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Asexual reproduction: an alternative for the propagation and conservation of papaya (*Carica papaya* L.) native to Guerrero, Mexico

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ABSTRACT

Objective: Evaluate the effect of three types of substrate and different shoot lengths on the rooting of *Carica papaya* L. shoots.

Design/methodology/approach: This experiment followed a completely randomized design with a 2×3 factorial arrangement. Shoots of 30 and 40 cm of length were collected in March 2018 from the lateral branches of papaya plants (*Carica papaya* L.) native to various regions of Guerrero. Shoots were placed in different substrates: 1) sand, 2) Peat Moss®, and 3) sand and Peat Moss® mixture (70:30, v/v). Sixty days after planting, rooting percentage, root length, plant height, stem diameter, number of leaves, crown diameter, biomass fresh weight, biomass dry weight, root fresh weight, and root dry weight were evaluated. Data were analyzed through an analysis of variance and a mean difference test (Tukey, $p \leq 0.05$).

Results: The rooting of 40- and 30-cm shoots was 60 and 50%, respectively. Plants with the highest height, number of leaves, root length, and crown diameter derived from 40-cm shoots. Sand was the best substrate for rooting, where plants with higher fresh biomass were significantly developed.

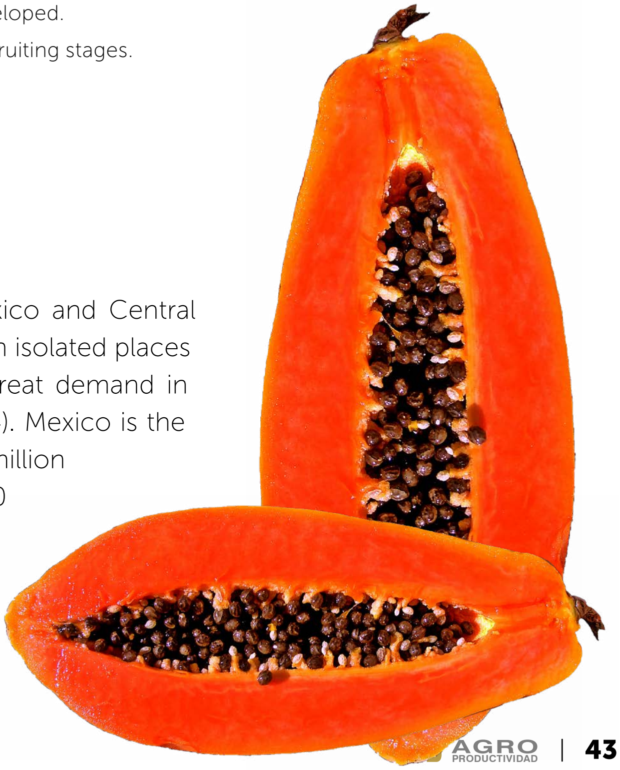
Study limitations/implications: Continue study during the flowering and fruiting stages.

Findings/conclusions: This protocol allows the rooting of papaya shoots.

Keywords: *Carica papaya*, rooting, shoots, root length, substrate.

INTRODUCTION

Papaya (*Carica papaya* L.) originated in southern Mexico and Central America. It is possible to find wild populations in isolated places in these regions. Papaya is an economically important crop with great demand in the national and international markets (Fuentes and Santamaría, 2014). Mexico is the third country worldwide with the highest papaya production, one million 40 thousand tons/year, and the first exporter with more than 163,000 tons/year (FAOSTAT, 2019).



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Several researchers around the world work on the preservation of genetic variability. More than 30 *Carica* sp. collections exist worldwide to preserve, characterize, and evaluate germplasm (Dantas *et al*., 2002).

The most used propagation method is by seed; this is an easy and inexpensive approach that takes advantage of the fact that fruits generally have hundreds of seeds. However, this method has some disadvantages, such as genetic and phenotypic heterogeneity (Jiménez, 2002). Papaya is a dioecious species with female and hermaphrodite flowers in different plants. In the market, the preferred fruits come from hermaphrodite plants. Since sex can only be determined by the plant's flowers, which appear after the fifth month of germination, propagation by open-pollinated seeds decreases production value (Giampan *et al*., 2005). Moreover, the sarcotesta, the mucilaginous external layer of the seeds, can cause dormancy, which results in dehydration-sensitive seeds that easily lose viability (Tokuhisa *et al*., 2007). This problem could be overcome by vegetative propagation of shoots and grafts; these simple, fast, and inexpensive techniques preserve the genotypic characteristics of mother plants (Giampan *et al*., 2005; Hartmann *et al*., 1990).

Papaya can be propagated by the rooting of shoots; these asexually propagated plants show greater uniformity, shorter fruiting time, shorter fruiting height, and higher yield per productive cycle (Ruíz-López, 2016). This propagation method allows the selective multiplication of plants with desired characteristics, such as hybrids, hermaphrodite plants free of pests and diseases, among others (Allan, 1990; Giampan *et al*., 2005). Additionally, the plants obtained using this propagation method start flowering shortly after plantation; thus, fruiting occurs earlier than in the plants obtained by seed propagation (Giampan *et al*., 2005; Grana, 2000; Reuveni and Shlesinger, 1990). A common problem of asexual propagation is shoot rooting (Grana, 2000).

Hidaka *et al*. (2008), Ruíz-López (2016), and Yu *et al*. (2000) reported that shoot length affects the vigor of the generated plant. Additionally, solutions containing auxins, such as indole-3-butyric acid (IBA), or combinations of stimulating hormones significantly affect the rooting and survival of papaya shoots.

It is possible to secure higher shoot rooting percentages and plant quality through the evaluation and control of different factors, such as type of substrate, age of the mother plant, shoot length and type, and the dose of growth regulators (Hartmann *et al*., 2002; Rivera-Rodríguez *et al*., 2016). Biotechnology could be an alternative tool for the rapid propagation of elite papaya plants, as well as the commercial scale introduction of new varieties or hybrids (Posada-Pérez, 2016). Therefore, this study aimed to evaluate the effect of three types of substrate and different shoot lengths on the rooting of *Carica papaya* L. shoots.

MATERIALS AND METHODS

Collection. The vegetative material (shoots) was collected from the lateral branches of papaya plants (*Carica papaya* L.) native to several Guerrero regions. Shoots were stored in properly labeled plastic bags and kept in a cooler during their transportation to the planting site. Twenty-four hours after collection, shoots were processed in the nursery and fruit propagation laboratory (18° 20' 39.4", N 99° 30' 09.7" W).

Propagation material. Shoots of 30 and 40 cm and a diameter of 1.5 cm were collected. Leaves were cut with a previously disinfected scalpel; only a couple of them were left at the apical part. The material was washed with running water and immersed for 5 min in a 1.5 g L^{-1} fungicide solution (Mancozeb 80 WP) to reduce contaminating microorganisms. Subsequently, shoots were submerged for 10 min into a Rootex® solution (2.0 g L^{-1}); Rootex® is both a fertilizer and a rooting agent. These prepared shoots were then planted in the substrate 5 cm deep in 59×19×16 cm disinfected plastic pots with three 2-cm diameter perforations at the bottom. Three types of substrates were used: 1) sand, 2) Peat Moss®, and 3) sand and Peat Moss® mixture (70:30, v/v). Substrates were sterilized with steam for 30 min.

Pots were kept in a nursery at field capacity and irrigated every three days. Additionally, Rootex® (a combination of rooting promoting amino acids, organic acids, and nutrients) was applied three times (2.0 g L^{-1}), at 24 h, 8, and 16 days after planting. During the experiment, the nursery temperature was 25±4 °C, relative humidity ranged from 70-80%, and plants were subjected to a 12±1 h photoperiod. These variables were recorded every two hours using a Hobo® U12 data logger.

Evaluated variables. Sixty days after establishing the experiment, rooting percentage (RP), root length (RL), plant height (PH), stem diameter (SD), number of leaves (NL), crown diameter (CD), biomass fresh weight (BFW), biomass dry weight (BDW), root fresh weight (RFW), and root dry weight (RDW) were evaluated.

Experimental design and statistical analysis. This experiment followed a completely randomized design with a 2×3 factorial arrangement. The studied factors were: three types of substrate (sand, Peat Moss®, and mix of both) and two shoot lengths (40 and 30 cm), as well as the interaction of both factors. Three shoots (replicates) per pot were placed using only one substrate type (factor 1) and shoot length (factor 2). Thus, six combinations were studied, with four repetitions (pots) per combination, 24 experimental units in total. The experimental units were randomized using the "design.ab" procedure in the statistical program "R" to ensure the independence of the observations. Error normality was determined using the Shapiro-Wilk test, and Barttlet's test was used to evaluate the homogeneity of variances. An analysis of variance and a Tukey mean difference test ($p \leq 0.05$) were carried out using the statistical software SAS® V.9.4 for the study factors and evaluated variables.

RESULTS AND DISCUSSION

Rooting. There were no significant differences in the rooting of papaya shoots between the different substrates ($p \leq 0.05$). However, the rooting of the 40-cm long shoots was significantly higher (Figure 1).

Moreover, all the experimental units had a positive response to the treatments (number of roots, root length, and biomass dry and fresh weight). This demonstrates that asexual propagation is a viable option for establishing mass reproduction and conservation units of papaya plants. This could be due to the positive action of the rooting agent Rootex® as an exogenous growth regulator. Under natural conditions, auxins intervene in root formation (Cuisance, 1988). The use of auxins is important in species with rooting difficulties (Hartmann *et al*., 2002). Vargas-Simón *et al*. (1999) reported higher rooting percentages, number of roots, and root length in icaco plants (*Chrysobalanus icaco* L.) treated with 10 000 mg·L^{-1} of indole-3- butyric acid (Radix 10 000®). González-Pulido *et al*. (2019) evaluated the effect of the cutting type, treatment with a rooting agent (Radix 10 000®), and fertilization in *Acer negundo*. Their results indicated differences between the different cutting types; the use of the rooting agent resulted in larger buds and higher dry and fresh weights. The rooting agent/fertilizer interaction had a positive synergistic effect on the different evaluated variables.

Similar to our results, Ruíz-López (2016) reported that shoot length significantly affects plant vigor. Furthermore, he mentions that using a rooting agent, such as IBA, is critical for successfully establishing papaya shoots. Yu *et al*. (2000) reported that low IBA concentrations (2.5 Mm) result in satisfactory rooting percentages. Schmildt *et al*. (2016) reported significant rooting results in papaya cv. Calimán shoots treated with 1.5 to 2 ppm of IBA.

Root length, plant height, and crown diameter. The plants originated from 40-cm shoots were statistically taller, with a higher number of leaves, wider crown diameter, and longer root length (>20 cm) (Table 1).

Plants from the Sand*40-cm shoot interaction had the highest plant heights, number of leaves, crown diameter, and root lengths (Table 2). These results suggest collecting shoots of at least 40 cm long and

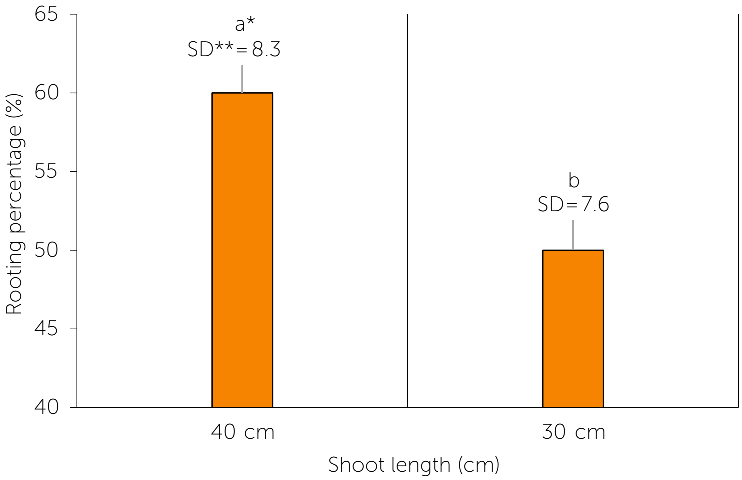

Figure 1. Rooting percentage of *Carica papaya* L. shoots according to shoot length. *Means with different letters are statistically different according to the Tukey test ($p \leq 0.05$). ** SD=Standard Deviation.

Table 1. Effect of shoot length in *Carica papaya* L. plants.

Shoot length (cm)	PH*	SD	NL	CD	RL
40	45.58 a**	12.83 a	6.83 a	27.62 a	27.72 a
30	35.33 b	11.62 a	4.16 a	6.66 b	4.83 b

*PH=Plant height, SD=Stem diameter, NL=Number of leaves, CD=Crown diameter, RL=Root length. **Means with different letters in the same column are statistically different according to the Tukey test (p≤0.05).

Table 2. Effect of the multiple interactions of different substrates and shoot lengths on the plants developed from *Carica papaya* L. shoots.

Interaction (cm)	PH*	SD	NL	CD	RL
Sand - 40	46.50 a**	12.50 a	8.25 a	31.87 a	31.66 a
Sand - 30	35.00 b	13.25 a	3.75 b	7.12 bc	5.25 b
Peat Moss®- 40	44.75 a	13.00 a	7.50 ba	23.50 bac	27.50 a
Peat Moss®- 30	35.50 b	11.87 a	4.00 b	5.25 c	4.25 b
Mixture- 40	45.50 a	13.00 a	4.75 ba	27.50 ba	24.00 a
Mixture- 30	35.50 b	9.75 a	4.75 ba	7.62 bc	5.00 b

*PH=Plant height, SD=Stem diameter, NL=Number of leaves, CD=Crown diameter, RL=Root length. **Means with different letters in the same column are statistically different according to the Tukey test (p≤0.05).

carefully considering the type of rooting substrate used for propagation.

Rivera-Rodríguez *et al*. (2016) evaluated the effect of different substrates (perlite *vs*. peat and vermiculite mix), the mother plant age (12, 18, and 24 months), and the dose of IBA (0, 5000, and 10 000 ppm) on the rooting of *Pinus patula* shoots. They observed the highest rooting percentage with perlite; however, they obtained the highest survival, root length, and callus percentage in non-rooted shoots using the peat-vermiculite mix. Galindo-García *et al*. (2012) reported the rooting of poinsettia shoots using indolebutyric acid (IBA). IBA is particularly recommended in warm climates or when propagation is delayed.

Root, dry biomass, and fresh biomass weight. The statistical analysis and factorial design determined that sand was the best substrate; the fresh biomass weight was significantly higher using this substrate (Table 3).

Furthermore, this study determined that the 40-cm shoots resulted in plants with higher fresh and biomass weights (Table 4). The latter was confirmed in the factor interaction analysis (Table 5).

These results are like those reported by Cachique *et al*. (2011), who evaluated two substrate types, five IBA doses, and three shoot lengths to optimize the asexual propagation of sacha inchi (*Plukenetia volubilis* L.). Their factor interaction analysis indicated that the use of intermediate texture sand, 0.2% of IBA, and 8-cm shoots was the best combination for high rooting percentages and plant quality. Similarly, Boschini-Figueroa and Rodríguez (2002) reported the significantly (P≤0.01) successful rooting and establishment of white mulberry

Table 3. Effect of different substrates on the plants developed from *Carica papaya* L. shoots.

Substrate	BFW*	BDW	RFW	RDW
Sand	24.91 a**	5.19 a	3.21 a	0.63 a
Peat Moss®	17.87 ba	5.39 a	2.87 a	0.55 a
Mixture	16.00 b	3.98 a	2.43 a	0.46 a

*BFW=Biomass fresh weight, BDW=Biomass dry weight, RFW=Root fresh weight, RDW=Root dry weight. **Means with different letters in the same column are statistically different according to the Tukey test (p≤0.05).

Table 4. Effect of shoot length in *Carica papaya* L. plants.

Stem length (cm)	BFW*	BDW	RFW	RDW
40	24.33 a**	6.25 a	3.75 a	0.73 a
30	14.86 b	3.45 b	1.93 b	0.36 b

*BFW=Biomass fresh weight, BDW=Biomass dry weight, RFW=Root fresh weight, RDW=Root dry weight. **Means with different letters in the same column are statistically different according to the Tukey test (p≤0.05).

Table 5. Effect of the multiple interactions of different substrates and shoot lengths on the plants developed from *Carica papaya* L. shoots.

Interaction (cm)	BFW*	BDW	RFW	RDW
Sand - 40	29.50 a**	7.02 a	4.12 a	0.77 a
Sand - 30	20.33 ba	3.36 a	2.30 a	0.50 a
Peat Moss®- 40	23.25 ba	5.85 a	4.00 a	0.77 a
Peat Moss®- 30	12.50 b	4.93 a	1.75 a	0.32 a
Mixture- 40	20.25 ba	5.90 a	3.12 a	0.65 a
Mixture- 30	11.75 b	2.06 a	1.75 a	0.27 a

*BFW=Biomass fresh weight, BDW=Biomass dry weight, RFW=Root fresh weight, RDW=Root dry weight. **Means with different letters in the same column are statistically different according to the Tukey test ($p \leq 0.05$).

(*Morus alba*) shoots using IBA, compared to untreated shoots.

CONCLUSIONS

This protocol allows the rooting of *C. papaya* shoots and their conservation by establishing mass production units. The 40-cm shoot and sand substrate interaction showed the highest rooting percentages. The proper management of propagation material during collection and the different intervening factors, such as substrate type, age of the mother plant, and use of growth regulators, are crucial for obtaining satisfactory results.

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