

Article

Response of Bell Pepper to Rootstock and Greenhouse Cultivation in Coconut Fiber or Soil

Neymar Camposeco-Montejo ¹, Valentín Robledo-Torres ^{1,*}, Francisca Ramírez-Godina ², Rosalinda Mendoza-Villarreal ¹, Miguel Ángel Pérez-Rodríguez ³ and Marcelino Cabrera-de la Fuente ¹

¹ Department of Horticulture, Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro 1923, Saltillo 25315, Coahuila, Mexico; neym_33K@hotmail.com (N.C.-M.); rosalindamendoza@hotmail.com (R.M.-V.); cafum7@yahoo.com (M.C.-d.l.F.)

² Department of Plant Breeding, Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro 1923, Saltillo 25315, Coahuila, Mexico; godramf@gmail.com

³ Department of Botany, Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro 1923, Saltillo 25315, Coahuila, Mexico; miguel_cbg@hotmail.com

* Correspondence: robledo3031@gmail.com; Tel.: +52-844-667-9590

Received: 6 May 2018; Accepted: 26 June 2018; Published: 4 July 2018



Abstract: Vegetable production in greenhouses is preferred when soil quality is degraded by high salinity or incidence of pests and diseases. In these soils with abiotic and biotic issues, it is a challenge to increase the yield and quality of fruits. The use of rootstocks and organic substrates are effective and environmentally friendly techniques to solve that challenge. The objective was to study the effect of rootstocks on yields and quality in bell peppers (*Capsicum annuum* L.) grown in either soil or coconut fiber substrate, in greenhouses. Using a randomized block design with three repetitions, the resulting treatment groups consisted of three rootstocks (Foundation-F1, Yaocali-F1, CLX-PTX991-F1 (Ultron), and non-grafted controls) with four hybrids as scions (Lamborghini, Bambuca, DiCaprio, and Ucumari). The yield of fruit per plant (YFP) and number of fruit per plant (NFP) obtained in coconut fiber were 85% and 55% greater, respectively, than in soil. The CLX-PTX991-F1 rootstock was superior to the hybrids without rootstock ($p \leq 0.05$) in YFP and NFP (30% and 19.5%, respectively). The Lamborghini hybrid had significantly greater YFP and NFP than the Ucumari. We concluded that the use of coconut fiber significantly improves the yields of bell pepper and that the use of rootstock improves plant vigor and plant yield.

Keywords: *Capsicum annuum*; graft; organic substrates; fruit quality; yields

1. Introduction

Currently, the human population is growing at an exponential rate, and consequently, the demand for food grows proportionally while the usable space for agricultural production diminishes as urban settlements grow. Mexico is no exception to this and the challenges of the global agricultural industry focus on developing new technologies that increase yields per unit of area and improve the quality of the resulting products. One of those technologies is grafting, a propagation technique that consists of joining one plant to another that is already rooted. A grafted plant consists of a rootstock that generally has no intrinsic agronomic value, but carries valuable biotic [1] or abiotic [2] resistance and tolerance genes, and the scion, which is a piece of stem or bud that is joined to the rootstock so that branches, leaves, flowers, and fruits develop [3].

Plant grafting has its beginning in Asia, in the 1920s. Currently, in countries such as Japan and South Korea, the use of grafting is increasing [4,5]. The industry of greenhouse vegetables shows

similar tendencies across the globe [6]. Watermelon, melon, cucumber, and tomato, among others, are grafted onto rootstocks with tolerance or resistance to soil pathogens [7,8]. The original purpose of plant grafting was to avoid diseases caused by soil parasites and pathogens, such as *Verticillium*, *Fusarium*, *Phytophthora*, *Ralstonia*, and *Nematodos* [9,10]. In actuality, grafting can also be used to mitigate abiotic stress [11,12], increase yields, improve fruit quality, and extend harvest times, as well as reduce agrochemical applications [13,14] and improve the commercial quality of crops [15,16], as it is effective at preventing soil-borne diseases and pests [17]. In eggplant, the rootstock used induced notable vigor and better yield [18,19], the latter of which favors the global reduction of dependency on synthetic agrochemicals [1]. As such, vegetable grafting represents a viable route [20] to ecologically friendly production. The importance of grafting is recognized in agricultural circles worldwide, as it is a very clean and effective technique whose use involves zero environmental impact when compared with commonly used soil treatments [1]. It has also been adopted as a safe and ecologically friendly means of production as it minimizes the need for chemical treatments against soil-borne pests and pathogens, and consequently reduces the absorption of undesirable agrochemical residues [5].

In previous reports from Mexico, grafts were used in poblano pepper using Crole Morelos-344 rootstock to counteract the effects of *Phytophthora capsici*, managing to reduce the incidence of the disease to 1%, allowing production even in areas of high incidence [21]. The same was seen with jalapeños, cayenne, and chilaca chilies [22]. The use of this technique with commercial varieties of bell pepper could significantly reduce the losses caused by *P. capsici* by up to 100% [23,24]. The use of grafting also increases yields by up to 50% [25,26], resulting in increased commercial yields [11]. Yield increases of up to 35% [17,27] and a 35% increase in the quality of exported tomatoes [16] have both been reported for grafted tomato. López et al. [28] reports increases of over 25% in fruit yield, with the use of abiotic stress tolerant rootstocks, making it a good adaptation strategy for abiotic stress conditions [29]. The selection of rootstock is incredibly important for improving the quality of the plants and the commercial quality of the fruit [30], as such there must exist sufficient compatibility between the rootstock and scion [6]. The benefits of using rootstocks in chili peppers is clear, however, and given the rise in popularity of the technique, it has become necessary to test all the different commercially available rootstocks. This must be done in order to find the combination of rootstock–scion capable of both satisfying the production volumes demanded by cultivators and meeting market quality requirements. To that end, the objective of the present study was to determine the effect of using three commercial rootstocks on the yield and fruit quality of four bell pepper hybrids.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Foundation F1 (Rijk Zwaan, De Lier, Holanda; has a powerful root system, size compact variety, and high generative power), Yaocali F1 (Enza Zaden, Enquizen, Holanda; has abundant root system, tolerance to *Phytophthora*, and salinity), and CLX-PTX991 F1 (Harris Moran, CLAUSE. SA. Rue Louis Saillant ZI La Motte 26800 Portes-Les-Valence France) were the three bell pepper rootstocks selected, while the four hybrid bell pepper scions, type blocky, were Lamborghini F1 (Rijk Zwaan); Bambuca F1 (Rijk Zwaan) yellow fruit, sizes L and XL, and thick walls; Dicaprio F1 (Enza Zaden) yellow fruit, sizes L and XL, and tolerant to cracking; and Ucumari F1 (Enza Zaden) orange fruit, sizes L and XL, thick walls and long shelf life. The experiment was conducted in a greenhouse with a wet wall and extractors for climate control and 30% shade belonging to the Universidad Autónoma Agraria Antonio Narro (UAAAN) Department of Horticulture in Saltillo, Coahuila (latitude 25°21'24" N, longitude 101°02'05" W, 1762 m a.s.l.) with a BS0hw (x) climate semidry arid, with average annual temperature of 18–22 °C and average annual precipitation of 342.4 mm (climate card No. 14R-VII, 1970). The environment within the greenhouse was controlled automatically by means of temperature sensors with minimum value of 16 °C and maximum values of 32 °C, with registers of 60–90% relative

humidity, and a light intensity of 800 to 1300 $\text{w m}^{-2} \text{s}^{-1}$. They were registered by means of a WatchDog 1450 datalogger from Spectrum Technologies Inc. (Aurora, IL, USA).

2.2. Graft Technique and Sowing of Plant Material

Rootstock and hybrid F1 scion seeds were sown in 200 cell polystyrene propagation trays with a 70:30 peat moss/perlite mix for germination. The hybrid scions were sown on 5 March 2017. The rootstock seeds were sown three days later in order to synchronize the stem diameters. Thirty-five days after sowing, when plants had reached a stem diameter of 2–2.4 mm, rootstocks and scions were joined by splice grafting [31]. The stems of rootstock and scion were cut at 45° with a new blade disinfected with 70% ethyl alcohol. Cut rootstock and scion stems were joined with 2.5 mm silicon clips to provide support. The workspace was kept at 22–28 °C, 80–90% relative humidity, and all grafting was done on tables disinfected with 50-ppm bleach. Grafted plants were kept in a grafting chamber at 22–25 °C, 80–90% relative humidity. For the first 48 h, they were kept in total darkness to avoid cellular oxidation at the union site, and to favor the process of cicatrization and graft joining. The following six days (24 h/day) were normal day–night cycles. Afterwards, the grafted plants were taken to the greenhouse (18–28 °C, 79–90% relative humidity) for adaptation and acclimatization.

2.3. Greenhouse Rooting in Soil and Coconut Fiber

The plants were transplanted 20 days after grafting. The silicon clips were kept on to provide support. Both grafted hybrids and non-grafted hybrids were grown in coconut fiber (the real density of coconut fiber was 1.48 g cm^{-3} and 76% for water retention capacity; total porous space was 92%; regarding chemical characteristics, the pH was 5.2 and the electrical conductivity of the saturation extract ranged from 1.5 to 2 dS m^{-1}) grow bags, with 15 cm between plants. They were also grown in soil, with 2.78% organic matter, pH 8.06, 43.7 ppm of phosphorus, 312 ppm of potassium, Ca 3101 ppm, Mg 375 ppm, Na 137 ppm, Fe 22.5 ppm, Zn 6.3 ppm, Mn 3.04 ppm, Cu 0.41 ppm, B 1.68 ppm, S 22.4 ppm, and N-NO₃ 70.5 ppm. In a microbiological analysis, *Fusarium* spp., *Alternaria* spp., *Phytium* spp., *hytophthora* spp. and *Rizoctonia* spp. were found. Their seedlings were also grown in raised soil beds with black plastic padding, with 30 cm distance between plants and 25 cm between doubled rows. In both cases, the distance between furrows was 1.80 m, resulting in a calculated plant density of 36,000 plants per hectare. The spatial distribution of the plants in the two culture media was different, because they are cultivated in this way in Mexico, in soil or coconut fiber. In both cultivation systems, the first experimental factor is the growth substrate (coconut fiber or soil), the rootstocks (Foundation F1, Yaocali F1, CLX-PTX991 F1 (Ultron), and non-grafted hybrid controls) are the second factor, and the hybrid scions (Lamborghini F1, Bambuca F1, Dicaprio F1, and Ucumary F1) are the third factor, yielding a 2 × 4 × 4 experimental design matrix.

During the 2017 spring–summer cycle, the experiment was set up in a completely randomized block design with three replicates. Each experimental unit consisted of four completely competent plants with two viable stems per plant. Standard cultivation procedures were followed. Steiner nutrient solution [32] was utilized in both cultivation sites for localized watering. In soil or coconut fiber, 1.5 L of water per day was applied in each adult plant. The watering schedule was as follows: 50% Steiner solution at the beginning of cultivation, 75% at 30 days after transplanting, and 100% upon initiation of flowering and fruiting until the end of the cycle. Salt drainage was promoted by overwatering by 20%. Weekly applications of 15.3% spirotetramat, 23.1% spiromesifen, 17% imidacloprid plus 12% cyfluthrin (1 mL/L), and 90% methomyl (1 g/L) were used to control thrips and white flies.

2.4. Measurement of Fruit Yield Parameters

The yield of fruit per plant (FYP) was estimated by weighing all the fruit from the four plants in each replicate on a SARTORIUS TS 1352Q37 digital balance and dividing that measurement by the number of plants considered. There were 11 harvests made, the first 90 days after transplanting, where only fruit with greater than 60% coloration was collected. Following weighing, the collected fruits were counted in order to estimate number of fruits per plant (NFP) harvested from each

experimental unit, taking into account the 11 harvests made throughout the cultivation cycle. The average fruit weight (AFW) was calculated by dividing the total fruit weight per repetition by the total number of fruits. The equatorial diameter (ED), fruit length (FL), and mesocarp thickness (MT) were estimated by measuring eight fruits per repetition at random from three harvests made 21 days apart after 18 August, with Autotec[®] digital Vernier calipers (Anyi Instrument Co. Ltd., Guilin, Guangxi, China).

2.5. Morphological Variable Measurement

The plant height (PH) was measured in four plants per useful plot, leaf length (LL) and leaf width (LW) were measured in four fully developed leaves, from each plant of the useful plot. The leaves were chosen at random, 50 cm above the ground, with a measuring tape marked in centimeters. The main stem diameter (SD) was measured in four plants per useful plot, at 5 cm above the ground, with Autotec[®] digital Vernier calipers.

2.6. Fruit Quality Measurement

Total soluble solid content was measured with an Atago N-1E[®] refractometer (National Industrial Supply, Temecula, CA, USA), and expressed in °Brix. Fruit firmness was evaluated with a FT-327 Fruit Pressure Tester penetrometer (max. capacity 13 kg) with a 3 mm point. The content of vitamin C was determined according to the official methodology of the Association of Official Agricultural Chemists (AOAC) [33] and expressed as milligrams of vitamin C per 100 g of fresh weight fruit (mg 100 g⁻¹).

2.7. Statistical Analysis

In the present study, the variables chosen for study (substrate, rootstock, and scion) gave rise to a 2 × 4 × 4 trifactorial (three way analysis of variance (ANOVA)) experimental design, with 32 treatments set up in a completely randomized block design with three repetitions. The data were statistically analyzed with SAS 9.1 (SAS Institute Inc., Cary, NC, USA) software and the means were compared with Tukey's test.

3. Results

3.1. Evaluation of Both Cultivation Systems (Coconut Fiber and Soil)

3.1.1. Yield Parameters

The FYP and NFP of bell pepper plants grown in coconut fiber were 85% greater (statistical significance, $p \leq 0.05$) than those of plants grown in soil (Table 1). The choice of cultivation system did not affect AFW, but did affect other fruit parameters. Plant height was 29% greater; leaf length and width were 23% and 7.8% greater, respectively; and main stem thickness was 23% greater in the coconut-fiber-grown plants (Table 1).

Table 1. Yield averages ($p \leq 0.05$) and morphological variables evaluated for bell pepper grown in coconut fiber and soil.

Cultivation System	FYP g	NFP	AFW g	PH cm	LL cm	LW cm	ST mm
Coconut fiber	3807.8a ±446.07	19.00a ±1.53	203.81a ±23.22	134.33a ±6.00	20.58a ±0.67	10.32a ±0.23	18.27a ±0.28
Soil	2052.2b ±214.34	10.24b ±0.91	200.42a ±11.62	103.87b ±6.31	16.68b ±0.72	9.57b ±0.33	14.86b ±1.54
VC (%)	23.41	21.76	13.19	14.35	7.17	7.96	12.58

VC = variation coefficients; FYP = fruit yield per plant; NFP = number of fruits per plant; AFW = average fruit weight; PH = plant height; LL = leaf length; LW = leaf width; ST = stem thickness; ± = standard deviation.

There was significant interaction between cultivation system and rootstock in the fruit length. Table 2 shows that the length of the fruit obtained from each rootstock had a significantly different behavior in each culture system studied.

Table 2. Interaction between cultivation system and rootstock in the fruit length.

	Non-Grafted	Foundation	Yaocali	CLX-PTX991
Coconut fiber	73.94 ± 3.16	73.86 ± 10.78	84.89 ± 3.94	75.42 ± 3.78
Soil	84.98 ± 4.30	77.07 ± 5.75	82.75 ± 4.06	79.21 ± 6.89

3.1.2. Morphological Variables

Significant differences were observed for all morphological variables measured in plants grown on coconut fiber substrate, as compared with those grown in soil. When grown in soil, there were increases in FL (5.2%) and MT (5.4%) in comparison with the coconut fiber, which were significantly different ($p \leq 0.05$), whereas fruit from plants grown in coconut fiber had significantly higher values ($p \leq 0.05$) in FED (5.6% greater) on soil (Table 3).

Among cultivation systems and rootstock, a significant interaction ($p \leq 0.05$) was observed in the stem thickness. The three rootstocks had similar stem thickness when they were developed in coconut fiber, however, the thickness of the stems developed in soils was very contrasting, especially the Foundation F1 rootstock, which had a poor soil behavior (Table 4).

The interaction between the variety and rootstock in the width of the leaf, shows that this characteristic was different in each variety and changes significantly among the rootstocks studied (Table 5).

Table 3. Fruit quality averages (Tukey, $p \leq 0.05$) evaluated in bell pepper grown in coconut fiber and soil.

Cultivation System	FL mm	FED mm	MT mm	Vit. C mg·100 g ⁻¹	TSS °Brix	FF kg cm ⁻²
Coconut fiber	77.03b ±4.58	87.51a ±1.51	6.79a 0.21	137.19a ±8.34	5.72a ±0.21	15.98a ±0.12
Soil	81.01a ±3.06	82.87b ±1.93	6.44b ±0.13	133.08a ±5.32	5.73a ±0.27	16.21a ±0.12
VC (%)	6.75	6.52	8.20	15.46	11.14	4.67

VC = variation coefficients; FL = fruit length; FED = fruit equatorial diameter; MT = mesocarp thickness; Vit. C = vitamin C content; TSS = total soluble solids; FF = fruit firmness; ± = standard deviation.

Table 4. Interaction between cultivation system and rootstock in stem thickness.

	Non-Grafted	Foundation	Yaocali	CLX-PTX991
Coconut fiber	17.10 ± 0.337	18.77 ± 1.19	18.56 ± 1.151	18.66 ± 0.678
Soil	13.88 ± 0.973	12.86 ± 1.116	16.66 ± 1.201	16.02 ± 1.481

Table 5. Interaction between the rootstock and variety in the width (cm) of the pepper leaf.

Rootstocks	Grafts				Average
	Lamborghini	Dicaprio	Bambuca	Ucumari	
Non-grafted	9.92	9.29	10.02	9.69	9.73b
Foundation	10.27	9.96	9.10	10.08	9.85ab
Yaocali	11.02	9.54	10.46	10.39	10.35a
CLX-PTX991	10.75	9.31	10.37	8.96	9.84ab
Average	10.49a	9.52b	9.98ab	9.78b	

3.1.3. Fruit Quality

With respect to the fruit quality parameters evaluated, neither vitamin C content, total soluble solids, nor fruit firmness were affected by choice of cultivation substrate (Table 3).

3.2. Rootstock Evaluation

3.2.1. Yield Parameters for Rootstocks and Non-Grafted Hybrids

The use of rootstocks Yaocali F1 y CLX-PTX991 F1 resulted in a significant increase ($p \leq 0.05$) in FYP. The CLX-PTX991 F1 rootstock excelled, demonstrating a 30% average increase over the non-grafted hybrids, though its performance was statistically equivalent to the other rootstocks. The NFP also increased significantly (19.5%, $p \leq 0.05$) with CLX-PTX991 rootstock, compared with non-grafted hybrids. AFW increased with the use of the Yaocali F1 rootstocks by 21.6% and 19.9%, respectively, compared with the fruit from non-grafted hybrids, which also exceeded 19.7% in the AFW obtained with the rootstock Foundation F1.

Only Yaocali was significantly different from non-grafted plants for PH, LL, and LW (see Table 4), so not all the rootstocks were superior as stated by authors. The Yaocali rootstock stood out amongst the others, promoting increases in PH (12.2%), LL (8.8%), LW (6.4%), and ST (13.7%), as compared with non-grafted hybrids (Table 6).

Table 6. Yield averages and parameters evaluated for non-grafted bell pepper hybrids and Foundation F1-, Yaocali F1-, and CLX-PTX991 F1-grafted hybrids.

	FYP	NFP	AFW	PH	LL	LW	ST
Rootstocks	g		g	cm	cm	cm	mm
Non-grafted	2502.6b ±570.19	13.40b ±3.49	184.30b ±4.94	114.60b ±12.76	18.03b ±1.57	9.72b ±0.35	15.49c ±1.61
Foundation	2817.6ab ±1011.42	14.97ab ±5.56	186.95b ±5.61	113.91b ±16.64	18.26b ±2.54	9.85ab ±0.65	15.81bc ±2.95
Yaocali	3135.2a ±1045.06	14.09ab ±4.10	224.19a ±7.31	128.63a ±14.46	19.60a ±1.87	10.35a ±0.25	17.61a ±0.95
CLX-PTX991	3264.6a ±884.65	16.02a ±4.27	213.02a ±9.00	119.25ab ±16.77	18.64ab ±1.80	9.84ab ±0.28	17.34ab ±1.32
VC (%)	23.41	21.76	13.19	14.35	7.17	7.96	12.58

VC = variation coefficients; FYP = fruit yield per plant in grams; NFP = number of fruits per plant; AFW = average fruit weight; PH = plant height; LL = left length; LW = leaf width; ST = stem thickness; ± = standard deviation.

3.2.2. Morphological Variables in Grafted and Non-Grafted Plants

The use of the Yaocali rootstock also resulted in significant increases ($p \leq 0.05$) in fruit length compared with non-grafted hybrids (5.5%) and the Foundation-grafted hybrids (11%) and in 8.4% compared with the rootstock CLX-PTX991 F1. FED and MT parameters were not significantly affected by the use of rootstock (Table 7).

Table 7. Morphological variable and values fruit quality parameter averages (Tukey, $p \leq 0.05$) in non-grafted and with rootstock.

	FL	FED	MT	Vit. C	TSS	FF
Rootstocks	mm	mm	mm	mg·100 g ⁻¹	°Brix	kg·cm ⁻²
Non-grafted	79.46b ±5.52	85.16a ±3.24	6.53a ±0.14	141.69a ±8.29	5.95a ±0.04	16.06a ±0.14
Foundation	75.47b ±1.60	83.04a ±2.04	6.54a ±0.08	128.43 a ±0.50	5.82ab ±0.005	15.93a ±0.11

Table 7. Cont.

	FL	FED	MT	Vit. C	TSS	FF
Rootstocks	mm	mm	mm	mg·100 g ⁻¹	°Brix	kg·cm ⁻²
Yaocali	83.82a ±1.07	85.77a ±3.3	6.72a ±0.44	130.01a ±0.65	5.81ab ±0.14	16.17a ±0.03
CLX-PTX991	77.31b ±1.89	86.79a ±0.68	6.67a ±0.03	140.40a ±1.22	5.33b ±0.07	16.22a ±0.17
VC (%)	6.75	6.52	8.20	15.46	11.14	4.67

VC = variation coefficients; FL = fruit length; FED = fruit equatorial diameter; MT = mesocarp thickness; Vit. C = vitamin C; TSS = total soluble solids; FF = fruit firmness; ± = standard deviation.

3.2.3. Fruit Quality in Grafted and Non-Grafted Plants

Vitamin C and FF of the fruit were not significantly affected by grafting or rootstock (Table 7). Non-grafted hybrids demonstrated a significant increase (Tukey, $p \leq 0.05$) in total soluble solids (TSS) content (11.6%) as compared with the quantity observed in CLX-PTX991 rootstock.

3.3. Hybrid Scion Evaluation

3.3.1. Hybrid Scions Yield Parameters

The Lamborghini F1 and Dicaprio F1 hybrids displayed statistically significant increases ($p \leq 0.05$) in FYP (32.9%) and NFP (21.9%). Bambuca F1 was also superior to Ucumari to FYP with 22%. With respect to the parameters of AFW (>16.3%) and plant height, the Dicaprio hybrid was significantly taller (>14%) than the Ucumari hybrid. Although, in leaf length and width, the Lamborghini hybrid presented with the highest differences (8.4% and 7.1%, respectively) over the Ucumari F1 hybrid. However, Ucumari and Dicaprio were statistically equal in LW and ST (Table 8).

Table 8. Yield averages (Tukey, $p \leq 0.05$) and parameters evaluated in four commercial bell pepper hybrids.

	FYP	NFP	AFW	PH	LL	LW	ST
Hybrids	g		g	cm	cm	cm	mm
Lamborghini	3197.0a ±1293.77	15.79a ±5.43	194.91b ±24.79	116.2ab ±21.27	19.30a ±2.06	10.48a ±0.64	16.76a ±2.50
Dicaprio	3168.0a ±772.90	15.12ab ±3.91	218.98a ±22.12	126.29a ±16.54	18.45ab ±1.71	9.52b ±0.47	16.18a ±1.61
Bambuca	2950.0a ±986.43	14.61ab ±4.79	206.33ab ±21.77	123.16ab ±17.73	18.95a ±1.99	9.98ab ±0.62	16.80a ±2.60
Ucumari	2404.9b ±940.11	12.95b ±5.11	188.31b ±21.02	110.73b ±17.16	17.81b ±2.85	9.78b ±1.05	15.89a ±2.12
VC (%)	23.41	21.76	13.19	14.35	7.17	7.96	12.58

VC = variation coefficients; FYP = fruit yield per plant in grams; NFP = number of fruits per plant; AFW = average fruit weight; PH = plant height; LL = left length; LW = leaf width; ST = stem thickness; ± = standard deviation.

3.3.2. Hybrid Scion Morphological Variables

With regard to FL, and FED, the Dicaprio hybrid was significantly superior to the Ucumari F1 307 and Lamborghini F1. The thickness of the mesocarp was statistically similar between all four chosen 308 hybrids (Table 9).

3.3.3. Hybrid Scion Fruit Quality

The vitamin C content in the Ucumari F1 hybrid—an orange-colored bell pepper—was greater than 17.3% ($p \leq 0.05$), as compared with the Bambuca F1 hybrid, which produces yellow fruit.

The content of Vit. C of Lamborghini F1 also exceeded the Bambuca F1 hybrid by 16.2%. This tendency was seen again in the TSS content, as the Ucumari F1 hybrid exceeded Bambuca F1 by 34.2%. As for fruit firmness, Ucumari F1 and Lamborghini F1 were statistically equivalent to each other, both surpassing Bambuca F1 by 4.7%, (Table 9).

Table 9. Morphological variable and fruit quality parameter averages (Tukey, $p \leq 0.05$) in four commercial bell pepper hybrids.

	FL	FED	MT	Vit. C	TSS	FF
Hybrids	mm	mm	mm	mg·100 g ⁻¹	°Brix	kg·cm ⁻²
Lamborghini	75.58b ±6.12	85.61ab ±4.09	6.80a ±0.31	143.21a ±15.29	5.85b ±0.32	16.44a ±0.36
Dicaprio	81.95a ±3.60	88.46a ±4.32	6.68a ±0.3932	129.65ab ±8.57	5.12c ±0.33	15.80ab ±0.54
Bambuca	83.67a ±4.83	85.23ab ±2.48	6.48a ±0.26	123.16b ±9.05	5.09c ±0.39	15.70b ±0.33
Ucumari	74.87b ±6.73	81.46b ±4.74	6.50a ±0.47	144.51a ±16.10	6.83a ±0.47	16.44a ±0.32
VC (%)	6.75	6.52	8.20	15.46	11.14	4.67

VC = variation coefficients; FL = fruit length; FED = fruit equatorial diameter; MT = mesocarp thickness; Vit. C = vitamin C; TSS = total soluble solids; FF = fruit firmness; ± = standard deviation.

4. Discussion

The 85% improvement in FYP, NFP, FED, and MT was a result of the increased plant vigor seen in the coconut-fiber-grown plants, which also demonstrated greater plant height, leaf length and width, and stem thickness. The overall better plant development can be attributed to the physicochemical characteristics of the substrate, such as the gas exchange rate, water retention capacity, porous space, pH, apparent density, cationic exchange capacity, and nutrient availability, which provide better growth conditions than those found in agricultural soils [34,35]. It has been demonstrated that, depending on the nature of the substrate, they can influence to a greater or lesser extent the complex process of mineral nutrition in plants, and favor the development and final yield of plants [36,37]. The use of different growth substrates in protected cultivation also provides the potential to reduce supplied mineral nutrients by 25–50% without affecting the final yield [36]. Cultivation on 100% coconut fiber has the added benefit of significantly increasing the dry biomass of leaves, stems, and roots [38].

The significant differences between rootstocks demonstrate that although they all improve the hybrid scions' characteristics, each rootstock has variations in their genetics and the vigor they show in their environmental interactions, which they transmit to their grafted scions. The larger values in FYP, NFP, and AFW are because the grafted plants were more vigorous than their non-grafted counterparts. The 20% increase in NFP in CLX-PTX991-grafted plants was thanks to this increased vigor and the greater carrying capacity it confers. Their final yields also reflected this increase because the yield is determined by the number of harvested fruits per unit of area and their individual sizes [39]. The increases in average fruit weight and fruit length of up to 21.6% and 5.5%, respectively, from using the Yaocali F1 or CLX-PTX991 F1 rootstocks, can be attributed to the overall greater plant growth and development that they promote [25], indicated by the 20% and 6.5%, respectively, increases in each parameter. Given the vigor that rootstock cultivars generally possess, frequently characterized by more efficient water and nutrient absorption [5,29], similar results are reported for grafted cucumber [40] and grafted tomato [17], as well as a 30% increase in dry biomass production and greater accumulation of mineral elements in the aerial parts of the plant [41]. Our recorded yield increases were only slightly higher than the 25% increase reported by Lopez et al. [28], and less than the up to 50% yield increases described by Muñoz et al. [42] and Sánchez et al. [25] for bell pepper. The latter result

was achieved with the Facinato and Janette hybrids grafted onto the Terreno F1 rootstock. Taken together, these results indicate that grafting onto rootstock could be a viable technique for sustainable horticulture in the future. Other reports of improving bell pepper yields through grafting [43,44] suggest that the robustness of the rootstock could be a result of greater salt tolerance, which allows an increase in commercial yields [11]. Previous studies with grafted tomato have reported a 30% [17], and up to 35% [27], yield increase, as well as a reduction in the incidence of disease. Improved vigor due to rootstock grafting was also evident in the values observed for plant height, leaf length, leaf width, and stem thickness. All of those morphological characteristics translate into a greater capacity for assimilation, absorption, and nutrient transport through the rootstock roots up to the sites of high demand when they are greater than normal [13,45,46].

The fruit quality parameters such as FED, MT, vitamin C content, and fruit firmness were not affected by the use of different rootstocks. As such, it can be inferred that the environment negligibly affects them or that the greenhouse environment did not exert sufficient pressure to induce significant changes in the grafted scions. Sánchez et al. [25], who affirm that neither fruit firmness nor total soluble solid content are affected by rootstock grafting, reported similar results. Regardless, the environmental pressure was sufficient to induce smaller fruit size and greater TSS content in non-grafted hybrid bell pepper plants. The increase in vitamin C content is consistent with the 33% increase in grafted tomatoes [47], which indicates that environmental root modifications favor its accumulation and subsequent expression in fruit quality. Conversely, it has been [13] reported that TSS content and titratable acid content are not affected by rootstock grafting, although this could be an indication that some fruit quality parameters are affected more by the foliar area, not the root system [48], even though these same parameters increase in the presence of osmotic stress [49] or mycorrhizas [15]. As it stands, finding the ideal combination of rootstock and scion that both increases yields and the commercial quality of the fruit is not an easy task, especially in the face of an ever-growing market demand and consumer demand for higher nutritive quality food products.

The significant interactions between cultivation system and rootstock or cultivation system and graft are inferred to be due to genetic differences in the rootstocks or grafts, which provide differences in the ability to adapt to each of the farming systems. Leaf width was affected significantly by the rootstock and variety interaction, because of genetic differences that can induce changes in root or stem characteristics, and mobilize different amounts of water and mineral salts, which are reflected in morphological or anatomical changes of the plant.

Among the bell pepper hybrids chosen as the scions, there are strong genetic differences that were fortified by the vigor of the used rootstocks. After comparison of the scion figures for FYP and NFP, the Lamborghini, Dicaprio, and Bambuca hybrids exhibited the greatest genetic potential for yield and yield parameters, compared with the Ucumari hybrid. The selection of the vegetative material for commercial production is vitally important, as that is what assures optimal production and success of the agricultural project. The lack of interaction between the hybrid scion and cultivation substrate or rootstock suggests that the genetics of the chosen hybrid have a greater effect on fruit quality parameters, as supported by investigators [48], who report the scion genotype as having greater influence than the rootstock.

5. Conclusions

The use of coconut fiber as the cultivation substrate allows for significant increases in bell pepper fruit yields when compared with soil-based production.

The vigor and tolerance to abiotic and biotic stresses conferred by the use of bell pepper rootstocks led to yield increases of up to 30% when compared with cultivation of non-grafted bell pepper.

In order to achieve high yields of bell pepper fruits, the use of genetically superior rootstock varieties with proven vigor and tolerance to abiotic and biotic stresses, as well as scions with strong genetic potential for high yields, is essential. In the present study, the CLX-PTX991 rootstock and the Lamborghini F1, Dicaprio F1, and Bambuca F1 hybrid scions emerged as the best candidates.

Interactions between rootstocks and grafts, as well as culture systems, induce different responses, in morphological characteristics or performance components. Therefore, it is necessary to find compatible genotypes to achieve the best fruit yields.

The lack of interaction between the graft and rootstocks suggests that the genetics of the chosen hybrid have a greater effect on the quality parameters of the fruit, since these were not affected by the rootstock.

The highest yield of fruit in the plants grown in coconut fiber, is inferred was due to the greater vigor of these plants, manifested by the greater height of the plant, length and width of the leaf and thickness of the stem. These characteristics favor a greater capacity of assimilation of light, absorption and transport of nutrients through the roots of the rootstocks to places of high demand.

Author Contributions: Conceptualization, V.R.-T. and M.C.-d.I.F.; Methodology, M.Á.P.-R.; Software, N.C.-M. and M.Á.P.-R.; Formal Analysis, R.M.-V.; Investigation, N.C.-M. and F.R.-G.; Writing-Original Draft Preparation, N.C.-M.; Writing-Review & Editing, V.R.-T.; Supervision, V.R.-T.; Project Administration, F.R.-G. and V.R.-T. All authors were responsible for processing information and manuscript writing. All authors read and approved the final manuscript.

Funding: UAAAN for the economic support to the project 2158, and to the CONACyT for the Scholarship awarded to carry out the project.

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

References

- King, S.R.; Davis, A.R.; Zhang, X.; Crosby, K. Genetics, breeding and selection of rootstock for solanaceae and cucurbitaceae. *Sci. Hortic.* **2010**, *127*, 106–111. [[CrossRef](#)]
- Zhao, X.; Guo, Y.; Huber, D.J.; Lee, J. Grafting effects on postharvest ripening and quality of 1-methylcyclopropene-treated muskmelon fruit. *Sci. Hortic.* **2011**, *130*, 581–587. [[CrossRef](#)]
- Hartmann, H.T.; Kester, D.E.; Davies, F.T.; Geneve, R.L. *Plant Propagation*; Prentice Hall: Upper Saddle River, NJ, USA, 1997; pp. 873–875, ISBN 9780136792352.
- Sakata, Y.; Ohara, T.; Sugiyama, M. The history and present state of the grafting of cucurbitaceous vegetable in Japan. *Acta Hortic.* **2007**, *731*, 159–170. [[CrossRef](#)]
- Lee, J.M.; Kubota, C.; Tsao, S.J.; Bie, Z.; Hoyos, E.P.; Morra, L.; Oda, M. Current status of vegetable grafting: Diffusion, grafting techniques, automation. *Sci. Hortic.* **2010**, *127*, 93–105. [[CrossRef](#)]
- Ren, Y.; Guo, S.; Shu, S.; Xu, Y.; Sun, Y. Isolation and expression pattern analysis of CmRNF5 and CmNPH3L potentially involved in graft compatibility in cucumber/pumpkin graft combinations. *Sci. Hortic.* **2018**, *227*, 92–101. [[CrossRef](#)]
- Lopes, C.A.; Mendonca, J.L. Reaction of accessions of two species of “jurubeba” as rootstocks to protect tomato plants against bacterial wilt. *Hortic. Bras.* **2016**, *34*, 356–360. [[CrossRef](#)]
- Sakata, Y.; Ohara, T.; Sugiyama, M. The history of melon and cucumber grafting in Japan. *Acta Hortic.* **2008**, *767*, 217–228. [[CrossRef](#)]
- Louws, J.F.; Rivard, L.C.; Kubota, C. Grafting fruiting vegetables to manage soilborne pathogens, foliar pathogens, arthropods and weeds. *Sci. Hortic.* **2010**, *127*, 125–146. [[CrossRef](#)]
- Rouphael, J.; Schwarz, D.; Krumbein, A.; Colla, G. Impact of grafting on product quality of fruit vegetables. *Sci. Hortic.* **2010**, *127*, 172–179. [[CrossRef](#)]
- Penella, C.; Nebauer, S.G.; López, G.S.; Quiñonez, A.; Calatayud, A. Grafting pepper onto tolerant rootstocks: An environmental-friendly technique overcome water and salt stress. *Sci. Hortic.* **2017**, *226*, 33–41. [[CrossRef](#)]
- Oztekin, G.; Tüzel, Y.; Gül, A.; Tüzel, I.H. Effects of grafting in saline conditions. *Acta Hortic.* **2007**, *761*, 349–355. [[CrossRef](#)]
- Colla, G.; Rouphael, Y.; Cardarelli, M.; Salerno, A.; Rea, E. The effectiveness of grafting to improve alkalinity tolerance in watermelon. *Environ. Exp. Bot.* **2010**, *68*, 283–291. [[CrossRef](#)]
- Schwarz, D.; Rouphael, Y.; Colla, G.; Venema, J.H. Grafting as a tool to improve tolerance of vegetables to abiotic stresses: thermal stress, water stress and organic pollutants. *Sci. Hortic.* **2010**, *127*, 162–171. [[CrossRef](#)]
- Oztekin, G.B.; Tuzel, Y.; Tuzel, H.I. Does mycorrhiza improve salinity tolerance in grafted plants? *Sci. Hortic.* **2013**, *149*, 55–60. [[CrossRef](#)]

16. Báez, V.E.P.; Carrillo, F.J.A.; Báez, S.M.A.; García, E.R.S.; Valdez, T.J.B.; Contreras, M.R. Resistant Rootstocks Utilization for *Fusarium* Control (*Fusarium oxysporum* f. sp. *lycopersici* Snyder & Hansen race 3) in Tomato (*Lycopersicon esculentum* Mill) under Shade Conditions. *Rev. Mex. Fitopatol.* **2010**, *28*, 111–123.
17. Álvarez, H.J.C. Agronomic performance and incidence of diseases in tomato grafted plants (*Solanum lycopersicum* L.). *Acta Agron.* **2012**, *61*, 117–125.
18. Sabatino, L.; Iapichino, G.; Maggio, A.; D'Anna, E.; Bruno, M.; D'Anna, F. Grafting affects yield and phenolic profile of *Solanum melongena* L. landraces. *J. Integr. Agric.* **2016**, *15*, 1017–1024. [[CrossRef](#)]
19. Mena, P.Y.M.; Mesa, C.N.C.; Estrada, S.E.I.; García, V.Y. Evaluation of resistance to *Prodidiplosis longifila* Gagné (Diptera: Cecidomyiidae) in cultivated and wild tomato genotypes. *Acta Agron.* **2014**, *63*, 181–190. [[CrossRef](#)]
20. Ezziyani, M.; Pérez, S.C.; Requena, M.E.; Sid, A.A.; Candela, M.E. Efecto del sustrato y la temperatura en el control biológico de *Phytophthora capsici* en pimiento (*Capsicum annuum* L.). *Anales Biol.* **2005**, *27*, 119–126.
21. García, R.M.A.; Chiquito, A.E.; Loeza, L.P.D.; Godoy, H.H.; Villordo, P.E.; Pons, H.J.L.; González, C.M.M.; Anaya, L.J.L. Production of ancho chili graft on criollo de Morelos 334 for the control of *Phytophthora capsici*. *Agrociencia* **2010**, *44*, 701–709.
22. Osuna, Á.P.; Aguilar, S.P.; Fernández, P.S.; Godoy, H.H.; Corral, D.B.; Flores, M.J.P.; Borrego, P.A.; Olivas, E. Injertos en chiles tipo Cayene, jalapeño y chilaca en el noroeste de Chihuahua, México. *Rev. Mex. Cienc. Agríc.* **2012**, *3*, 739–750.
23. Guijón, L.C.; González, G.P.A. Estudio regional de las enfermedades del chile (*Capsicum annum* L.) y su comportamiento temporal en el sur de Chihuahua. *Rev. Mex. Fitopatol.* **2001**, *19*, 49–56.
24. Rico, G.L.; Medina, R.S.; Muñoz, S.C.I.; Guevara, O.L.; Guevara, G.R.G.; Guerrero, A.B.Z.; Torres, P.I.; Rodríguez, G.R. Detección de *Phytophthora capsici* Leonian en plantas de chile (*Capsicum annuum* L.) mediante PCR. *Rev. Mex. Fitopatol.* **2004**, *22*, 1–6.
25. Sánchez, C.E.; Torres, G.A.; Flores, C.M.A.; Preciado, R.P.; Marquez, Q.C. Use of rootstocks on the yield, fruit quality and resistance to *Phytophthora capsici* Leonian in bell peppers. *Rev. Electrón. Nova Sci.* **2015**, *7*, 227–244.
26. Marquez, P.A.; Sánchez, C.E. Influence of the variety, rootstock and harvest time on the quality and maturity indices in Bell pepper. *Nova Sci.* **2017**, *19*, 1–23.
27. Chew, M.Y.L.L.; Gaytan, M.A.; Espinoza, A.J.J.; Reta, S.D.G.; Reyes, J.I.; Chew, M.R.G.; Ramírez, F.R. Planta de tomate injertada bajo condiciones de invernadero: Rendimiento y calidad de fruto. *Agrofaz* **2012**, *12*, 31–38.
28. López, M.J.; Galvez, L.A.; Porras, I.; Brotons, M.J.M. Injerto en pimiento (*Capsicum annuum*): Beneficios y rentabilidad de su uso. *ITEA* **2012**, *20*, 1–20. [[CrossRef](#)]
29. Lopez, M.J.; Galvez, L.A.; Del Amor, F.; Albacete, A.; Pérez, A.F. Selecting vegetative/generative/dwarfing rootstocks for improving fruit yield and quality in water stressed sweet pepper. *Sci. Hortic.* **2014**, *214*, 9–17. [[CrossRef](#)]
30. Huang, W.; Liao, S.; Haiyan, L.; Khaldun, A.B.M.; Wang, Y. Characterization of the growth and fruit quality of tomato grafted on a woody medicinal plant, *Lycium Chínense*. *Sci. Hortic.* **2015**, *197*, 447–453. [[CrossRef](#)]
31. Johnson, S.; Miles, C.; Kreider, P.; Roozen, J. *Injerto de Verduras; Berenjena y Tomate*; Publicacion de la Extension de la Universidad Estatal de Washington FS052E: Washington, DC, USA, 2011.
32. Steiner, A.A. The influence of chemical composition of a nutrient solution on the production of tomato plants. *Plant Soil* **1966**, *24*, 454–466. [[CrossRef](#)]
33. AOAC. *The Official Methods of Analysis of AOAC (Association of Official Analytical Chemists) International*, 17th ed.; AOAC International: Gaithersburg, MD, USA, 2005.
34. López, B.J.; Méndez, M.A.; Pliego, M.L.; Aragón, R.E.; Lourdes, R.M. Agronomic evaluation of substrates in pepper seedlings 'onza' (*Capsicum annuum*) in greenhouse. *Rev. Mex. Cienc. Agríc.* **2013**, *6*, 1139–1150.
35. Ortega, M.L.D.; Sánchez, O.J.; Ocampo, M.J.; Sandoval, C.E.; Salcido, R.B.A.; Manzo, R.F. Effect of different substrates on the growth and yield of tomato (*Lycopersicum esculentum* mill) under greenhouse conditions. *Ra Ximhai* **2010**, *6*, 339–346.
36. Cruz, C.E.; Sandoval, V.M.; Volke, H.V.H.; Can, C.A.; Sánchez, E.J. Mixtures of substrates and nutrient solution concentration effect on growth and yield of tomato. *Rev. Mex. Cienc. Agríc.* **2012**, *3*, 1361–1373.
37. De Grazia, J.; Tittonell, P.A.; Chiesa, A. The effect of substrates with compost and nitrogenous fertilization on photosynthesis, precocity and pepper (*Capsicum annuum*) yield. *Cienc. Investig. Agrar.* **2006**, *34*, 195–204. [[CrossRef](#)]

38. Valles, R.G.J.; Lugo, G.J.G.; Rodríguez, G.Z.F.; Díaz, T.L.T. Effects of substrate and plant spacing on growth of pepper (*Capsicum annuum* L.) plants in hydroponic system without cover. *Rev. Fac. Agron.* **2009**, *26*, 159–178.
39. Sánchez-del Castillo, F.; Moreno-Pérez, E.D.C.; Vázquez-Rodríguez, J.C.; González-Núñez, M.Á. Population densities and blunting levels for contrasting varieties of greenhouse tomatoes. *Rev. Chapingo. Ser. Hortic.* **2017**, *23*, 167–174.
40. González, G.H.; Ramirez, G.F.; Ortega, O.O.; Benavides, M.A.; Robledo, T.V.; de la Cabrera, F.M. Use of chitosan-PVA Hidrogels with copper nanoparticles to improve the growth of grafted watermelon. *Molecules* **2017**, *22*, 1031. [[CrossRef](#)]
41. San Bautista, A.; Calatayud, A.; Nabuer, A.G.; Pascual, B.; Marto, J.V.; López, G.S. Effects of simple and double grafting melon plants on mineral absorption, photosynthesis, biomass and yield. *Sci. Hortic.* **2011**, *130*, 575–580. [[CrossRef](#)]
42. Muñoz, M.E.; Sánchez, C.E.; Flores, C.M.A.; Sida, A.J.P. ¿Puede el Portainjerto Incrementar la Producción en Variedades de Pimiento Morrón? Memorias del XL Congreso Nacional de la Ciencia del Suelo 2015, Año Internacional de los Suelos: Crear Conciencia en la Sociedad Para el Manejo Sostenible del Suelo. 2015, pp. 210–214. Available online: http://www.smcsmx.org/files/congresos/2015/4_DIVISION_2.pdf (accessed on 8 February 2018).
43. Colla, G.; Roupheal, Y.; Cardarelli, M.; Temperini, O.; Rea, E.; Salerno, A.; Pierandrei, F. Influence of grafting on yield and fruit quality of pepper (*Capsicum annuum* L.) grown under greenhouse conditions. *Acta Hortic.* **2008**, *782*, 359–363. [[CrossRef](#)]
44. Del Amor, F.M.; López, M.J.; González, A. Effect of photoselective sheet and grafting technique on growth, yield, and mineral composition of sweet pepper plants. *J. Plant Nutr.* **2008**, *31*, 1108–1120. [[CrossRef](#)]
45. Savvas, D.; Colla, G.; Roupheal, Y.; Schwarz, D. Amelioration of heavy metal and nutrient stress in fruit vegetables by grafting. *Sci. Hortic.* **2010**, *127*, 156–161. [[CrossRef](#)]
46. Khah, E.M.E.; Kakava, A.; Mavromatis, D.; Chachalis, G.; Goulas, G. Effect of grafting on growth and yield of tomato (*Lycopersicon esculentum* Mill.) in greenhouse and open-field. *J. Appl. Hortic.* **2006**, *8*, 3–7.
47. Chávez, M.C.; Sánchez, E.; Muñoz, M.E.; Sida, A.J.P.; Flores, C.M.A. Bioactive compounds and antioxidant activity in different grafted varieties of bell pepper. *Antioxidants* **2015**, *4*, 427–446. [[CrossRef](#)] [[PubMed](#)]
48. Martínez, R.M.M.; Estañ, M.T.; Moyano, E.; García, A.J.O.; Flores, F.B.; Campos, J.F.; Al, A.M.J.; Flowers, T.J.; Bolarín, M.C. The effectiveness of grafting to improve salt tolerance in tomato when an “excluder” genotype is used as scion. *Environ. Exp. Bot.* **2008**, *63*, 392–401. [[CrossRef](#)]
49. Flores, F.B.; Sánchez, B.P.; Estañ, M.T.; Martínez, R.M.M.; Moyano, M.B.; Campos, J.F.; García, A.J.O.; Egea, M.I.; Fernández, G.N.; Romojaro, F.; et al. The effectiveness of grafting to improve tomato fruit quality. *Sci. Hortic.* **2010**, *125*, 211–217. [[CrossRef](#)]



© 2018 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).