and an orange coloration. The fruits have high resistance to TM.

2.3. Establishment of the Experiment

The seeds of grafts and rootstocks were sown in 200-cell polystyrene seedling trays, using a germination medium consisting of a mixture of Premier Sphagnum Peat moss (Premier Horticulture Inc. Quakertown, PA, USA) and perlite mineral (Hortiperl, Termolita, Santa Catarina, N.L, Mx) in a 4:1 ratio, respectively.

Grafting was performed 33 days after sowing when the materials exhibited high synchronization in stem thickness. The grafting technique used was the "cleft" method under aseptic conditions. The procedure involved making a "barb" cut on the hybrid plant and a cleft on the cotyledon leaves of the rootstock. The cleft cut was made in the center of stem, which was 1.5 cm long. Subsequently, the barb of the hybrid plant was inserted into the cleft, and a 2.5 mm silicone clip was used to provide support. Subsequently, they were placed in a rooting chamber that provided favorable temperature conditions of 24 ± 2 °C and relative humidity of $83 \pm 4\%$. For the first 48 h, they were kept in darkness to prevent cellular oxidation at the grafting site and promote the healing and acclimatization process. The following 6 days consisted of regular day–dark cycles (24 h/day). The healing and acclimation phase was achieved within a period of 16 days, resulting in a total of 50 days from sowing to acclimation.

The transplanting in the greenhouse was undertaken directly into the soil, using a raised-beds system with a plant-to-plant spacing of 33 cm, resulting in a density of 3.7 plants per square meter. Each bed was covered with black plastic mulch, and a drip irrigation system was used. The crop management followed standard procedures for cultivation. The nutrient solution used was based on the formulation proposed by Steiner [31] and applied as follows: 50% at the beginning of cultivation, 75% after 30 days of transplanting and 100% once flowering and fruiting started until the end of the cycle. Cultural practices such as pruning, tutoring, irrigation, pest control, weed management, disease control and harvesting at commercial maturity (75%) were performed during the crop development, starting 93 days after transplanting.

2.4. Evaluations2.4.1. Crop Yield

The evaluated variables were total fruit yield (TFY), which was determined at the time of each harvest by weighing all the fruits produced per m^{-2} and summing them throughout the crop cycle, expressed in kg m^{-2} . Total number of fruits per m^{-2} (NF), were counted and summed throughout the crop cycle. Average fruit weight (AFW) was estimated by dividing the total fruit weight between the total number of fruits, and expressed in grams (g). The variable fruit length (FL), expressed in centimeters (cm), and the equatorial fruit diameter (EFD), expressed in millimeters (mm), were measured using a digital precision vernier caliper (AutoTECTM) on a random sample of 5 fruits from each treatment at each harvest. These variables were estimated in 12 harvests conducted at intervals of 10–12 days.

2.4.2. Commercial Classification

The fruits were classified based on size and commercial weight, obtaining the following categories: (S) small: with an average weight from 135 to 155 g; (M) medium: from 157 to 175 g; (L) large: from 176 to 195 g; (XL) extra-large: from 196 to 223 g. This classification was based on the "Mexico Calidad Suprema" seal, which specifies the number of fruits that should be included in a 25 lb. box depending on the size [32]. For this purpose, a NOVAL EK5055E digital scale was used, as well as a digital vernier caliper to determine the FL and EFD.

2.4.3. Fruit Quality

The variables evaluated for these parameters were determined in fresh fruits at Laboratory of Plant Nutrition and Tissue Culture of the Department of Horticulture at UAAAN.

Vitamin C

The content of ascorbic acid (CAA) was determined according to the official methodology of A.O.A.C [33], and estimated in mg 100 g⁻¹, using a visual color change titration method with 2,6-dichlorophenol-indophenol. This method involved the reduction of the redox indicator by ascorbic acid.

Total Soluble Solids (TSS)

This variable was expressed in °Brix and quantified in the juice extracted from the fruits by placing a drop of the juice on the optical sensor of a HANNA HI-96801[®] refractometer.

Fruit Firmness (FF)

Fruit firmness was determined using a Wagner F-10 digital penetrometer, which had a force of 500 g and a 2.5 mm diameter probe. For this, the cuticle was removed from the equatorial part of the fruit, the penetrometer was inserted with a single impulse, and two readings per fruit were taken (Kg·cm⁻²). These variables were measured on 5 randomly selected fruits from each experimental unit, in each harvest conducted.

Morphological Characterization

The mesocarp thickness (MT) and plant height (PH) were evaluated to identify the morphological changes induced by the rootstock in the bell pepper plants. The MT was assessed using a digital precision vernier caliper, measuring 5 fruits from each harvest, randomly sampled from each experimental unit, and reported in millimeters (mm). At 150 days after transplanting, the PH was measured on 5 plants from each experimental unit using a flexometer, and reported in centimeters (cm).

2.5. Statistical Analysis

The experiment was established under a randomized complete block design with a 4 × 3 factorial arrangement, resulting in 12 treatments with 3 repetitions: ((1) Avante non-grafted; (2) Avante grafted onto Foundation; (3) Avante grafted onto UAN; (4) Avante grafted onto Yaocali; (5) Ucumari non-grafted; (6) Ucumari grafted onto Foundation; (7) Ucumari grafted onto UAN; (8) Ucumari grafted onto Yaocali; (9) DiCaprio non-grafted; (10) DiCaprio grafted onto Foundation; (11) DiCaprio grafted onto UAN; (12) DiCaprio grafted onto Yaocali). Each experimental unit consisted of nine plants, of which only seven were considered as usable plot to avoid edge effects. Homogeneity of variance between treatments was tested using the Chi-Square test described by Bartlett [34]. The normal distribution of observations of each treatment was determined by the modified Shapiro–Wilk test [35], with levels of significance of p < 0.05. The data obtained were analyzed using statistical software SAS [36], and a mean comparison was performed (Tukey test, $p \le 0.05$).

3. Results

3.1. Yield Components

The analysis of variance did not detect significant differences among the rootstocks, but differences were observed among the grafts (Table 1). The hybrid DiCaprio showed the highest yields (28.78 kg m⁻²), surpassing the Ucumari, which had the lowest yield (23.27 kg m⁻²), by 19.14%. Meanwhile, the hybrid Avante was 9.38% less productive than the DiCaprio. No significant differences were found in the rootstock–hybrid interaction.

	TFY	AFW	NF
_	${ m kg}{ m m}^{-2}$	g	Fruits m ⁻²
Rootstock			
UAN	25.9 a	189.6 b	137.1 a
Foundation	25.06 a	191.6 b	131.3 a
Yaocali	28.68 a	214.9 a	133.4 a
Non-grafted	24.36 a	199 ab	123.4 a
Anova $p \leq$	0.128 ns	0.001	0.5 ns
Hybrids			
Avante	26.08 ab	204.14 a	128.6 a
DiCaprio	28.78 a	204.64 a	124.5 a
Ucumari	23.27 b	187.65 b	140.8 a
Anova $p \le$	0.007	0.005	0.144 ns
Interaction	0.73 ns	0.63 ns	0.724 ns
CV (%)	15.0	6.5	15.2
Error	15.31	168.43	396.13

Table 1. Effects between the rootstock, hybrids and their interaction on variables: yield (kg m⁻²), average fruit weight (g) and number of fruits per m⁻² of bell pepper.

Values with different letters per column indicate statistical differences with Tukey's means test $p \le 0.05$. TFY: total yield, AFW: average fruit weight, NF: number of fruits, C.V. %: coefficient of variation.

3.2. Average Fruit Weight (AFW)

The analysis of variance detected significant differences among rootstocks and hybrids for the AFW (Table 1). The Yaocali rootstock was 13.34% higher than the UAN rootstock, which had the lowest average fruit weight. Similarly, the DiCaprio and Avante hybrids showed the highest AFW, significantly surpassing the Ucumari hybrid by 9% and 8%, respectively. The Ucumari produced the lowest average fruit weight (187.6 g).

3.3. Number of Fruits per m^{-2} (NF)

Based on the analysis of variance, no significant differences were observed among the treatments, indicating that this variable was not affected by rootstock and hybrid factors.

3.4. Commercial Classification

The use of rootstocks significantly influenced the size and/or classification of the bell pepper fruits (XL, L and S), as well as the interaction for the XL commercial size, showing highly significant differences ($p \le 0.05$). This indicated that one or more of the evaluated rootstocks modified the fruit size.

3.4.1. Commercial Classification (XL)

With the use of rootstocks, we could positively modify the fruit size. The mean comparison (Tukey, $p \le 0.05$) showed significant differences between these and the non-grafted genotypes (Table 2). The Yaocali rootstock significantly exceeded the UAN rootstock by 63.9% and the Foundation rootstock by 31.5%, which only produced 1.83 and 2.28 XL fruits per m⁻², respectively. In addition, in the mean comparison analysis, significant differences were detected due to the graft effect, showing that the fruits of the DiCaprio hybrid significantly exceeded the Ucumari hybrid by 231.5%, which had the lowest number of fruits (1.08 fruits per m⁻²).

Table 2. Effect of treatments on the number of fruits that comply with commercial classification according to the "Mexico Supreme Quality" seal.

	XL	L	М	S				
	Extra-Large	Large	Medium	Small				
	(Fruits per m ⁻²)							
Rootstock								
UAN	1.83 b	18.80 b	24.2 a	24.8 a				
Foundation	2.28 b	19.60 b	23.6 a	20.1 ab				
Yaocali	3.00 a	26.50 a	20.6 a	16.2 b				
Non-grafted	2.44 b	20.60 b	21.9 a	16.3 ab				
Anova $p \leq$	0.0003	0.021	0.44 ns	0.034				
Hybrids								
Avante	2.50 b	20.91 b	47.41 a	38.67 a				
DiCaprio	3.58 a	26.50 a	47.41 a	32.75 a				
Ucumari	1.08 c	16.87 b	43.41 a	45.00 a				
Anova $p \le$	0.0001	0.0008	0.62 ns	0.09 ns				
Interaction	0.0001	0.16 ns	0.17 ns	0.93 ns				
CV (%)	19	24	22	33				
Error	0.89	111.93	102.03	170				

Values with different letters per column indicate statistical differences with Tukey's means test $p \le 0.05$.

3.4.2. Commercial Classification (L)

In this category, there was also a marked difference between the use and absence of rootstocks. The Yaocali rootstock produced the highest number of size L fruits (26.5 fruits per m⁻²), surpassing the UAN rootstock by 40.95%, which showed the lowest fruit yield (18.8 fruits per m⁻²) in this classification, and 35.2% higher than the Foundation rootstock, which were not significantly different from the non-grafted genotypes. Meanwhile, the DiCaprio hybrid was 57.1% higher than the Ucumari graft and 26.7% higher than the Avante graft (Table 2).

3.4.3. Commercial Classification (M)

Regarding the M category, all treatments resulted in significantly equal fruit quantities, indicating that the production of size M fruits was not affected by the effect of any graft and/or rootstock (Table 2).

3.4.4. Commercial Classification (S)

For this commercial classification, the UAN rootstock was 53.1% higher than the Yaocali rootstock, which stood out for producing the highest number of L and XL fruits. On other hand, three grafts under study yielded equal quantities of S category fruits (Table 2).

3.4.5. Rootstock \times Graft Interaction

Figure 1 shows mean the values obtained in the number of XL fruit per m⁻², for rootstock/hybrid's factors regarding the XL-sized fruits, reflecting the variability among hybrids once they were grafted. The Avante/Yaocali treatment was significantly superior by 57% compared to the non-grafted hybrid. The same occurred with the Ucumari hybrid without a rootstock, which was surpassed by 228% by the Ucumari/Yaocali treatment in fruit production for this commercial category (XL). The opposite situation was observed in the DiCaprio hybrid, which, when grafted, showed significant decreases with all studied rootstocks, with the DiCaprio/UAN treatment presenting the most pronounced decrease, with a 65% lower yield compared to the non-grafted hybrid. The DiCaprio graft outperformed Ucumari and their interactions by 231.5% and Avante and their interactions by 142.8% for the extra-large (XL) fruit production, being the best performing hybrid for this classification.



Figure 1. Interaction detected between rootstock*hybrid*number fruits XL per m⁻². The vertical lines in each bar correspond to standard deviation. Means with same letters in each bar are not statistically different (Tukey $p \le 0.05$). AVA = Avante without grafting; A-F = Avante/Foundation; A-UA = Avante/UAN; A-Y = Avante/Yaocali; UCU = Ucumari without grafting; U/F = Ucumari/Foundation; U-UA = Ucumari/UAN; U-Y = Ucumari/Yaocali; DIC = DiCaprio without grafting; D-F = DiCaprio/Foundation; D-UA = DiCaprio/UAN; D-Y = DiCaprio/Yaocali.

3.5. Fruit Length (FL)

In the analysis of variance applied to this parameter, no significant differences were detected among the rootstocks. However, there were significant differences among hybrids in relation to the FL (Table 3). The Avante hybrid significantly exceeded the Ucumari hybrid by 10.85% in terms of the FL, with the Ucumari hybrid exhibiting the shortest FL.

Table 3. Effect between study factors on variables; fruit quality and morphological characteristics of three grafted bell pepper varieties.

	FL	EFD	CAA	TSS (°Brix) _	FF	MT	РН
-	(cm)	(cm)	(mg 100 g ⁻¹)		(kg cm ⁻²)	(mm)	(m)
Rootstock							
UAN	8.05 a	7.91 a	127.10 a	6.70 a	7.38 a	6.87 a	1.21 ab
Foundation	8.06 a	8.57 a	124.41 a	6.50 a	7.33 a	7.03 a	1.19 ab
Yaocali	8.60 a	8.03 a	124.81 a	6.89 a	7.34 a	7.06 a	1.32 a
Non-grafted	8.23 a	7.89 a	121.22 a	6.64 a	7.22 a	6.85 a	1.14 b
Anova $p \leq$	0.48 ns	0.29 ns	0.88 ns	0.6 ns	0.84 ns	0.52 ns	0.019
Hybrids							
Avante	8.78 a	8.256 a	132.47 a	6.98 a	7.99 a	7.44 a	1.23 ab
DiCaprio	8.01 ab	8.179 a	116.84 a	5.52 b	6.79 b	6.78 b	1.28 a
Ucumari	7.92 b	7.873 a	123.85 a	7.54 a	7.16 b	6.64 b	1.13 b
Anova $p \le$	0.036	0.5 ns	0.075 ns	0.0001	0.0001	0.0001	0.009
Interaction	0.184 ns	0.90 ns	0.015	0.55 ns	0.27 ns	0.40 ns	0.78 ns
CV (%)	10.1	10.3	12.7	9.2	5.43	5.3	9.3
Error	0.693	0.693	250	0.377	0.158	0.136	0.012

Values with different letters per column indicate statistical differences with Tukey test $p \le 0.05$. FL: fruit length; EFD: equatorial fruit diameter; CAA: Vitamin C; TSS: total soluble solids; FF: fruit firmness: MT: mesocarp thickness; PH: plant height; CV %: coefficient of variation.

3.6. Equatorial Fruit Diameter (EFD)

The analysis of variance did not detect significant differences among the rootstocks and hybrids, indicating that all grafts obtained similar fruit diameters and that the rootstocks did not have an influence on this variable (Table 3).

3.7. Vitamin C (CAA)

The analysis of variance did not detect significant differences due to study factors, rootstock and hybrid, on CAA (Table 3). However, significant differences were found in the interaction between the rootstocks and hybrids, indicating considerable variability among the hybrids once they were grafted. In Figure 2, it can be observed that the Avante hybrid was not affected by the effect of each rootstock; therefore, the CAA was the same between the grafted and non-grafted hybrids. However, the DiCaprio hybrid showed significant differences between being grafted and non-grafted, and also exhibited a differentiated response to each of the rootstocks used. The DiCaprio/Yaocali treatment reflected the most pronounced decrease (-28.4%) compared to the non-grafted DiCaprio hybrid, which had highest CAA. On the other hand, the non-grafted Ucumari hybrid had the lowest content and was significantly different from the same hybrid grafted onto any of the studied rootstocks. The Ucumari/Foundation treatment showed the highest increase at 32% compared to the non-grafted hybrid.



Figure 2. Interaction detected between rootstock*hybrid*Vitamin "C" quantification (mg ascorbic acid 100 g⁻¹ of fresh weight) in bell pepper fruits, for each treatment. The vertical lines in each bar correspond to standard deviation. Means with same letters in each bar are not statistically different (Tukey, 0.05). AVA: Avante without grafting; A-F: Avante/Foundation; A-UA: Avante/UAN; A-Y: Avante/Yaocali; DIC: DiCaprio without grafting; D-F: DiCaprio/Foundation; D-UA: DiCaprio/UAN; D-Y: DiCaprio/Yaocali; UCU: Ucumari without grafting; U-F: Ucumari/Foundation; U-UA: Ucumari-UAN; U-Y: Ucumari/Yaocali.

3.8. Total Soluble Solids (TSS)

The analysis of variance (Table 3) did not detect significant differences among the rootstocks; however, there were significant differences among the hybrids regarding the content of TSS. The Avante and Ucumari hybrids behaved significantly similarly, although the latter exhibited the highest content, surpassing the DiCaprio hybrid by a significant 36.6%.

3.9. Fruit Firmness (FF)

The analysis of variance for this variable (Table 3) did not detect significant differences among the rootstocks, but it did reveal differences among the hybrids or grafts. In the mean comparison, the Avante hybrid performed significantly better than the other two hybrids and exhibited 17.67% more firmness than the DiCaprio hybrid, which had lowest firmness level at 6.79 kg cm⁻².

3.10. Mesocarp Thickness (MT)

No significant differences were found due to the effect of rootstocks regarding variable MT, although significant differences were detected due to the effect of grafts. In the mean comparison (Table 3), the Avante hybrid was significantly different from the DiCaprio and Ucumari hybrids, as it surpassed the Ucumari by 12%, which had the lowest mesocarp thickness at 6.64 mm.

3.11. Plant Height (PH)

For this parameter, the analysis of variance reflected significant differences due to the effect of rootstocks and hybrids. Based on the mean comparison analysis (Tukey $p \le 0.05$), it was observed that the Yaocali rootstock was significantly superior to the non-grafted hybrids. For the grafted plants, the DiCaprio hybrid exhibited a significantly greater height than the Ucumari, surpassing it by 13.27% (Table 3).

4. Discussion

4.1. Yield Components

Grafting is an increasingly demanded technology for protected greenhouse crops [37] and has been widely adopted worldwide to reduce the risk of soil-borne diseases [38]. In recent years, grafting has been used not only to address phytopathological issues and external factors negatively affecting crops, but also to improve crop productivity and fruit quality characteristics [39]. However, some research indicates otherwise. For example, Flores et al. [40] reported a decrease in yield in grafted tomatoes. Pérez-Grajales et al. [41] agreed with the findings of the present study, as they did not find significant differences in the yield of grafted bell peppers. Cosme et al. [42] mentioned that two grafting methods they used did not increase the yield and quality of watermelon but provided greater plant vigor and disease tolerance. Medranda [43] reported no significant differences in size, weight and fruit quality between grafted and non-grafted watermelon on pumpkin rootstock.

On the other hand, there is research indicating that the use of rootstocks in vegetables is a strategy that improves the agronomic quality of fruit and increases crop productivity by up to 30% [44]. Therefore, choosing appropriate rootstock is very important for crop yield. It has been reported that non-grafted plants tend to have lower yields [45] compared to those using a vigorous rootstock. This is in line with Gisbert-Mullor et al. [46], who mentioned that grafting is a technique capable of improving yields of traditional pepper varieties without reducing their quality. Other studies have observed that grafting in tomato crops increased the number of marketable fruits per plant by up to 30%, but this increase comes at the expense of a significant decrease in the vitamin C content, phenolic compounds and antioxidant capacity [47]. On the other hand, García-Bañuelos et al. [48] reported yield increases of up to 2.95 times due to the effect of rootstocks. This could be attributed to the fact that grafted plants tend to have a larger root exploration area in the soil; thus, having a greater capacity for water and nutrient absorption [49,50], or it could be a direct effect of the rootstock on nitrogen absorption and plant metabolism [51]. However, this effect was not reflected in this study, highlighting the importance of carefully selecting the materials used, as grafting between different genotypes of cultivars and rootstocks involves complex physiological, biochemical and molecular mechanisms that affect the graft formation, compatibility and crop yield [52]. However, the success rate not only depends on the varieties used but also on the growing conditions, grafting technique employed, degree of concentration, work speed, experience, skill and the ability of workers, as the production of grafted plants is a labor-intensive task that is conducted manually and can become repetitive and exhausting [37]. Furthermore, in Mexico, rootstock seeds are imported, expensive and limited [53], making it crucial to pay close attention to these details.

The size of the fruit is very important as it determines the type of market. For example, the market prefers high-quality fruits of XL and L sizes, while the domestic market is not strict regarding calibers [54]. The effect of both rootstocks and grafting significantly influenced the size of the fruits, particularly in the XL category, which is suitable for the international market. However, there are fruits that do not conform to the standard (extra-small) but can be used for industrial purposes. Physiologically, fruit development involves a redirection of assimilate translocation towards reproductive organs [55], which aligns with the findings of previous studies [56], suggesting that rootstocks can promote a greater root volume, providing the stem with greater vigor and the ability to enhance elite fruit yield, as the vigor of pattern influences graft expression [13,57]. On the other hand, Doñas-Ucles et al. [58] reported improvements in fruit size in Italian sweet peppers (Palermo/Tresor) using rootstocks during two years of evaluation. Sánchez Solana [45] obtained similar results and found a positive effect of the rootstock on the fruit size. Furthermore, Palacio and Sánchez [14] found significant differences in fruit weight in the Jeanette variety grafted with Terra-no during two different harvest seasons, showing increases of over 5% compared to the non-grafted plants of the same variety. This difference is crucial for distinguishing between L and XL-sized fruits. Regarding the rootstockgraft interaction, it was observed that in the Avante and Ucumari hybrids, the rootstocks

significantly influenced the attainment of larger fruits, indicating that Yaocali as a rootstock for both hybrids exhibited positive compatibility. This corroborated information provided by the seed company, which stated that Yaocali induces greater vigor, production and fruit quality. This is further supported by a study by Camposeco-Montejo et al. [28], who reported average pepper weights of 224.19 g using Yaocali. However, the DiCaprio hybrid was negatively influenced by grafting, as the rootstock's effect decreased the fruit size. This suggested physiological incompatibility, lack of cellular recognition, the presence of growth regulators or incompatibility toxins between the rootstock and graft [59]. While no differences were found between the grafts in terms of commercial size (S), there were differences among the rootstocks, which somehow favored the increase in vegetative or generative characteristics of the hybrids [60]. The UAN rootstock favored the development of more generative plants, resulting in smaller fruits. This reflected the effect of rootstock in an imbalance in the fruit size, making management of grafted plants more complex in terms of size regulation. The treatment that achieved a better balance between generative and vegetative vigor was the Foundation/DiCaprio, which favored the production of fruits of higher commercial quality and increasing yield compared to other grafted and non-grafted hybrids.

4.2. Fruit Quality

Pepper is a rich source of vitamins, especially high levels of vitamin C (ascorbic acid), an antioxidant compound with favorable health effects [45,61]. In this study, the average vitamin C content among the treatments was 125 mg 100 g^{-1} of fresh weight, showing the following pattern: orange-colored fruits had the highest content, followed by red-colored fruits, and finally, yellow-colored fruits had the lowest content. However, the content was relatively lower compared to other reports. A previous investigation by García et al. [62] reported 209.04 mg 100 g⁻¹ of fresh weight in red peppers and 165.26 mg 100 g⁻¹ in green peppers. Similarly, a study by Moran et al. [63] obtained 166.10 mg 100 g^{-1} in red peppers and 125.80 mg 100 g^{-1} in yellow peppers. The low contents in this study, as indicated by Hernandez-Fuentes et al. [64], could have been influenced by the harvest period. The fruits were harvested at 75% maturity or 50% coloration [65] to achieve a longer shelf life and comply with the "Mexico Calidad Suprema" recommendations [32]. Additionally, the relationship between the materials, including the quality of the pepper fruits influenced by the grafting and rootstock [66], could explain the observed vitamin C content in each of treatments (Figure 2). Each graft showed a different scenario once grafted, which could be attributed to the distinct characteristics of the plant materials that significantly influenced the increase or decrease in one or several traits, depending on the compatibility between the rootstock and graft. Some authors reported both increases and decreases in the grafting effect, and this study demonstrated that both the rootstocks and hybrids exhibited different behaviors among them. The effect of the rootstock favored an increase in the vitamin C content in at least two of the evaluated hybrids, while a third hybrid showed decreases with each of the grafted rootstocks, which aligns with the findings of Zhu et al. [67], who mentioned that ascorbic acid increases due to rootstock. Similarly, Gisbert et al. [68] reported that the use of Foc and Charlot rootstocks did not affect the vitamin C content. In contrast, other studies have reported a 14-20% decrease in the vitamin C content in tomato fruits from plants grafted onto "Beaufort F1" and "Maxifort F1" compared to non-grafted treatment [69]. This coincides with López-Marín et al. [60], who indicated that the use of rootstocks resulted in a decrease in the vitamin C content. Some studies on tomatoes have demonstrated that the use of different nutrient solutions and different graft combinations significantly affect the ascorbic acid content [9].

On the other hand, a high content of total soluble solids is associated with a greater capacity for nutrient absorption or translocation, which is a desirable quality in rootstocks [55]. In this study, it was observed that the °Brix content was significantly affected by the hybrids, more than by the rootstocks. Therefore, the higher content of soluble solids was mainly determined by the genetic constitution of the graft, as pointed out by Martínez-Rodríguez, et al. [57], who mentioned that this characteristic is primarily determined by the genotype of hybrid.

However, there may be other environmental or nutritional factors that can influence the TSS content. Alternatively, these effects may be related to a vegetative incompatibility, which refers to alterations in the transport of metabolites through the phloem and a modification of physiological processes through hormonal synthesis [48]. Therefore, further study is needed to better understand the behavior of different varieties on each rootstock. For the above, soluble solids and titratable acidity, have been affected by grafting with varying results. A study by Muramatsu [70] reported the use of a rootstock resistant to Fusarium wilt, but it caused a decrease in the fruit quality and the total soluble solids, which coincides with Huang et al. [71], who found that soluble solids and fruit flavor significantly decreased in watermelon plants grafted onto squash. Additionally, in the study by Barrett et al. [72], there were no differences found in these parameters between grafted and nongrafted plants. This was similar to Djidonou et al. [73], who mentioned that grafting with interspecific tomato rootstocks maintained the content of soluble solids and total titratable acidity in their fruits. Flores et al. [40] mentioned that grafted tomato plants produced fruits with higher levels of both acidity and soluble solids. Similar findings were reported for Djidonou et al. [74], where it was quoted that the grafting effect with interspecific hybrid rootstocks in tomato drove consistently small but significant increases in the soluble solids. Therefore, the materials used as rootstocks influenced the grafting process in different ways. Based on these observations, the grafting process will also influence one or more variables as a result of genetic expression.

4.3. Morphological Characteristics

In this research, no interaction was found between the rootstock–grafts. Therefore, the rootstock did not influence the vigor of the graft, resulting in increased plant height. The plant height of both the rootstocks and grafts was a manifestation of the genotype interacting with the environment rather than an interaction between them. Similar results were found by Hernández-Gonzales et al. [75], as they did not observe a significant effect of the rootstock on the final height, although other authors found significant differences among treatments due to the rootstock effect. For example, López-Marín et al. [60] found significant differences between treatments, due to the effect of the rootstock in pepper. Similarly, Caradonia et al. [76] found that grafted cherry tomatoes in a greenhouse increased the plant height compared to the control plants. The same author [77] found that organic tomato achieved greatest plant height with grafted plants. Lee and Oda [78] suggest that increased height can be an effect of the expression of the rootstock on the graft. Although rootstock may have a greater capacity for water and mineral salt absorption, which benefits the development of the graft, certain characteristics of the graft are more determined by the genotype rather than the rootstock, such as plant height.

5. Conclusions

The use of rootstocks in bell pepper production represents a viable alternative for producing L and XL-sized fruits, which hold greater value in the export market. Grafted plants have shown improvements in the yield of fruits in these categories. However, careful selection of rootstocks and grafts is crucial in order to achieve the desired results, as not all combinations yield adequate compatibility.

The Yoacali rootstock and the DiCaprio hybrid produced the largest-sized fruits (L-XL), but their trend changed when they were grafted. The DiCaprio was the hybrid that achieved the highest overall yield due to the production of heavier fruits and/or those with high commercial classification. Once grafted, the treatment that achieved a better balance between generative and vegetative vigor was the Foundation/DiCaprio, which favored the production of fruits with higher commercial quality compared to other grafted and non-grafted hybrids. On the other hand, the UAN rootstock resulted in a higher number of fruits per square meter, but most of them fell into the small classification.

The grafted plants improved the quantity of fruits of higher classification; however, they did not have a significant influence on the yield and/or quality of fruits, which was

attributed to fact that there was no compatibility between them. It should be noted that some rootstocks may not be able to establish themselves and adapt adequately to biotic and abiotic stresses, or on contrary, that present a high tolerance to root diseases, so they could not express themselves fully, finally failing in the objective of improving the yields and quality of the fruits. Therefore, further studies are recommended to search for the best rootstock–hybrid compatibility in order to express the greatest vigor of the plant.

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