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Article

# Promising rootstocks and peculiar handling of the grafting technique for papaya

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## Abstract

Papaya has the potential to be grafted, derived from the fact that papaya populations develop, under edaphoclimatic and phytosanitary pressure, characteristics that can be used as rootstocks. Therefore, the standardization of a grafting method for papaya is required; however, in the early stage, it is important to evaluate the development of seedlings for the formation of grafted plants. The objectives were to evaluate the field behavior of different promising papaya materials, synchronize seedling development of prospective materials in the use of rootstocks and grafts, and develop a grafting method for papaya. Trials were conducted for each aspect in 2018. In the first experiment, five papaya genotypes were established under an experimental design. Morphological and productive variables were recorded. In the second experiment, materials derived from the previous experiment were used, a commercial one and papaya as a control, and plant height, stem diameter, and number of leaves were recorded. In the third experiment, different grafting methods were tested. Three trials were formed. The results were remarkable; the genotypes showed development capacity, adaptability, and productivity, and that they are potential lines for use as papaya rootstocks. In addition, it is possible to synchronize the seeding to generate quality grafted plants; the rootstock and graft seedlings reached maturity and size for grafting at 35 days. An effective procedure was also designed to graft papaya seedling under normal environmental conditions, called modified approach grafting, with 92.5% attachment in papaya.

## Palabras clave:

Carica papaya, papaya ecotypes, plant vigor, rootstock..

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## Introduction

In the world, papaya is the potentially most productive fruit species and it is commercially produced all year round. Recently, Mexico ranked sixth among countries with the largest area and fourth in terms of production volume (FAOSTAT, 2021). Mexico's largest producing states together totaled 19 698 ha, with Veracruz, Colima, Michoacán, Oaxaca, Chiapas, and Guerrero standing out (SIAP-SADER, 2023).

*Carica papaya* belongs to the genus *Carica* and is the most notable of the six genera of the family Caricaceae (Badillo, 2000; Antunes *et al*., 2014). Territorially, its distribution is wide, but the qualities are little addressed because it is commonly observed that plants develop under edaphoclimatic and phytosanitary pressure with outstanding transmissible characteristics (Niklas and Marler, 2007), through hybridization or the use of rootstocks, but information on characterization is limited (Álvarez *et al*., 2019).

For that reason, the use of rootstocks offers an option for their establishment in soils under limiting and management conditions. Based on the above, asexual propagation through grafting makes it possible to improve papaya (Allan, 2007; Senthilkumar *et al*., 2014). The topic of papaya grafting is limited (Van-Hong *et al*., 2018; Lima *et al*., 2018), so some factors in the initial stage of seedlings, such as graft type, compatibility, vigor, post-graft management, and health, should be considered, based on other species (Kawaguchi *et al*., 2008).

Therefore, it is common to resort to using similar grafting methods, but, when testing them on papaya, the attachment is low. For that reason, the development of a papaya method will improve the production chain (Senthilkumar *et al*., 2016). To implement this strategy, it is important to choose seedlings that present an ideal condition. Thus, the objectives were to evaluate the field behavior of different promising papaya materials, synchronize seedling development of prospective materials as rootstocks, and develop a grafting method for papaya.

## Materials and methods

Three experiments were carried out at the Valle de Apatzingán Experimental Field (CEVA, for its acronym in Spanish) of the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP, for its acronym in Spanish), in Antúnez, Michoacán, Mexico. The climate is classified as $Bs_1$ (García, 2004).

### Evaluation of promising materials for use as rootstocks

In October 2018, an experiment was established with materials of outstanding qualities for use as papaya rootstocks under an experimental design of randomized complete blocks. There were five treatments and eight replications (one plant represented one replication). The treatments consisted of the following papaya ecotypes: 1) Robusta papaya with quality; 2) Robusta papaya without quality (RWQ, RNQ); 3) wild papaya green petiole; 4) wild papaya purple petiole (WPG, WPP) and 5) Maradol papaya (MP) as control.

Agronomic management was carried out considering the technological package recommended in the region (Coria *et al*., 2017). At 208 days after transplantation, plant height, stem circumference, number and weight of fruits, number of seeds, germination (%) and plant vigor were evaluated. For this last variable, an assessment scale was designed with modifications to what was proposed by Barchuk and Díaz (2000) (Table 1).

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**Table 1. Ordinal scale used to assess plant quality.**

| | |
|---|---|
| 1. | Vigorous plant: normal plant, balanced leaf structure, intense green leaf, spaced and uniform internodes, adequate terminal shoot, buds and flower attached to the stem. |
| 2. | Plant with good vigor: normal plant, balanced leaf structure, green leaf, spaced and uniform internodes, normal terminal shoot, buds, and flower attached to the stem, some spaces on stems with no fruits. |
| 3. | Plant with little vigor: abnormal plant, unbalanced leaf structure, light green leaves, short internodes, compacted terminal shoot, limited presence of buds and flowers and several spaces on stems with no fruits. |
| 4. | Less vigorous plant: weak plant, unbalanced leaf structure, light green leaves, compacted internodes, compacted and reduced terminal shoot, without buds and flower attached to the stem. |
| 5. | Stunted plant: weak plant, unbalanced leaf structure, undeveloped leaves of clear appearance, compacted internodes, reduced terminal shoot, no signs of flower buds. |

Analyses of variance (Anova) were performed for the variables, except for the variable of plant vigor, which was analyzed with the non-parametric Kruskal-Wallis test. The analyses with differences were compared with Tukey and Mann-Whitney tests ($p \leq 0.05$). The SAS (2002) and PAST 3.2 (Hammer, 2018) programs were used, respectively.

## Assessment of genotype seedlings for rootstock use

This evaluation was carried out at the nursery of the CEVAMEX, INIFAP, during January and April 2019. The rootstocks used were two papaya materials derived from the previous test, Robusta with quality (RWQ) and Robusta without quality (RNQ), and one commercial material, Crispin (Carigen®). The Tainung genotype (TP) was used as a graft. The experimental design was randomized complete blocks of four treatments with 15 replications (seedlings).

The seeds were sown in a 7.8 x 12.4 cm plastic bag containing moistened commercial substrate Growing Mix IVM® (Canadian Sphagnum Peat Moss). Three seedling production trials were developed. Management consisted of daily irrigation supplemented with nitrogen fertilizer and the seedlings were kept free of insects with applications of chemical products ai. cypermethrin and fungicide ai. tribasic copper sulfate, as well as humic and fulvic acids.

The development lasted approximately 35 days and plant height, stem diameter, and number of leaves were recorded. The data were analyzed with the statistical program of SAS (2002) through an Anova and comparison of means by Tukey ($p \leq 0.05$).

## Evaluation of grafting techniques

Papaya seedlings were produced in a similar way to the previous test in a nursery covered with anti-aphid mesh on walls and shade mesh on top. Robusta with quality (RWQ) was used as rootstock, which was chosen based on its behavior shown in the previous evaluation; the Maradol genotype (MP) was used as a graft. Three trials were carried out 15 days apart and their data records were averaged.

Seedling management was the same as reported in the previous test. The methods tested were: similar approach grafting (Lee and Oda, 2003) fixed with metal tape and with forceps (AGFT and AGFF, respectively), similar barb grafting (Lee and Oda, 2003) fixed with metal tape and with forceps (BGFT and BGFF), and modified approach grafting (MAG).

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The latter consisted of removing the apical part of the rootstock at the time of forming the tongues by cuts in both stems and fastening with metal tape, the root system of the graft was removed six days later. The percentage of attachment was recorded based on the number of grafted plants and the number of plants attached. An Anova was performed after transforming the data to the arcsine of the square root of the proportion.

The statistical program was SAS (2002) and Tukey's mean comparison ($p \leq 0.05$). The vigor of the grafted seedlings was also assessed, measured after one week necessary for the generation of the union callus, and after three and six days post-cut, based on the scale in Table 1, using the Kruskal-Wallis test and the Mann-Whitney comparison ($p \leq 0.05$) with the PAST 3.2 program (Hammer, 2018).

# Results

## Evaluation of promising materials for use as rootstocks

The Anova performed showed statistically significant differences in the development variables (Table 2). In the variable of plant height, WPG and WPP plants were 18% taller than RWQ and RNQ; MP plants showed an intermediate behavior (Table 2).

**Table 2. Morphological characterization of genotypes for use as rootstocks.**

| Treatments | Development variables | | | | | | |
|---|---|---|---|---|---|---|---|
| | Plant height (cm)† | Stem circumference (cm) | Plant vigor§ | Fruits/ plant | Fruit weight (kg) | Num. of seeds/ fruit | Germination (%) |
| RWQ | 173c | 40ab | 1.8bc | 23.2b | 2.09a | 209.4b | 66.04a |
| RNQ | 172.4c | 41.6ab | 2.2ab | 16b | 1.86a | 215b | 62.23 a |
| WPG | 200.4b | 48.8a | 1.2c | 23b | 0.3c | 307a | 53.64a |
| WPP | 221.2a | 45.2ab | 1.4bc | 39.6a | 0.12c | 259.2ab | 53.19a |
| MP | 172.6c | 35b | 2.8a | 19.4b | 1.08b | 110c | 69.96a |
| C.V. | 3.83 | 12.5 | 21.12 | 23.4 | 13.97 | 15.84 | 22.5 |
| Significance | *** | *** | ** | *** | *** | *** | NS |

† Means within columns followed by the same letter do not differ (Tukey, 0.05); §Kruskal-Wallis (non-parametric test); CV= coefficient of variation; NS= not significant; $^{**}= p \leq 0.01$; $^{***}= p \leq 0.0001$.

It is important to note that it is desirable to have low height materials since they have a more resistant support structure. The stem circumference variable was higher in WPG plants and the RWQ, RNQ, and WPP plants were similar (Table 2). The fruits of MP had the smallest circumference (Table 2). An important characteristic of this variable is that stem thickness represents a condition that favors handling during grafting operations at the seedling stage.

WPG plants were more vigorous than RWQ and WPP, whereas MP was the significantly less vigorous treatment (Table 2). In fruit set, WPP plants stand out with more than 48% set; the rest of the treatments had between 16 and 23 set fruits (Table 2). Regarding fruit weight, the analyses of RWQ and RNQ plants showed the highest fruit weight compared to WPG and WPP plants, which showed the smallest fruits (Table 2). The number of seeds per fruit was higher in WPG and WPP plants than in RWQ and RNQ plants (Table 2); this can be explained by the 'ferality' that WPG and WPP still retain. No differences were detected in germination (Table 2).

## Assessment of genotype seedlings for rootstock use

At 35 days later, rootstock and graft seedlings reached adequate maturity for grafting. Significant differences were detected in plant height and stem diameter; the number of leaves did not show differences (Table 3). As observed, the height of plants in treatment C exceeded RWQ and RNQ plants, whereas the plants in TP treatment were lower.

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**Table 3. Development variables of papaya seedlings of different genotypes.**

| Treatments | Height (cm)[†] | Stem diameter (cm) | Leaves (num.) |
|---|---|---|---|
| RWQ | 6.59 ab | 0.17 a | 5.03 a |
| C | 6.81 a | 0.14 ab | 4.73 a |
| RNQ | 5.62 bc | 0.14 ab | 4.9 a |
| TP | 5.16 c | 0.12 b | 5.36 a |
| CV | 5.87 | 10.07 | 6.61 |
| Significance | *** | * | NS |

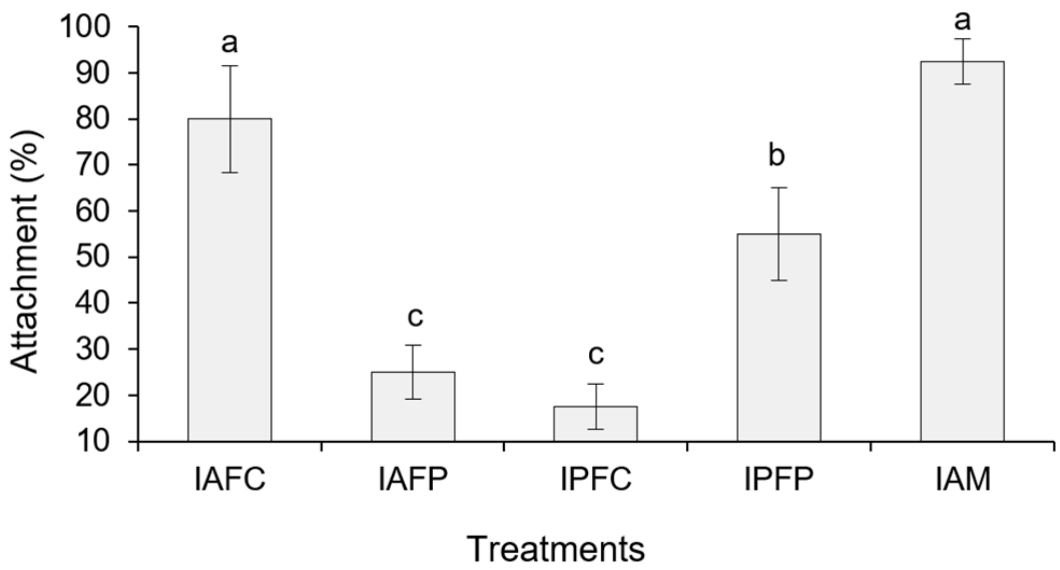
[†] Means within columns followed by the same letter do not differ (Tukey, 0.05); CV= coefficient of variation; NS= not significant; * = $p \leq 0.05$; *** = $p \leq 0.0001$.

On the other hand, the stem diameter variable reflected statistical differences. Thus, the RWQ, C, and RNQ treatments were the ones with the highest stem thickness; however, the best treatment for stem diameter was RWQ, with 0.17 cm. Likewise, the C and RNQ treatments were statistically the same as the TP treatment. For its part, the variable of number of leaves did not present differences (Table 3).

## Evaluation of grafting techniques

The percentage of attachments in MAG and AGFT was 92.5% and 80%, respectively; therefore, the first treatment exceeds the expectations of a horticultural graft (Figure 1). The AGFF, BGFT, and BGFF plants showed the lowest attachment percentages (Figure 1).

**Figure 1. Attachment of grafting methods for papaya. Each bar symbolizes the meaning of n= 40 and SD. Different le tters indicate differences (Tukey, 0.05).**




On the other hand, the MAI and AGFT plants showed the highest seedling vigor compared to the rest of the treatments (Figure 2).

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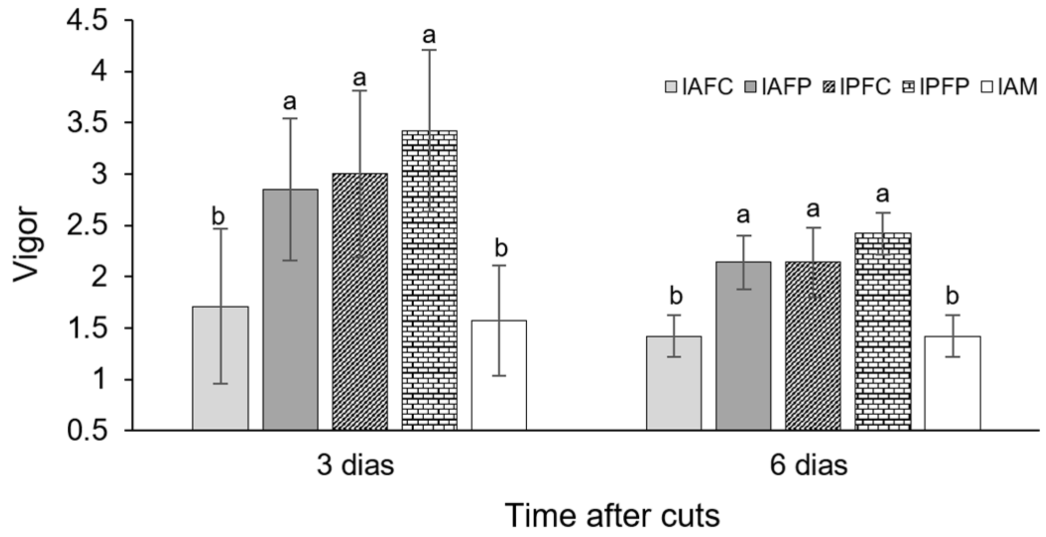
**Figure 2. Vigor of grafted seedlings in two observation times. Each bar symbolizes the meaning and SD. Different lett ers indicate differences (Tukey, 0.05).**




## Discussion

The materials called RWQ and RNQ showed a development similar to the control treatment, mainly in morphological variables and some productive variables. This condition is important since the similarity of sizes allows these parameters to be standardized to ensure the success of grafts. As Niklas and Marler (2007) point out, different materials are considered a source of natural wealth and are of agronomic interest for improvement and agricultural development purposes (Hunter and Heywood, 2011).

In terms of fruit weight, the RWQ and RNQ treatments had large fruits, of 2.09 and 1.8 kg; the WPG and WPP treatments also present smaller but outstanding fruits. In this regard, Manshardt (2014) reports papaya populations with a fruit weight of less than 100 g, which is similar to what was found in the present study. Nonetheless, in the number of seeds, the fruits of WPG and WPP, although smaller in size, had the highest number of seeds, so it is possible that the materials of these treatments still retain a level of ferality.

Nevertheless, the seed is a distinguishable characteristic since wild plants contain more seeds than cultivated populations, but smaller in size (Paz and Vázquez-Yanes, 1998). Germination was similar between treatments; however, this will depend on environmental factors; in this regard, Soriano-Melgar *et al*. (2016) reported germination percentages between 10 and 47%. As for plant vigor as a variable that reflects the state and size of the plants, the rootstocks were very stable.

Regarding the assessment of the initial development of papaya seedling for use as rootstock and graft, it is important to note that regardless of whether seedlings are desired for grafting or not, quality seedlings must be produced. For this, the choice of growth media must be adequate as they will directly influence the germination, rooting, and development of seedlings (Meena *et al*., 2017), even some additives to be supplied, mainly organic amendments and bioregulators, positively favor germination, development, and root quality (Desaid *et al*., 2017).

Therefore, in seedling production, in addition to the variable of plant height as an indicator of state, it is important that the seedlings have a large stem diameter, thus facilitating the manipulation of seedlings, cuts and fixation of devices (Kumar and Sanket, 2017), a trait shown by the RWQ, C,

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and RNQ treatments. On the other hand, approach grafting and barb grafting techniques (Lee and Oda, 2003) are the ones with the highest affinity; nevertheless, there are several factors involved that facilitate the quality of the grafts.

In fact, in papaya, an implicated and important factor is bacterial contamination (Allan *et al*., 2010); however, it did not occur in this study. As it was observed, the attachment per graft operation under the AGFT and MAG methods reached values of 80% and 92.5%, respectively, and the grafted seedling tended to show more seedling vigor, thus ensuring success in projections of quality papaya seedling production on a larger scale.

The other treatments, including barb grafting, also responded in papaya, but with a low percentage of attachment and lower post-graft seedling vigor, so it is possible to improve them with other strategies (Van-Hong and Chung-Ruey, 2018). The disadvantage of barb grafting is that temperature and humidity control are necessary while the approximation grafting method can be performed without necessarily meeting these conditions; nonetheless, seedling quality is highly influenced by growth media (Agbo and Omaliko, 2006).

It should be noted that this method of barb grafting is generally employed in other species (Haghighi *et al*., 2016); however, it responded well in papaya, but it is noteworthy that the development conditions did not involve temperature and humidity control, as detailed in other species and strictly as established in the literature (Hassell *et al*., 2008), which makes this work interesting since even one of the barb grafts showed attachment. Regarding grafting in other species, the uniformity and vigor of the seedlings increase over the recovery time and they are more resistant to biotic and abiotic stresses (Leonardi and Romano, 2004), which is similar to the results obtained.

## Conclusions

The genotypes showed development capacity and productivity, so they are lines with potential for use as rootstocks since the morphological characteristics resemble the size shown by the commercial materials subject to grafting. Regarding the seedling state, the rootstock materials presented appropriate development in all variables, so it is possible to program their sowing establishment in order to synchronize the grafting process in papaya seedlings.

In relation to the grafting method for papaya, modified approximation grafting (AMI) was outstanding due to its high percentage of attachment (92.5%); likewise, the seedlings grafted under this method were vigorous three and six days after cutting.

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